ISM2016
The Golden Jubilee Annual Meeting of the Israel Society for Microscopy
May 31st – June 2nd, 2016
Haifa, Israel

BOOK OF ABSTRACTS
The Israel Society for Microscopy wishes to dedicate the ISM Golden Jubilee meeting to the memory of its founders.

- **Prof. David Danon - President** (1921-2015) - דנון דוד פרופ׳
- **Prof. Olga Stein - Vice President** (1925-2016) - שטיין אולגה פרופ׳ - סגנית יו״ר
- **Dr. Yehuda Marikovsky** (Morgenbesser) - מזכיר - יודה יודה מרקובסקי
- **Prof. Moshe Wollman** (1914-2009) - וולמן משה פרופ׳ - ירו וולמן
- **Dr. Shimon Klein** (1922-1984) - קלין שמעון פרופ׳ - ד׳ קלין שמעון
- **Prof. Jack Gross** (1921-1994) - גרוס ג׳ק פרופ׳ - פרופ׳ ג׳ק גרוס
- **Mr. Natan Orgal** (1928-2000) - אורגל נטן ירו - נטן אורגל
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**TIMETABLE**

**Location of Sessions:**
4th floor: Halls A,B,C, Exhibition, Registration, Lunch
5th floor: Workshops - Halls D,E,F including lunch for workshop participants

**MS** = Materials Science  **LS** = Life Sciences

### TUESDAY, MAY 31, 2016

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tbody>
<tr>
<td>08:30-09:30</td>
<td>Registration, Light Refreshments, Vendors Exhibition, Poster Mounting</td>
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</tr>
<tr>
<td>09:30-10:00</td>
<td>OPENING SESSION</td>
<td>Hall A</td>
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<tr>
<td>10:00-10:45</td>
<td>PLENARY SESSION 1: David A. Muller, USA</td>
<td>Hall A</td>
</tr>
<tr>
<td>10:45-11:15</td>
<td>Coffee Break and Vendors Exhibition</td>
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<tr>
<td>11:15-12:00</td>
<td>PLENARY SESSION 1 (cont’d): Ernst H.K. Stelzer, Germany</td>
<td>Hall A</td>
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<tr>
<td>12:00-12:30</td>
<td>SOUND BITE SESSION</td>
<td>Hall A</td>
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<tr>
<td>12:30-13:30</td>
<td>Lunch, Vendors Exhibition and Parallel Lunch Workshops</td>
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<tr>
<td>12:30-13:30</td>
<td>POSTER SESSION A; DEVICE FABRICATION COMPETITION &amp; MICROGRAPH COMPETITION</td>
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<tr>
<td>13:30-14:30</td>
<td>ISM GENERAL ASSEMBLY (Hall C)</td>
<td></td>
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<tr>
<td>14:00-16:10</td>
<td>Advances in Electron Microscopy Imaging</td>
<td>Hall A</td>
</tr>
<tr>
<td>16:10-16:40</td>
<td>Coffee Break and Vendors Exhibition</td>
<td></td>
</tr>
<tr>
<td>16:40-18:20</td>
<td>POSTER SESSION B; DEVICE FABRICATION COMPETITION &amp; MICROGRAPH COMPETITION</td>
<td></td>
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<tr>
<td>18:00-18:45</td>
<td>“Mysteries of the Unseen World” Movie by FEI &amp; National Geographic</td>
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<tr>
<td>19:15-22:00</td>
<td>CONFERENCE DINNER – Sponsored by FEI (Optional)</td>
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### WEDNESDAY, JUNE 1, 2016

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<th>Time</th>
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<tbody>
<tr>
<td>08:30-09:00</td>
<td>Registration, Light Refreshments, Vendors Exhibition and Poster Mounting</td>
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<tr>
<td>09:00-09:15</td>
<td>PLENARY SESSION 2: Paula da Fonseca, UK</td>
<td>Hall A</td>
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<tr>
<td>09:45-10:15</td>
<td>Coffee Break and Vendors Exhibition</td>
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<tr>
<td>10:15-11:00</td>
<td>PLENARY SESSION 2 (cont’d): David N. Seidman, USA</td>
<td>Hall A</td>
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<tr>
<td>11:00-11:30</td>
<td>SPECIAL JUBILEE SESSION 1: Martin Kessel, Israel</td>
<td>Hall A</td>
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<tr>
<td>11:30-12:00</td>
<td>SOUND BITE SESSION</td>
<td>Hall A</td>
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<tr>
<td>12:00-13:00</td>
<td>Lunch, Vendors Exhibition and Parallel Lunch Workshops</td>
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<tr>
<td>12:00-13:00</td>
<td>POSTER SESSION B; DEVICE FABRICATION COMPETITION &amp; MICROGRAPH COMPETITION</td>
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<tr>
<td>14:00-15:40</td>
<td>Advances in Spectroscopy and STM</td>
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<tr>
<td>15:40-16:10</td>
<td>Coffee Break &amp; Vendors Exhibition</td>
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<tr>
<td>16:10-17:50</td>
<td>&quot;Mysteries of the Unseen World&quot; Movie by FEI &amp; National Geographic</td>
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<tr>
<td>18:00-18:45</td>
<td>18:50 Departure from the Conference venue entrance lobby</td>
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## THURSDAY, JUNE 2, 2016

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<tr>
<td>08:30-09:00</td>
<td>Registration, Light Refreshments and Vendors Exhibition</td>
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<tr>
<td>09:00-09:45</td>
<td>PLENARY SESSION 3: Peter Rez, USA Hall A</td>
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<tr>
<td>09:45-10:15</td>
<td>Coffee Break and Vendors Exhibition</td>
</tr>
<tr>
<td>10:15-11:00</td>
<td>PLENARY SESSION 3 (cont’d): Hari Shroff, USA Hall A</td>
</tr>
<tr>
<td>11:00-12:00</td>
<td>SPECIAL JUBILEE SESSION 2:</td>
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<td></td>
<td>Hans J. Tanke, The Netherlands; Susan I. Anderson, UK</td>
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<tr>
<td>12:00-13:20</td>
<td>Lunch, Vendors Exhibition and Parallel Lunch Workshops</td>
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<tr>
<td>12:00-13:15</td>
<td>Lunch Workshop 7 Protochips Inc.</td>
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<td>Lunch Workshop 8 Danyel Biotech for GE Healthcare</td>
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<td></td>
<td>Lunch Workshop 9 Gatan (AVBA)</td>
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<tr>
<td>13:20-15:00</td>
<td>MS7 - Hall A</td>
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<td></td>
<td>In Situ Electron Microscopy</td>
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<tr>
<td>15:00-15:30</td>
<td>CLOSING SESSION:</td>
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<tr>
<td></td>
<td>Hall A</td>
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<tr>
<td></td>
<td>Concluding remarks; Best Poster, Best Micrograph and Best Device Fabrication Competitions Awards</td>
</tr>
<tr>
<td>15:45</td>
<td>Conference Tour of Haifa (sponsored by the Municipality of Haifa)</td>
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<td></td>
<td>Assemble for departure at the Conference venue entrance lobby</td>
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</table>

### Transportation to/from the Conference Venue

**To the Conference Venue:**
- 08:15 from the Leonardo Hotel
- 08:25 from the Haifa Youth Hostel

**Return:** 10 minutes after last session

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**Transportation to/from the Conference Venue**

**Tuesday, Wednesday, Thursday**

- 08:15 from the Leonardo Hotel
- 08:25 from the Haifa Youth Hostel

**Return:** 10 minutes after last session
Dear Colleagues,

We are happy and honored to welcome you to ISM2016 – a three-day conference celebrating the Golden Jubilee Meeting of the Israel Society for Microscopy (ISM).

ISM2016 is the largest meeting held by the ISM since it hosted the 6th European Congress on Electron Microscopy in Jerusalem 40 years ago, in 1976. The meeting is preceded by a workshop on In-situ Electron Microscopy and its program includes 6 plenary lectures and 14 parallel sessions, covering a wide pallet of subjects and microscopy techniques and applications in Materials Science as well as in Life Sciences. More than 80 posters will be displayed and some of them will be presented orally in sound bite sessions. Three special Golden-Jubilee lectures will take us to a tour from our past, describing the history of ISM, to present days, presenting the development of fluorescence light microscopy during the last 50 years and then to the future – education of our young generation.

We are thankful to our colleagues from abroad, coming from more than 14 countries to celebrate with us and share their knowledge. We also thank our partners from the industry, and from the academia for their financial support and for making this meeting possible. You are encouraged to take advantage of the exceptionally large vendor exhibition, which includes more than 22 booths, displaying cutting edge technology, presented by experts from Israel as well as from abroad.

The word Jubilee stems, most probably from the Hebrew word Yovel, יובל, meaning a ram. According to very ancient Hebrew laws (not practiced at least since 2,000 years ago), the 50th year, or the jubilee year, is a sacred year of freedom, in which slaves are freed. We hope that the ISM jubilee meeting will be a celebration of freedom of the mind as well as a celebration of the renaissance we experience in so many field of microscopy. We also hope that this meeting will be a fulcrum in our activities, leading to further development of microscopy in Israel and to flourishing collaborations with our colleagues abroad.

We hope that you will enjoy this meeting, find it fruitful and be inspired by the talks, posters and various activities.

On behalf of the organizing committee,

Eyal Shimoni    Yaron Kauffmann
Chair, ISM       Secretary, ISM
ORGANIZING COMMITTEES

General Organizing Committee
Eyal Shimoni, Chairperson, Weizmann Institute of Science
Yaron Kauffmann, Secretary, Technion-Israel Institute of Technology
Zahava Barkay, Treasurer, Tel Aviv University
Yuval Garini, Bar-Ilan University
Aryeh Weiss, Bar-Ilan University
Louisa Meshi, Ben-Gurion University
Edith Suss-Toby, Technion-Israel Institute of Technology
Dganit Danino, Technion-Israel Institute of Technology
Maya Bar-Sadan, Ben-Gurion University
Amit Kohn, Tel Aviv University
Inna Popov, The Hebrew University of Jerusalem

Local Organizing Committee
Yaron Kauffmann, Technion-Israel Institute of Technology
Edith Suss-Toby, Technion-Israel Institute of Technology
Dganit Danino, Technion-Israel Institute of Technology
Wayne D. Kaplan, Technion-Israel Institute of Technology
Maayan Duvshani-Eshet, Technion-Israel Institute of Technology
Ellina Kesselman, Technion-Israel Institute of Technology

International Advisory Board
Rafal Dunin-Borkowski, Research Centre Jülich, Jülich, Germany
Alberto Diaspro, Istituto Italiano di Tecnologia (IIT), Genoa, Italy
Alasdair Steven, National Institutes of Health (NIH), Bethesda, Maryland, USA

Life Science Scientific Committee
Edith Suss-Toby, Chair, Technion-Israel Institute of Technology
Aryeh Weiss, Bar-Ilan University
Dganit Danino, Technion-Israel Institute of Technology
Eyal Shimoni, Weizmann Institute of Science
Yuval Garini, Bar-Ilan University
Ilan Tsarfaty, Tel Aviv University

Materials Science Scientific Committee
Amit Kohn, Chair, Tel Aviv University
Inna Popov, The Hebrew University of Jerusalem
Yaron Kauffmann, Technion-Israel Institute of Technology
Peri Landau, NRCN
The Israel Society for Microscopy gratefully acknowledges the support and assistance rendered by the following:

**Platinum Sponsors**

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- PicoTech
- TESCAN
- Polytec

**Gold Sponsors**

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- danyelBIOTECH
- IDEA Group
## SPONSORS & EXHIBITORS (continued)

### Silver Sponsors

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<td><img src="image" alt="Bruker" /></td>
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*The Business of Science*
# SPONSORS & EXHIBITORS (continued)

## Sponsors from the Industry

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<tr>
<th>Applied Materials</th>
<th>Dens Solutions</th>
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<tr>
<td><img src="image" alt="Jerusalem Nano Bible" /></td>
<td><img src="image" alt="Protochips" /></td>
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## Research and Academic Institutes

<table>
<thead>
<tr>
<th>Technion Israel Institute of Technology</th>
<th>RBNI The Russel Berrie Nanotechnology Institute Technion – Israel Institute of Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Moshe Yanai Foundation for International Scientific Relations</td>
<td>The Nancy and Stephen Grand Technion Energy Program (GTEP)</td>
</tr>
<tr>
<td>The Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging</td>
<td>Lorry I. Lokey Interdisciplinary Center for Life Sciences &amp; Engineering, Technion</td>
</tr>
<tr>
<td>The Ilse Katz Institute for Nanoscale Science &amp; Technology Ben-Gurion University of the Negev</td>
<td>The Benoziyo Fund for the Advancement of Science</td>
</tr>
<tr>
<td>Wolfson Applied Materials Research Center</td>
<td>Bar Ilan Institute for Nanotechnology &amp; Advanced Materials</td>
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<tr>
<td>Weizmann Institute of Science</td>
<td>The Kimmel Center for Nanoscale Studies (KCNS)</td>
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<td><img src="image" alt="BINA logo" /></td>
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GENERAL INFORMATION

Meeting Venue
Sammy Ofer Stadium Conference Center
2, Rutenberg Street
Haifa, Israel

Internet
Free WI-FI will be available at all meeting areas. To connect, use SOpress, password stadium1

Registration and Hospitality Desk
Diesenhaus-Unitours will operate the registration and hospitality desk throughout the conference, daily, from 08:30 until the end of the last session.

Registration Envelope
The envelope you received upon registration contains your name badge, tickets to lunches and the optional Conference Dinner, if purchased as well as a voting slip for the Micrograph Competition. Please wear the badge throughout the meeting and present the relevant ticket at each function.

Speakers and Session Chairpersons
Session chairs and speakers are requested to meet with each other 10 minutes prior to the commencement of their respective sessions, in the session hall. In order to make it possible for participants to plan attendance at different parallel sessions, it is essential that the timetable be strictly adhered to. Please take into consideration that the last 5 minutes of each talk are reserved for questions.

Data Projection
Please bring your presentation on a USB flash drive (disk-on-key) to the AV technician in your session hall, at least one hour before the start of your session and have him load it on the meeting’s computer. If you wish to use your own portable computer, please check it with him during a break prior to your session.

Instructions for Poster Presenters
Please hang your poster on the final board number listed in this program.
Poster presenters are required to mount and remove their posters as follows:

Tuesday - Poster Session A:
Mount from 08:30. Remove at the end of the day.

Wednesday - Poster Session B:
Mount from 08:30. Remove by the end of the Meeting on Thursday.
Conference Program - Tuesday, May 31

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
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<tr>
<td>08:30 - 09:30</td>
<td>Registration, Light Refreshments, Vendors Exhibition, Poster Mounting</td>
</tr>
<tr>
<td>09:00 - 10:00</td>
<td>OPENING SESSION (Hall A)</td>
</tr>
<tr>
<td>Session Chair:</td>
<td>Eyal Shimoni, ISM Chairperson</td>
</tr>
<tr>
<td>09:30 - 10:00</td>
<td>Prof. Dan Shechtman and Prof. Wayne D. Kaplan, Technion - Israel Institute of Technology - Greetings, opening remarks and presentation of the ISM Honorary Fellowship and the Lev Margulis Prizes</td>
</tr>
<tr>
<td>10:00 - 10:45</td>
<td>PLENARY SESSION #1 (Hall A)</td>
</tr>
<tr>
<td>Session Chairs:</td>
<td>Wayne D. Kaplan, Technion - Israel Institute of Technology, Haifa Adi Salzberg, Technion - Israel Institute of Technology, Haifa</td>
</tr>
<tr>
<td>10:00 - 10:45</td>
<td>Plenary Lecture: David A. Muller, Cornell University, USA MEASURING PHYSICAL AND ELECTRONIC PROPERTIES AT THE NANOSCALE</td>
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<tr>
<td>10:45 - 11:15</td>
<td>Coffee Break and Vendors Exhibition</td>
</tr>
<tr>
<td>11:15 - 12:00</td>
<td>Plenary Lecture: Ernst H.K. Stelzer, Goethe-Universität Frankfurt, Germany SUPER-RESOLUTION AND LIGHT SHEET-BASED FLUORESCENCE MICROSCOPY</td>
</tr>
<tr>
<td>12:00 - 12:30</td>
<td>SOUND BITE SESSION (Hall A)</td>
</tr>
<tr>
<td>Session Chair:</td>
<td>Louisa Meshi, Ben-Gurion University of the Negev, Beer Sheva</td>
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<tr>
<td>12:00 - 12:30</td>
<td>Posters Sound Bites</td>
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<tr>
<td>12:30 - 13:30</td>
<td>LUNCH SESSION</td>
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<tr>
<td>13:30 - 14:30</td>
<td>PARALLEL SESSIONS</td>
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<tr>
<td>Poster Session A (Hall A)</td>
<td>LIFE SCIENCES POSTERS (PA-1-17) MATERIALS SCIENCE POSTERS (PA-18-36)</td>
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<tr>
<td>DEVICE FABRICATION COMPETITION (PA-37-40)</td>
<td>MICROGRAPH COMPETITION (PA-41-54) VENDORS EXHIBITION</td>
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<tr>
<td>14:30 - 16:10</td>
<td>LIFE SCIENCES Posters (MS1)</td>
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<tr>
<td>14:30 - 16:10</td>
<td>Life Science LS1</td>
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<td>16:10 - 16:40</td>
<td>Life Science LS2</td>
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<tr>
<td>14:30 - 16:10</td>
<td>LIFE SCIENCE Posters (LS1)</td>
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<td>16:40 - 18:20</td>
<td>LIFE SCIENCE Posters (LS2)</td>
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<td>14:30 - 16:10</td>
<td>LIFE SCIENCE Posters (LS3)</td>
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<tr>
<td>16:40 - 18:20</td>
<td>LIFE SCIENCE Posters (LS3)</td>
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<td>14:30 - 16:10</td>
<td>LIFE SCIENCE Posters (LS3)</td>
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<tr>
<td>16:40 - 18:20</td>
<td>LIFE SCIENCE Posters (LS3)</td>
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## Parallel Sessions - Tuesday, May 31

<table>
<thead>
<tr>
<th>Time</th>
<th>Session A (Hall A)</th>
<th>Session B (Hall B)</th>
<th>Session C (Hall C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:30 - 15:00</td>
<td>Lothar Houben, Weizmann Institute of Science, Rehovot, Germany</td>
<td>Kwanghun Chung, Massachusetts Institute of Technology (MIT), USA</td>
<td>Roland Fleck, King’s College London, UK</td>
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<tr>
<td></td>
<td>ENERGY-FILTERED AND LOW-VOLTAGE CHROMATIC ABERRATION-CORRECTED HIGH-RESOLUTION TEM</td>
<td>SCALABLE PROTEOMIC IMAGING OF INTACT BIOLOGICAL SYSTEMS</td>
<td>UNDERSTANDING INTRACELLULAR ORGANIZATION IN CELLS AS REVEALED BY 3D ELECTRON MICROSCOPY</td>
</tr>
<tr>
<td>15:00 - 15:20</td>
<td>Ute Kaiser, Ulm University, Germany</td>
<td>Adi Schejter Bar-Noam, Technion - Israel Institute of Technology, Haifa</td>
<td>Moran Shalev Ben Ami, Weizmann Institute of Science, Rehovot</td>
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<td>PERFORMANCE AND FIRST RESULTS OF THE C/C-CORRECTED 20-80KV SALVE MICROSCOPE</td>
<td>TWO-PHOTON IN VIVO IMAGING OF THE MOUSE RETINA</td>
<td>INVESTIGATIONS OF EUKARYOTIC TRANSLATION MACHINERIES THROUGH SINGLE PARTICLE CRYO-EM</td>
</tr>
<tr>
<td>15:20 - 15:40</td>
<td>Roy Shiloh, Tel Aviv University, Tel Aviv</td>
<td>Vyacheslav Kalchenko, Weizmann Institute of Science, Rehovot</td>
<td>Elad Milrot, Weizmann Institute of Science, Rehovot</td>
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<tr>
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<td>PROSPECTS FOR ELECTRON BEAM ABERRATION CORRECTION USING SCULPTED PHASE MASKS</td>
<td>A SIMPLE K-Omega ADAPTIVE METHOD TO REGISTER DYNAMIC MICROSCOPY IMAGE SEQUENCES</td>
<td>THE BACTERIOPHAGE LIKE INFECTION OF THE LARGE EUKARYOTIC INFECTING VIRUS</td>
</tr>
<tr>
<td>15:40 - 16:10</td>
<td>Semyon Shofman, El-Mul Technologies, Ltd., Rehovot</td>
<td>Jackie Schiller, Technion - Israel Institute of Technology, Haifa</td>
<td>Dorit Hanein, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA</td>
</tr>
<tr>
<td></td>
<td>ENHANCING THE DETECTION OF VERTICALLY EMITTED SECONDARY ELECTRONS IN FIB SYSTEM BASED ON 3D ELECTRON TRAJECTORY SIMULATIONS</td>
<td>USING TWO PHOTON MICROSCOPY FOR BRAIN RESEARCH</td>
<td>NANO SCALE CHARACTERIZATION OF LEADING EDGE PROTRUSIONS OF WHOLE MAMMALIAN CELLS: A QUANTITATIVE CORRELATIVE CRYO-LIGHT AND ELECTRON CRYO-TOMOGRAPHY STUDY</td>
</tr>
</tbody>
</table>
## Parallel Sessions - Tuesday, May 31

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>LS3 / Hall A</th>
<th>MS2 / Hall B</th>
<th>MS3 / Hall C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16:40 - 17:10</strong></td>
<td><strong>MICROSCOPY IMAGE ANALYSIS TOOLS</strong></td>
<td>Invited</td>
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<td>Chrysanthe Preza, The University of Memphis, Memphis, TN, USA</td>
<td>Gitti Frey, Technion – Israel Institute of Technology, Haifa</td>
<td>Jordi Arbiol, Institut Català de Nanociència Nanotecnologia, Bellaterra, CAT, Spain</td>
</tr>
<tr>
<td></td>
<td><strong>ADVANCES IN COMPUTATIONAL IMAGING FOR QUANTITATIVE 3D FLUORESCENCE MICROSCOPY</strong></td>
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<tr>
<td><strong>17:10 - 17:30</strong></td>
<td><strong>QUANTITATIVE ANALYSES OF PROTEIN ORGANIZATION IN VIVO</strong></td>
<td>Avital Steinberg, Weizmann Institute of Science, Rehovot</td>
<td>Maor Ram-On, Technion – Israel Institute of Technology, Haifa</td>
<td>Nir Kedem, Weizmann Institute of Science, Rehovot</td>
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<td><strong>17:30 - 17:50</strong></td>
<td><strong>THREE-DIMENSIONAL RECONSTRUCTION FROM CRYO-ELECTRON MICROSCOPE IMAGES OF SINGLE PARTICLES IN FOURIER SPACE USING A NEW PROGRAM, ICR3D</strong></td>
<td>Edward Morris, Institute of Cancer Research, UK</td>
<td>Luba Kolik Shmuel, Technion – Israel Institute of Technology, Haifa</td>
<td>Hadar Nahor, Technion – Israel Institute of Technology, Haifa</td>
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<tr>
<td><strong>17:50 - 18:20</strong></td>
<td><strong>SINGLE MOLECULE APPROACHES FOR STUDYING GENE EXPRESSION IN INTACT MAMMALIAN TISSUES</strong></td>
<td>Shailev Itzkovitz, Weizmann Institute of Science, Rehovot</td>
<td>Oren Reggew, Ben-Gurion University of the Negev, Be’er Sheva</td>
<td>Magnus Garbrecht, Linköping University, Linköping, Sweden</td>
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<td><strong>USING HIGH RESOLUTION ELECTRON MICROSCOPY FOR STRUCTURE-PERFORMANCE CORRELATION IN ORGANIC AND HYBRID SOLAR CELLS</strong></td>
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<td></td>
<td><strong>EFFECT OF POLYELECTROLYTE STIFFNESS AND SOLUTION pH ON THE NANOSTRUCTURE OF COMPLEXES FORMED BY CATIONIC AMPHIPHILES AND NEGATIVELY CHARGED POLYELECTROLYTES</strong></td>
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<td></td>
<td><strong>ONE-DIMENSIONAL SELF-ASSEMBLY OF DIACETYLENIC PHOSPHOLIPID INTO TUBULAR STRUCTURES</strong></td>
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<td></td>
<td><strong>DISCERNING INTERFACE ATOMIC STRUCTURE BY PHASE CONTRAST IN STEM</strong></td>
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<tr>
<td></td>
<td><strong>THERMAL STABILITY AND MICROSTRUCTURAL EVOLUTION OF NITRIDE-BASED METAL/SEMICONDUCTOR SUPERLATTICES</strong></td>
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</tbody>
</table>
### Conference Program – Wednesday, June 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:30 - 09:00</td>
<td>Registration, Light Refreshments, Vendors Exhibition, Poster Mounting</td>
</tr>
<tr>
<td>09:00 - 09:45</td>
<td><strong>PLENARY SESSION #2 (Hall A)</strong></td>
</tr>
<tr>
<td>Session Chairs:</td>
<td>Dganit Danino, Technion - Israel Institute of Technology, Haifa</td>
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<tr>
<td></td>
<td>Yossi Rosenwaks, Tel Aviv University, Tel Aviv</td>
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<tr>
<td></td>
<td>Plenary Lecture: Paula da Fonseca, Cambridge Biomedical Campus, UK</td>
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<tr>
<td>09:45 - 10:15</td>
<td>Coffee Break and Vendors Exhibition</td>
</tr>
<tr>
<td>10:15 - 11:00</td>
<td>Plenary Lecture: David N. Seidman, Northwestern University, USA</td>
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<td></td>
<td>CONTRIBUTIONS OF ATOM-PROBE TOMOGRAPHY TO THE SCIENCE AND DEVELOPMENT OF ALUMINUM-BASED SUPERALLOYS WITH COARSERING AND CREEP RESISTANCE UP TO 673 K</td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td><strong>SPECIAL JUBILEE SESSION #1 (Hall A)</strong></td>
</tr>
<tr>
<td>Session Chair:</td>
<td>Ruth Sperling, The Hebrew University of Jerusalem</td>
</tr>
<tr>
<td>11:30 - 12:00</td>
<td>Posters Sound Bites</td>
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<tr>
<td>12:00 - 13:00</td>
<td>Lunch and Vendors Exhibition</td>
</tr>
<tr>
<td></td>
<td>Lunch Workshops (5th floor)</td>
</tr>
<tr>
<td>13:00 - 14:00</td>
<td><strong>PARALLEL SESSIONS</strong></td>
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<tr>
<td></td>
<td><em>Poster Session B (Hall A)</em></td>
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<tr>
<td></td>
<td>LIFE SCIENCES POSTERS (PB-1-16)</td>
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<tr>
<td></td>
<td>MATERIALS SCIENCE POSTERS (PB-17-37)</td>
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<tr>
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<td>DEVICE FABRICATION COMPETITION (PA-37-40)</td>
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<td>MICROGRAPH COMPETITION (PA-41-54)</td>
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<td>VENDORS EXHIBITION</td>
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<tr>
<td>14:00 - 15:40</td>
<td>Life Science LS4</td>
</tr>
<tr>
<td>15:40 - 16:10</td>
<td>Coffee Break and Vendors Exhibition</td>
</tr>
<tr>
<td>16:10 - 17:50</td>
<td>Materials Science MS4</td>
</tr>
<tr>
<td>18:00 - 18:45</td>
<td>&quot;Mysteries of the Unseen World&quot; - Movie by FEI &amp; National Geographic</td>
</tr>
<tr>
<td>19:15 - 22:00</td>
<td>Conference Dinner - Sponsored by FEI (Optional)</td>
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<td>Presentation of the FEI Company Award &amp; Artistic program</td>
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<td></td>
<td>18:50 Assemble for departure at the Conference venue entrance lobby - Ticket required.</td>
</tr>
</tbody>
</table>
### Parallel Sessions - Wednesday, June 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>LS4 / Hall A</th>
<th>MS4 / Hall B</th>
<th>MS5 / Hall C</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td><strong>CORRELATIVE LIGHT &amp; ELECTRON MICROSCOPY</strong></td>
<td>Invited</td>
<td>Invited</td>
<td>Invited</td>
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<tr>
<td>14:00</td>
<td>Correlate Light and Electron Microscopy in Cell Biology</td>
<td>Bruno Humbel, University of Lausanne, Switzerland</td>
<td>Mathieu Kociak, CNRS/Université Paris Sud, Orsay, France</td>
<td>Ute Kolb, Johannes Gutenberg-University, Mainz, Germany</td>
</tr>
<tr>
<td>14:30</td>
<td><strong>ADVANCES IN SPECTROSCOPY AND STM</strong></td>
<td>Invited</td>
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<tr>
<td>14:30</td>
<td>Biomineralization Pathways in a Foraminifer Revealed Using a Novel Correlative Cryo-Fluorescence-SEM-EDS Technique</td>
<td>Gal Mor Khalifa, Weizmann Institute of Science, Rehovot</td>
<td>Mahdi Halabi, Ben-Gurion University of the Negev, Beer Sheva</td>
<td>Ran Elian Alshbul, Ben-Gurion University of the Negev, Beer Sheva</td>
</tr>
<tr>
<td>14:50</td>
<td><strong>SHAPE AND PHASE CONTROL OF NANOCRYSTALLINE ( \pi )-SnS AND ( \pi )-SnSe – NEW CUBIC PHASES IN THE TIN CHALCOGENIDE SYSTEM</strong></td>
<td>Invited</td>
<td>Invited</td>
<td>Invited</td>
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<tr>
<td>15:10</td>
<td><strong>ARRAY TOMOGRAPHY BASED DIRECT 3D-CLEM APPROACH</strong></td>
<td>Invited</td>
<td>Invited</td>
<td>Invited</td>
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<tr>
<td>15:10</td>
<td>Array Tomography Based Direct 3D-CLEM Approach</td>
<td>Irina Kolotau, University of Lausanne, Lausanne, Switzerland</td>
<td>Yossi Rosenwaks, Tel Aviv University, Tel Aviv</td>
<td>Louisa Meshi, Ben-Gurion University of the Negev, Beer Sheva</td>
</tr>
<tr>
<td>Time</td>
<td>Topic</td>
<td>MS6 / Hall A</td>
<td>LS5 / Hall B</td>
<td>LS6 / Hall C</td>
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<tr>
<td>16:10 - 16:40</td>
<td>LOW DIMENSIONAL MATERIALS</td>
<td>VARIABLE TEMPERATURE STM STUDIES OF INDIVIDUAL MAGNETITE NANOCRYSTALS</td>
<td>IMAGING OF BIOLOGICAL SPECIMEN WITH LIQUID STEM, 3D STEM, IN SITU STEM, AND ABBERRATION CORRECTED STEM. RESOLVING THE LOCATIONS OF INDIVIDUAL PROTEINS WITHIN THE CONTEXT OF INTACT CELLS</td>
<td>“GREAT EXPECTATIONS” OR CHALLENGES FOR TRANSMISSION ELECTRON MICROSCOPY IN THE IDENTIFICATION OF THE DIVERSE POPULATION OF EXTRACELLULAR VESICLES</td>
</tr>
<tr>
<td></td>
<td>Session chair</td>
<td>Gil Markovich, Tel Aviv University, Tel Aviv</td>
<td>NM – Leibniz Institute for New Materials, Saarbrücken, Germany</td>
<td>Agnes Kittel, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary</td>
</tr>
<tr>
<td>16:40 - 17:00</td>
<td>MATERIALIZED NANOAGENTS: INSIGHTS INTO THE ATOMIC SCALE</td>
<td>MAYA BAR SADAN, BEN-GURION UNIVERSITY OF THE NEGEV, BEER-SHEVA</td>
<td>RESOLVING NEW ULTRASTRUCTURAL FEATURES OF CYTOKINETIC ABSCISSION WITH SOFT-X-RAY CRYO-TOMOGRAPHY</td>
<td>LIVE IMAGING OF APOPTOTIC CELL CLEARANCE IN DROSOPHILA EMBRYO</td>
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<tr>
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<td>Nanoporous single crystals of gold</td>
<td>MARIA KOIFMAN KHristosov, TECHNION – ISRAEL INSTITUTE OF TECHNOLOGY, HAIFA</td>
<td>OMER WAGNER, BAR-ILAN UNIVERSITY, RAMAT-GAN</td>
<td>OMER WAGNER, BAR-ILAN UNIVERSITY, RAMAT-GAN</td>
</tr>
<tr>
<td>17:00 - 17:20</td>
<td>DIMENSIONALITY MATTERS: DIMENSIONALITY EFFECTS ON OPTOELECTRONIC BEHAVIOR OF SEMICONDUCTOR NANOCRYSTALS</td>
<td>URI BANIN, THE HEBREW UNIVERSITY OF JERUSALEM</td>
<td>AMOS DANIELI, BAR-ILAN UNIVERSITY, RAMAT-GAN</td>
<td>LIVE CELL IMAGING OF CELL CYCLE TRANSITIONS</td>
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<tr>
<td></td>
<td>Invited</td>
<td>URI BANIN, THE HEBREW UNIVERSITY OF JERUSALEM</td>
<td>AMOS DANIELI, BAR-ILAN UNIVERSITY, RAMAT-GAN</td>
<td>LIVE CELL IMAGING OF CELL CYCLE TRANSITIONS</td>
</tr>
<tr>
<td>17:20 - 17:50</td>
<td>Label-free nonlinear photoacoustic nanoscopy and spectroscopy</td>
<td>URI BANIN, THE HEBREW UNIVERSITY OF JERUSALEM</td>
<td>AMOS DANIELI, BAR-ILAN UNIVERSITY, RAMAT-GAN</td>
<td>LIVE CELL IMAGING OF CELL CYCLE TRANSITIONS</td>
</tr>
</tbody>
</table>
# Conference Program - Thursday, June 2

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Details</th>
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<tbody>
<tr>
<td>08:30 - 09:00</td>
<td>Registration, Light Refreshments, Vendors Exhibition</td>
</tr>
<tr>
<td><strong>PLENARY SESSION #3 (Hall A)</strong></td>
<td></td>
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</tbody>
</table>
| **Session Chairs:** | Michael Elbaum, Weizmann Institute of Science  
Natalie Elia, Ben-Gurion University of the Negev, Beer Sheva |
| 09:00 - 09:45 | Plenary Lecture: Peter Rez, Arizona State University, USA  
PUSHING BACK THE FRONTIERS OF ELECTRON MICROSCOPY: THEORY AS A GUIDE |
| 09:45 - 10:15 | Coffee Break and Vendors Exhibition |
| 10:15 - 11:00 | Plenary Lecture: Hari Shroff, National Inst. of Biomedical Imaging & Bioengineering, Bethesda, USA  
HIGH SPEED BIOLOGICAL IMAGING AT AND BEYOND THE DIFFRACTION LIMIT |
| **SPECIAL JUBILEE SESSION #2 (Hall A)** | |
| **Session Chairs:** | Yuval Garini, Bar-Ilan University, Ramat-Gan  
Einat Zelinger, The Hebrew University of Jerusalem |
| 11:00 - 11:30 | Hans J. Tanke, Leiden University Medical Center, The Netherlands  
HOW ADVANCED MICROSCOPY DEVELOPED IN 50 YEARS TO ANALYSE THE MOLECULAR MACHINERY IN CELLS |
| 11:30 - 12:00 | Susan I. Anderson, University of Nottingham School of Medicine, UK  
EDUCATION AND OUTREACH AT THE ROYAL MICROSCOPICAL SOCIETY: DEVELOPING AND IMPLEMENTING A PROGRAMME FOR ALL- FROM SCHOOLS EDUCATION TO CPD FOR PROFESSIONAL MICROSCOPISTS |
| **LUNCH SESSION** | |
| 12:00 - 13:20 | Lunch and Vendors Exhibition  
Lunch Workshops (5th floor) |
| **PARALLEL SESSIONS** | |
| 13:20 - 15:00 | Materials Science  
Life Science |
| MS7 | LS7 |
| **CLOSING SESSION (Hall A)** | |
| **Session Chair:** | Eyal Shimoni, ISM Chairperson |
| 15:00 - 15:30 | Concluding remarks  
Best Poster, Best Micrograph & Best Device Fabrication Awards |
| 15:45 | Departure for Conference Tour of Haifa (Sponsored by the Haifa municipality).  
Assemble for departure at the Conference venue entrance lobby |
<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Location</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:20</td>
<td>OPPORTUNITIES AND LIMITATIONS OF ELECTRON MICROSCOPY IN LIQUID ENVIRONMENTS FOR IN SITU STUDIES OF CRYSTAL GROWTH AND DISSOLUTION</td>
<td>MS7 / Hall A</td>
<td>Roland Kröger, University of York, UK</td>
</tr>
<tr>
<td>13:50</td>
<td>DIRECT OBSERVATIONS OF INTERACTIONS BETWEEN WATER AND SINGLE WS2 NANOTUBES IN SITU SEM AND AFM STUDIES</td>
<td>MS7 / Hall A</td>
<td>Ifat Kaplan-Ashiri, Weizmann Institute of Science, Rehovot</td>
</tr>
<tr>
<td>14:10</td>
<td>SYNTHESIS AND CHARACTERIZATION OF NANO-SCALE BUILDING BLOCKS WITH ELECTRONIC AND STRUCTURAL HETEROGENEITY BY POST-SYNTHESIS MODIFICATIONS</td>
<td>LS7 / Hall C</td>
<td>Pavel Sidorenko, Technion – Israel Institute of Technology, Haifa</td>
</tr>
<tr>
<td>14:30</td>
<td>IN-SITU NANOSCALE WETTABILITY STUDY</td>
<td>Invited</td>
<td>Natalie Elia, Ben-Gurion University of the Negev, Beer Sheva</td>
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<tr>
<td>14:30</td>
<td>INVITED</td>
<td>Invited</td>
<td>Boaz Pokroy, Technion – Israel Institute of Technology, Haifa</td>
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<tr>
<td>14:30</td>
<td>INVITED</td>
<td>Invited</td>
<td>Yuval Garini, Bar-Ilan University, Ramat-Gan</td>
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<tr>
<td>13:20</td>
<td>SUPER-RESOLUTION FLUORESCENCE IMAGING BY dSTORM</td>
<td>LS7 / Hall C</td>
<td>Markus Sauer, University of Würzburg, Germany</td>
</tr>
<tr>
<td>13:50</td>
<td>USING TEMPORAL CORRELATIONS OF FLUORESCENCE INTENSITY TO IMPROVE SINGLE MOLECULE LOCALIZATION MICROSCOPY</td>
<td>LS7 / Hall C</td>
<td>Shachar Schidorsky, The Hebrew University of Jerusalem</td>
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<tr>
<td>14:10</td>
<td>SINGLE-SHOT Ptychography, overcoming the scanning-limited temporal resolution and also demonstrate sparsity-based subwavelength Ptychographic microscopy approach, overcoming the Abbe spatial resolution limit</td>
<td>LS7 / Hall C</td>
<td>Pavol Sidor, Technion – Israel Institute of Technology, Haifa</td>
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<tr>
<td>14:30</td>
<td>VISUALIZING ESCRT-MEDiated Mammalian Cell ABSciSSION AT NANOSCALE RESOLUTION</td>
<td>Invited</td>
<td>Natalie Elia, Ben-Gurion University of the Negev, Beer Sheva</td>
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</tbody>
</table>

Parallel Sessions - Thursday, June 2, 2016
Tuesday, May 31, 2016

13:30-14:30 Poster Session A

**Life Sciences**

**PA-1** VISUALIZING ESCRT MEDIATED ABCISCISSION DURING EARLY DEVELOPMENT IN LIVE ZEBRAFISH EMBRYOS  
Shai Adar, Ramon Birnbaum, Natalie Elia  
Ben-Gurion University of the Negev, Beer-Sheva, Israel

**PA-2** 3D VISUALIZATION OF BIOLOGICAL TISSUES FROM MICROMETER TO NANOMETER SCALE USING CRYO-FIB-SEM  
Anat Akiva¹, Netta Vidavsky¹, Lia Addadi², Steve Weiner¹, Andreas Schertel²  
¹Weizmann Institute of Science, Rehovot, Israel  
²Carl Zeiss Microscopy GmbH, Oberkochen, Germany

**PA-3** TRACKING shRNA-MEDIATED INHIBITION OF mRNA TRANSCRIPTIONAL ACTIVITY IN THE NUCLEUS OF SINGLE CELLS  
Shira Avivi¹, Amir Mor¹, Iris Dotan², Dani Canaani², Yaron Shav-Tal¹  
¹Bar-Ilan University, Ramat-Gan, Israel  
²Tel Aviv University, Tel Aviv, Israel

**PA-4** COMPUTERIZED CELL FLOW-PROPERTIES ANALYZER (CFA) – INSTRUMENT AND METHODOLOGY FOR VISUALIZATION AND CHARACTERIZATION OF RED BLOOD CELL FLOW PROPERTIES  
Gregory Barshtein, Felix Tsipis, Saul Yedgar  
The Hebrew University of Jerusalem-Medical School, Jerusalem, Israel

**PA-5** THE LOCATION AND INTERACTIONS OF NXF1/Tap DURING mRNA EXPORT EXAMINED AT HIGH RESOLUTION WITHIN INDIVIDUAL NUCLEAR PORES  
Rakefet Ben-Yishay, Amir Mor, Amit Shraga, Asaf Ashkenazy, Yuval Garini, Yaron Shav-Tal  
Bar-Ilan University, Ramat-Gan, Israel

**PA-6** MULTI-SCALE CHARACTERIZATION OF PLATELET-MATRIX ADHESION  
Melanie Bokstad Horev¹, Tova Volberg¹, Revital Zarka¹, Sharon Wolf¹, Hagit Hauschner², Uri Seligsohn², Benjamin Geiger¹  
¹Weizmann Institute of Science, Rehovot, Israel  
²Sheba Medical Center, Tel-Hashomer, Israel

**PA-7** DETECTION OF ISOLATED METAL ATOMS ON PROTEIN BY CRYO-SCANNING TRANSMISSION ELECTRON MICROSCOPY  
Nadav Elad, Giuliano Bellapadrona, Lothar Houben, Irit Sagi, Michael Elbaum  
Weizmann Institute of Science, Rehovot, Israel

**PA-8** IMAGING INTRACELLULAR MINERAL PHASES IN ALGAE USING CRYO-X-RAY TOMOGRAPHY  
Assaf Gal¹,², André Scheffel¹, Damien Faivre²  
¹Max-Planck Institute of Molecular Plant Physiology, Potsdam, Germany  
²Max-Planck Institute of Colloids and Interfaces, Potsdam, Germany
**Poster Session A (continued)**

PA-9  **IS THE ESCRT COMPONENT VPS4 A MICROTUBULE BINDING PROTEIN?**

*Ofir Gershony*, Ofer Shapira, Mehtap Abu-Qarn, Inbar Segal, Dikla Nachmias, Leah Gheber, Natalie Elia  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-10 **A MODEL SYSTEM FOR THE RECONSTITUTION OF THE CELLULAR ACTIN CORTEX**

*Or Gill*, Anne Bernheim-Groswasser  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-11 **RESOLVING MIXED MECHANISMS OF MOLECULAR SUB-DIFFUSION AT THE PLASMA MEMBRANE OF LIVE T-CELLS BY SINGLE PARTICLE TRACKING**

*Yonatan Golan*, Eilon Sherman  
*The Hebrew University of Jerusalem, Jerusalem, Israel*

PA-12 **DECIPHERING THE STRUCTURAL ORGANIZATION OF THE ESCRT COMPLEX DURING CYTOKINETIC ABSCESSION AT NANO SCALE**

*Inna Goland*¹, Shachar Sherman¹, Dikla Nachmias¹, Tali Dadosh², Natalie Elia¹  
¹*Ben-Gurion University of the Negev, Beer-Sheva, Israel*  
²*Weizmann Institute of Science, Rehovot, Israel*

PA-13 **BIOGENIC LIGHT INDUCED TUNEABLE PHOTONIC CRYSTALS**

*Dvir Gur*¹, Ben Leshem¹, Viviana Farstey², Dan Ornon¹, Steve Weiner¹, Lia Addadi¹  
¹*Weizmann Institute of Science, Rehovot, Israel*  
²*The Interuniversity Institute for Marine Sciences, Eilat, Israel*

PA-14 **INTRACELLULAR DYNAMICS AND LOCALIZATION OF β-CATENIN IN RESPONSE TO WNT SIGNALING DURING THE CELL CYCLE**

*Sarah Hasenson*, Pinhas Kafri, Yaron Shav-Tal  
*Bar-Ilan University, Ramat-Gan, Israel*

PA-15 **SUPER RESOLUTION MICROSCOPY ELUCIDATES THE INTERACTION BETWEEN ANAEROBIC RESPIRATORY COMPLEX II AND THE FLAGELLAR MOTOR**

*Anna Koganitsky*, Tali Dadosh, Vladimir Kiss, Michael Eisenbach  
*Weizmann Institute of Science, Rehovot, Israel*

PA-16 **SEARCH FOR COMPOUNDS STIMULATING MITOCHONDRIAL TURNOVER FOR TREATING TYPE 2 DIABETES**

*Noga Kozer*², Fernanda Cerqueira¹, Orian Shirhai¹  
¹*Ben-Gurion University of the Negev, Beer-Sheva, Israel*  
²*Weizmann Institute of Science, Rehovot, Israel*

PA-17 **SILICA DEPOSITION IN SORGHUM SILICA CELLS TAKES PLACE IN VIABLE CELLS**

*Santosh Kumar*¹, Yonat Milstein², Yaniv Brami², Rivka Elbaum¹  
¹*The Hebrew University of Jerusalem, Rehovot, Israel*  
²*B-nano Ltd., Rehovot, Israel*

**Materials Science**

PA-18 **INNOVATIVE PROCEDURES IN PARTICLE ANALYSIS: THE CORRELATIVE MICROSCOPY APPROACH**

*Uri Admon*¹, Ernesto Chinea-Cano², Aryeh Weiss³, Naida Dzigal², Itzhak Halevy¹, Eli Boblil¹  
¹*Nuclear Research Center Negev, IAEC, Beer-Sheva, Israel*  
²*IAEA, Vienna, Austria*  
³*Bar-Ilan University, Ramat-Gan, Israel*
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<tr>
<td>Snejana Bakardjieva¹, Jan Šubrt¹, Robert Klie²</td>
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<td>¹Institute of Inorganic Chemistry AS CR, Prague, Czech Republic</td>
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<td>²UIC Chicago, Chicago, IL, USA</td>
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<td><strong>PA-20</strong></td>
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<td>Maya Barzilay, Yachin Ivry</td>
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<td>Technion-Israel Institute of Technology, Haifa, Israel</td>
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<td>Ora Bitton¹, Kotni Santhosho¹, Lev Chuntonov², Gilad Haran¹</td>
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<td>¹Weizmann Institute of Science, Rehovot, Israel</td>
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<td>Aaron Brahami¹, Efrat Zlotkin-Rivkin¹, Benjamin Aroeti², Oleg Fedoseyeyv², Aaron Lewis¹,²</td>
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<td>¹The Hebrew University of Jerusalem, Jerusalem, Israel</td>
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<td>²Nanomics Imaging, Jerusalem, Israel</td>
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<td>Rajesh Chalasani¹, Alex Pekin², Alex Rabkin², Yuval Golan², Amit Kohn¹</td>
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<td>¹Tel Aviv University, Tel Aviv, Israel</td>
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<td>Keith Dicks</td>
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<td>Oxford Instruments NanoAnalysis, High Wycombe, UK</td>
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<td>Alla Duhin, Zahava Barkay, Alexandra Inberg, Noam Eliaz, Eliezer Gileadi</td>
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<td>Yael Etinger-Geller, Alexander Katsman, Boaz Pokroy</td>
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<td>Ofir Friedman, Alex Upcher, Tzvi Tempelman, Vladimir Ezersky, Yuval Golan</td>
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<td>Nimrod Gazit, Leonid Klinger, Eugen Rabkin</td>
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<td>Technion-Israel Institute of Technology, Haifa, Israel</td>
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<td>Gilad Hemo¹, Eddie Redmard¹, Yafit Fleger²</td>
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<td>¹SanDisk, Omer, Israel</td>
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<td>Poster Session A (continued)</td>
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| **PA-30** TRANSMISSION KIKUCHI DIFFRACTION IN THE SCANNING ELECTRON MICROSCOPE FOR IMAGING OF REVERTED AUSTENITE IN CUSTOM 465° STAINLESS STEEL  
Sigalit Ifergane¹,², Zahava Barkay¹, Ofer Beeri², Noam Eliaz¹  
¹Tel Aviv University, Tel Aviv, Israel  
²Nuclear Research Center Negev, Beer-Sheva, Israel |

| **PA-31** CATION EXCHANGE COMBINED WITH KIRKENDALL EFFECT IN THE PREPARATION OF SnTe/CdTe AND CdTe/SnTe CORE/SHELL NANOCRYSTALS  
Youngjin Jang, Diana Yanover, Richard Karel Capek, Arthur Shapiro, Nathan Grumbach, Yaron Kauffmann, Aldona Sashchiuk, Efrat Lifshitz  
Technion-Israel Institute of Technology, Haifa, Israel |

| **PA-32** DIRECT IMAGING OF CARBON NANOTUBES IN SUPER ACID SOLUTIONS AND LIQUID CRYSTALLINE PHASES  
Olga Kleinerman, Yachin Cohen, Yeshayahu Talmon  
Technion-Israel Institute of Technology, Haifa, Israel |

| **PA-33** "TRANSROTATIONAL" MICROCRYSTALS AND NANOSTRUCTURES DISCOVERED BY TRANSMISSION ELECTRON MICROSCOPY IN CRYSTALLIZING AMORPHOUS FILMS  
Vladimir Yu. Kolosov  
Ural Federal University, Ekaterinburg, Russia |

| **PA-34** SPACE CHARGE REGION AND DIFFUSION LENGTH OF CsPbBr₃ SOLAR CELLS  
Michael Kulbak, Nir Kedem, Gary Hodes, David Cahen  
Weizmann Institute of Science, Rehovot, Israel |

| **PA-35** SYNTHESIS OF CORE-SHELL MoS₂ FULLERENE-LIKE INCORPORATING GOLD NANOPARTICLE Au@IF-MoS₂  
Anna Lavie, Lothar Houben, Ronit Popovitz-Biro, Reshef Tenne  
Weizmann Institute of Science, Rehovot, Israel |

| **PA-36** THE EFFECT OF CARBON NANOTUBE PARAMETERS ON THEIR PHASE BEHAVIOR IN SUPER-ACID SOLUTIONS  
Lucy Liberman, Olga Kleinerman, Yeshayahu Talmon  
Technion-Israel Institute of Technology, Haifa, Israel |
Wednesday, June 1, 2016

13:00-14:00 Poster Session B

Life Sciences: PB-1 - PB-16
Materials Science: PB-17 - PB-37
Device Fabrication Competition: See Tuesday, Poster Session A, PA-37 - PA-40
Micrograph Competition: See Tuesday, Poster Session A, PA-41 - PA-54

Life Sciences

PB-1  POINT SPREAD FUNCTION ESTIMATION FROM PROJECTED SPECKLE ILLUMINATION
Nizan Meitav, Erez N. Ribak, Shy Shoham
Technion-Israel Institute of Technology, Haifa, Israel

PB-2  THE AAA1Pase VPS4 SHAPES EARLY STEPS IN CILIA FORMATION AND ALTERS CENTRIOLAR SATELLITES AND THE PERICENTRIOLAR MATERIAL
Dikla Nachmias¹, Carolyn Ott², Shai Adar¹, Shachar Sherman¹, Jennifer Lippincott-Schwartz², Natalie Elia³
¹Ben-Gurion University of the Negev, Beer-Sheva, Israel
²National Institute of Child Health and Human Development, Bethesda, MD, USA

PB-3  IMMUNOGOLD LABELING OF PHOSPHATIDYL-SERINE BY ANNEXIN V IN CRYO-TEM SPECIMENS
Maayan Nir-Shapira, Naama Koifman, Yeshayahu Talmon
Technion-Israel Institute of Technology, Haifa, Israel

PB-4  MINERAL SCAFFOLDS ENABLE THE MORPHOGENESIS OF BACTERIAL BIOFILMS
Yaara Openheimer-Shaanan, Vlad Brumfeld, Ilana Kolodkin-Gal
Weizmann Institute of Science, Rehovot, Israel

PB-5  ULTRASTRUCTURE STUDY OF BIOLOGICAL MICRO-INJECTION SYSTEM
Gady Piriatsinski, Jonathan Ben David, Yulia Pollak, Tamar Lotan
University of Haifa, Haifa, Israel

PB-6  PROBING THE MECHANICS OF THE HIV CAPSID
Ruben Ramalho¹, Sanela Rankovic¹, Jing Zhou², Christopher Aiken², Itay Rousso¹
¹Ben-Gurion University of the Negev, Beer-Sheva, Israel
²Vanderbilt University School of Medicine, Nashville, Tennesse, USA

PB-7  REVERSE TRANSCRIPTION MECHANICALLY INDUCES HIV-1 UNCOATING
Sanela Rankovic¹, Ruben Ramalho¹, Christopher Aiken², Itay Rousso¹
¹Ben-Gurion University of the Negev, Beer-Sheva, Israel
²Vanderbilt University School of Medicine, Nashville, Tennesse, USA

PB-8  IMPROVEMENT OF THE DECALCIFICATION PROCESS FOR ALL TYPES OF HARD MATERIAL IN THE ROUTINE HISTOLOGY
Ute Schmidt, Daniela Grabis
Merck KGaA, Darmstadt, Germany

PB-9  TAG-FREE LABELING OF CELLULAR PROTEINS IN LIVE CELLS WITH FLUORESCENT ORGANIC DYES
Tomer Schwartz, Noa Alush, Sarit Cohen, Dikla Nachmias, Eyal Arbely, Natalie Elia
Ben-Gurion University of the Negev, Beer-Sheva, Israel

PB-10  A CORRELATIVE STUDY OF OSTEOCLAST ADHESION TO BONE SURFACES
Michal Shemesh¹, Sefi Addadi³, Yonat Milstein², Lia Addadi¹, Benny Geiger¹
¹Weizmann Institute of Science, Rehovot, Israel
²B-nano Ltd., Rehovot, Israel

PB-11 STOMATAL CELL WALL CRYSTALLINITY: DISTINCTIVE STRUCTURAL PATTERNS IN DIVERSE PHYLOGENETIC GROUPS
Ilana Shtein1,2, Yaniv Shelef2, Ziv Marom2, Benny Bar-On3, Zoë A. Popper3, Einaat Zelinger2, Amnon Schwartz1, Smadar Harpaz-Saad1
1The Hebrew University of Jerusalem, Rehovot, Israel
2Ben-Gurion University of the Negev, Beer-Sheva, Israel
3National University of Ireland Galway, Galway, Ireland

PB-12 CORRELATIVE CRYO-SOFT X-RAY TOMOGRAPHY AND STORM STUDY OF CHOLESTEROL CRYSTAL EARLY FORMATION IN CELLS
Neta Varsano1, Sergey Kapishnikov2, Tali Dadash2, Eva Pereiro2, Xueting Jin4, Howard Kruth4, Leslie Leiserowitz1, Lia Addadi1
1Weizmann Institute of Science, Rehovot, Israel
2Helmholtz-Zentrum Berlin, Berlin, Germany
3ALBA Synchrotron Light Source, Barcelona, Spain
4National Institutes of Health, Bethesda, Maryland, USA

PB-13 3D VISUALIZATION OF BIOLOGICAL TISSUES FROM MICROMETER TO NANOMETER SCALE USING CRYO-FIB-SEM
Netta Vidavsky1, Anat Akiva1, Lia Addadi1, Steve Weiner1, Andreas Schertel2
1Weizmann Institute of Science, Rehovot, Israel
2Carl Zeiss Microscopy GmbH, Oberkochen, Germany

PB-14 CANCELLED

PB-15 AN INSIGHT INTO THE UNKNOWN TERRAIN OF SPERM STORAGE IN DROSOPHILA FEMALE USING CORRELATIVE MICROSCOPY
Einat Zelinger1, Vlad Brumfeld2, Katya Rechav2, Yael Heifetz1
1The Hebrew University of Jerusalem, Rehovot, Israel
2Weizmann Institute of Science, Rehovot, Israel

PB-16 MOLECULAR INTERACTIONS UNDERLYING SYMMETRY REDUCTION AND NON-HELICAL PERTURBATIONS IN THE BACTERIAL FLAGELLAR FILAMENT
Yair Ben Shaul1, Shlomo Trachtenberg1
1The Hebrew University of Jerusalem-Hadassah Medical School, Jerusalem, Israel

Materials Science

PB-17 INTRODUCING A NEW EDS DETECTOR DESIGN CAPABLE OF SUM 10NM ANALYTICAL SPACIAL RESOLUTION AND HIGH EDS SURFACE SENSITIVITY IN A FEG SEM
John Maddock, Simon Burgess, X. Li, James Holland
Oxford Instruments NanoAnalysis, High Wycombe, UK

PB-18 CHARACTERIZATION OF ALUMINA FIBERS
Rachel Marder1, Mili Gandman1, Galit Atiya1, Carmen Cerecedo2,3, Victor Valcárcel2,3, Wayne D. Kaplan1
1Technion-Israel Institute of Technology, Haifa, Israel
2Neoker S.L, Spain, Coruña, Spain
3Tsinghua Innovation Center, Dongguan, China

PB-19 MAXIMIZING THE POTENTIAL OF LAYERED COMPOUNDS FOR HYDROGEN PRODUCTION
Oren Meiron1, Lothar Houban2, Maya Bar Sadan1
1Ben-Gurion University of the Negev, Beer-Sheva, Israel
2Weizmann Institute of Science, Rehovot, Israel

PB-20 THE INFLUENCE OF SOLUTES ON GRAIN BOUNDARY MOBILITY IN ALUMINA
Ruth Moshe, Wayne D. Kaplan
Technion-Israel Institute of Technology, Haifa, Israel
PB-21  COMPOUND ELECTROSTATIC-MAGNETIC FINAL LENS SEM IMAGING FOR INCREASED VERSATILITY  
Daniel Phifer, Ernst Jan Vesseur  
FEI Company, Eindhoven, Netherlands

PB-22  RETRACTABLE PROJECTION LENS FOR STEM IN SEM; CONTINUING THE EVOLUTION OF LOW  
VOLTAGE STEM WITH THE HELIOS G4 FX  
Daniel Phifer  
FEI Company, Eindhoven, Netherlands

PB-23  IMPROVING THERMAL CONDUCTIVITY OF HYDROGENATED MAGNESIUM COMPACTS WITH THE  
AID OF CARBON NANOPARTICLES  
Larisa Popilevsky, Vladimir Skripnyuk, Yaron Amouyal, Eugen Rabkin  
Technion-Israel Institute of Technology, Haifa, Israel

PB-24  A METHOD OF RELIABILITY ASSESSMENT OF EFUSE ROM (READ ONLY MEMORY) IN CMOS BASED  
ASIC MICRO CONTROLLERS  
Eddie Redmard, Gilad Hemo  
SanDisk, Kfar Saba, Israel

PB-25  MICROSTRUCTURE EVOLUTION OF THE HAVAR ALLOY DURING COLD WORK  
Sergei Remennik, Shlomo Harouch2,3, Vladimir Ezersky2, Daniel Moreno3,  
Ido Silberman1, Yaniv Gelbstein2, Roni Zvi Shneck2  
1 Nuclear Research Center Negev, Beer-Sheva, Israel  
2 Ben-Gurion University of the Negev, Beer-Sheva, Israel  
3 Nuclear Research Center Soreq, Yavne, Israel

PB-26  THE GENERATION AND REGENERATION OF BIOLOGICAL WAX IN REGARDS TO SURFACE  
wettability on plant leaves  
Benjamin Rich, Boaz Pokroy  
Technion-Israel Institute of Technology, Haifa, Israel

PB-27  CANCELED

PB-28  TEM STUDIES OF Ni AND Mn FULL CONCENTRATION GRADIENT MATERIALS AS CATHODES FOR Li  
ION BATTERIES  
Hadar Sclar1, Judith Grinblat1, Evan Erickson1, Hana Bouzaglo1, Florian Schipper2, Chandan Ghanty1, Boris  
Markovsky1, Yang-Kook Sun2, Doron Aurbach2  
1 Bar-Ilan University, Ramat-Gan, Israel  
2 Hanyang University, Seoul, South Korea

PB-29  TRIPLE BEAM MULTI-MODAL ANALYSIS OF LITHIUM ION BATTERIES  
Rostislav Vana, Jiri Dluhos, Libor Sedlacek  
TESCAN BRNO, s.r.o., Brno, Czech Republic

PB-30  3D SHAPING OF ELECTRON BEAMS  
Roy Shiloh, Ady Arie  
Tel Aviv University, Tel Aviv, Israel

PB-31  STEPS AT INTERFACES IN SrTiO3 AND THEIR ROLE IN KINETIC PROCESSES  
Hadas Sternlicht1, Wolfgang Rheinheimer2, Alex Mehlmann1, Avner Rothchild1, Michael J. Hoffmann2, Wayne D.  
Kaplan1  
1 Technion-Israel Institute of Technology, Haifa, Israel  
2 Karlsruhe Institute of Technology, Karlsruhe, Germany
Poster Session B (continued)

PB-32  HIERARCHICAL CRYSTALLIZATION OF MEDICINES FROM NANOMICELLES
Tanya Turovsky¹, Dganit Danino¹, Yechezkel Barenholz²
¹Technion-Israel Institute of Technology, Haifa, Israel
²The Hebrew University of Jerusalem-Hadassah Medical School, Jerusalem, Israel

PB-33  MECHANISTIC INSIGHTS INTO CRYSTALLIZATION. PERYLENE DIIMIDE BASED ORGANIC NANOCRYSTALS IN AQUEOUS MEDIA
Haim Weissman, Yael Tsarfati, Shaked Rossene, Boris Rybtchinski
Weizmann Institute of Science, Rehovot, Israel

PB-34  OPTICAL CHARACTERIZATION OF A SINGLE WS₂ NANOTUBE
Lena Yadgarov¹,² Eitam Vinegrad³, Michael Mrejen², Ori Cheshnovsky¹, Haim Suchowski²
Tel Aviv University, Tel Aviv, Israel

PB-35  THE GEOMETRY AND MECHANICS OF SELF-ASSEMBLED CHIRAL NANOSTRUCTURE
Mingming Zhang¹, Doron Grossman¹, Luba Kolik², Dganit Danino², Eran Sharon¹
¹The Hebrew University of Jerusalem, Jerusalem, Israel
²Technion-Israel Institute of Technology, Haifa, Israel

PB-36  PHASE TRANSFORMATIONS IN EQUIATOMIC Al-Co-Cr-Fe-Ni HIGH ENTROPY ALLOY
Yatir Linden¹, Shai Salhov², Abraham Munitz², Malki Pinkas³, Louisa Meshi¹
¹Ben-Gurion University of the Negev, Beer-Sheva, Israel
²Nuclear Research Center Negev, Beer-Sheva, Israel
³Nuclear Research Center Negev, Beer-Sheva, Israel

PB-37  THE GENESIS OF ADIABATIC SHEAR BAND
Peri Landau¹, Shmuel Osovski², Arie Venkert³, Viera Gartnerova⁴, Daniel Rittel²
¹Nuclear Research Center Negev, Beer-Sheva, Israel
²Technion-Israel Institute of Technology, Haifa, Israel
³Nuclear Research Center Negev, Beer-Sheva, Israel
⁴Institute of Physics, Prague, Czech Republic
PA-37  DAVID (MICHELANGELO) NONLINEAR METAMATERIALS FOR HOLOGRAPHY  
Ora Bitton, Euclides Almeida, Yehiam Prior  
Weizmann Institute of Science, Rehovot, Israel

PA-38  OUR GROUP INVESTIGATED THE ELECTRICAL AND MECHANICAL PROPERTIES OF  
THE P(VDF-TRFE-CFE) ELECTRO ACTIVE POLYMER AND DEVELOPED CUSTOM  
PROCESSING TOWARDS INTEGRATING WITH CONVENTIONAL FABRICATION  
TECHNOLOGIES  
Moti Ben-David, Leeya Engel, Ramon Axelrod, Yosi Shacham-Diamand,  
Slava Krylov  
Tel Aviv University, Tel Aviv, Israel

PA-39  A PARALLEL AND SIMPLE FABRICATION OF MULTIPLE PHOTODETECTORS BASED ON GUIDED  
gROWTH OF ZnSe NANOWIRES  
Eitan Oksenberg, Ernesto Joselevich  
Weizmann Institute of Science, Rehovot, Israel

PA-40  FROM MICRO TO NANO: TOWARDS ELECTRICAL BIASING EXPERIMENTS  
Roy Shiloh, Ady Arie  
Tel Aviv University, Tel Aviv, Israel
PA-41  THE MICROTUBULES HIGHWAY MESH OF THE CELLULAR MILIEU  
**Tali Dadosh**, Liran Ben Yaakov  
*Weizmann Institute of Science, Rehovot, Israel*

PA-42  EXPERIMENTAL ELECTRON RONCHIGRAM AT THE EDGE OF A BN 001 CRYSTAL SHOWING KIKUCHI LINES WHEN THE PROBE IS WELL UNDERFOCUSED  
**Vladimir Ezersky**  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-43  DO YOU WANT TO BUILD A NANO SNOWMAN?  
**Youngjin Jang**  
*Technion-Israel Institute of Technology, Haifa, Israel*

PA-44  CARDIOMYOCYTE SHOWING COMPLEX SARCOMERIC PROTEIN NETWORK  
**Yair Lewis**, Izhak Kehat  
*Technion-Israel Institute of Technology, Haifa, Israel*

PA-45  MICROSCOPIC WONDERLAND  
**Ilana Shtein**  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-46  EVOLUTION IN THE MERISTEM  
**Einat Zelinger**, Leor Williams-Eshed  
*The Hebrew University of Jerusalem, Rehovot, Israel*

PA-47  ACTING FILAMENTS OF THE GIANT AMOEBA  
**Liran Ben Yaakov**  
*Weizmann Institute of Science, Rehovot, Israel*

PA-48  LITTLE SHOP OF HORRORS (CARNIVOROUS PLANT)  
**Ofir Friedman**  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-49  THE MICRO BRITISH ISLAND  
**Nimrod Gazit**, Eugen Rabkin  
*Technion-Israel Institute of Technology, Haifa, Israel*

PA-50  TAKE A GLASS OF WINE AND LETS CELEBRATE ISM’S 50TH ANNIVERSARY!  
**Gilad Hemo**, Eddie Redmard  
*SanDisk, Omer, Israel*

PA-51  BIG OR SMALL THEY ALL HAVE POWERS, ROSSES, DAISIES OR NANOFLOWERS  
**Oren Meiron**  
*Ben-Gurion University of the Negev, Beer-Sheva, Israel*

PA-52  REVERSE CONNECTION  
**Maria Koifman Khristosov**  
*Technion-Israel Institute of Technology, Haifa, Israel*

PA-53  SCIENTIFIC VOLCANO (IN THE NIGHT)  
**Vladimir Yu. Kolosov**  
*Ural Federal University, Ekaterinburg, Russia*

PA-54  HAMANTASCHEN  
**Hadar Nahor**, Wayne D. Kaplan  
*Technion-Israel Institute of Science, Haifa, Israel*
LUNCH WORKSHOPS

- All Workshops take place on the 5th floor and lunch for workshop participants will be served there, prior to the commencement of the workshop.
- Participation in the workshop required on-line registration to the workshop/s, as well as checking/adding your name on the list of participants on-site on the morning of the selected workshop, by the end of the morning coffee break.
- The number of participants in each workshop is limited and will be confirmed on-site.

Tuesday, May 31, 2016

12:30-12:45  Lunch – 5th floor
12:45-13:30  Workshop

Workshop 1  Hall D

FEI™  RECENT DEVELOPMENTS IN ELECTRON MICROSCOPY
WORKFLOWS FOR LIFE SCIENCES

Moderator: Ben Lich, FEI Company

Latest developments in the cryo-TEM workflow have brought the major structural biology technologies (NMR, XRD) closer together.

Now, finally, a continuum has been reached on all important aspects with regards to resolution and macromolecular scales which allows for the full deployment of the combination of these technologies.

We will discuss the future of structural biology based on the latest developments of the FEI workflow and its components.

For resin embedded biological material we introduced a novel workflow for high spatial resolution and throughput SEM volume imaging overcoming the resolution limits set by mechanical slicing by combining it with virtual sectioning. Virtual slicing is realized by FEI's proprietary Multi-Energy de-convolution SEM (MED-SEM), a non-destructive technique that allows high resolution reconstruction of the top layers of the sample.

We will discuss how MED-SEM can be utilized in the workflows that are currently used for resin embedded samples.

Workshop 2  Hall E

Nano Instruments Ltd.
Moderator: Philippe Ayasse, WITec GmbH

RISE Microscopy is a novel correlative microscopy technique which combines confocal Raman Imaging and Scanning Electron (RISE) Microscopy within one integrated microscope system.

This unique combination provides advantages for the microscope user with regard to comprehensive sample characterization: electron microscopy is an excellent technique for visualizing the sample surface structures in the nanometer range; confocal Raman imaging is an established spectroscopic imaging method used for the detection of the
chemical and molecular components of a sample with diffraction limited resolution. In contrast to existing combinations, where single Raman spectra are typically collected from few micrometer size areas, the RISE combination allows for the first time diffraction limited confocal Raman imaging on the same sample position as the SEM image was taken. It can also generate 3D-images and depth profiles to visualize the distribution of the molecular compounds within a sample volume. Both analytical methods are fully integrated into the RISE Microscope.

**Workshop 3**

**Hall F**

**DIGITAL IMAGING RE-INVENTED WITH NEW CAMERAS INCLUDING ONEVIEW AND K2**

**Gatan (AVBA)**

Moderators: Paul Spellward & Colleagues, Gatan Inc.

This workshop will discuss both modern scintillator based cameras and advanced direct detection systems, also with emphasis on speed for in situ work.

Digital cameras for TEMs were initially a film substitute, only used for final acquisition, because they were too slow for use whilst searching or focussing. In more recent times, cameras became faster and could be used less painfully in fast readout modes, at reduced resolution or from sub-areas. Finally, OneView has arrived. Gatan's new sensor technology allows a full 4K x 4K image to be constantly refreshed at 25 frames per second.

OneView has no compromises on read-out area or resolution and a very easy, streamlined user experience. Furthermore, this technology enables real time drift correction during image acquisition. Sharp images are available even from drifting or unstable samples. With the addition of the "in situ" option, this camera can be accelerated to >200 frames per second, opening new experimental possibilities for dynamic TEM studies.

The limitations of scintillator cameras for low dose work (whether from inherently fragile specimens or because the exposure needs to be so short to enable very fast imaging) are overcome by using direct (electron) detection cameras. Gatan’s K2 Summit camera is well established as the only commercially available, fast, counting direct detector. It can be used by for structural biology single particle and tomography work, within existing workflows (2-4 second exposures at 5-10eA-2). It has the best DQE of any such camera and brings additional benefits from dose fractionation and drift correction.

The world’s fastest TEM in situ camera is based on the K2 Summit. With additional processing power and storage, Gatan is offering the K2 IS (K2 In Situ) camera. This is already finding applications in fast imaging for structural and magnetic applications and also in fast STEM diffraction imaging, where whole new application fields are available, for example strain mapping. Some application examples will be presented.
FEI has achieved a further improvement in resolution and in contrast using a new FEI SEM equipped with a compound electrostatic-magnetic final lens. This new final lens design provides a resolution equal to 1.0 nm at 1 kV acceleration voltage. The compound final lens SEM combines a magnetic final lens in the pole piece, a magnetic immersion final lens and an electrostatic lens formed by the potential at the bottom of the column to achieve excellent contrast as well. Contrast improvement results from the independent in-lens detection of secondary (SE) and backscattered (BSE) electrons. Secondary electrons are separated and independently detected by in-lens and in-column detectors. With the new compound final lens, it is possible to further energy filter the backscattered electrons detected on the lower T1 in-lens detector. When high-loss (low energy) BSEs are filtered out, T1 provides extremely strong material contrast images formed by only low-loss (high energy) BSEs.

The Apreo SEM, equipped with the compound final lens and the in-lens and in-column detectors, improves both the imaging resolution and contrast performance. It allows researchers to capture the maximum amount of information from conductive as well as insulating samples, with the right detail and with the least amount of compromises.

As Silicon Drift Detectors (SDDs) have become the standard detectors for energy dispersive x-ray detection in the last years, special detector designs and concepts can bring the performance to the next level.

A special multi element concept is the XFlash® 5060FQ, an annular detector which can be placed between the pole piece and the sample in a standard SEM using a BSE detector like setup. This setup leads to an extremely large solid angle of more than 1 sr. This is a value which is typically 100 times larger than a 10mm² detectors in a conventional setup. Therefore extremely high count rates can be achieved easily even with low probe currents, and can be processed with four separate electronic channels in parallel, leading to maximum output count rate of more than 1,600,000 cps. These properties make the detector an ideal device for high speed mapping applications or analyzing beam sensitive samples.
ELECTRON ENERGY LOSS SPECTROSCOPY: THE MOST POWERFUL ANALYTICAL TECHNIQUE IN TEM

Gatan (AVBA)
Moderators: Paul Spellward & Colleagues, Gatan Inc.

This workshop will discuss the most powerful analytical technique in TEM: Electron Energy Loss Spectroscopy (EELS).

EELS, like EDS, can measure local composition. However, it is far more powerful than EDS because it brings additional information on local chemistry, valency, and a wealth of other details. For most elements, EELS has higher or much higher sensitivity and better signal to noise. Even for very heavy elements, recent comparisons show EELS at least as good as EDS. Even with new “large area” EDS systems, the collection efficiency for EELS is vastly higher and this is reflected in the quality of elemental “maps”.

The workshop will discuss EELS Spectrometer (Enfinium) and Gatan Imaging Filter (GIF Quantum) hardware and will introduce the new Gatan Microscopy Suite (GMS) 3 software. GMS3 includes new model-based quantification for EELS and EDS. Recently, advances in control of EDS systems from GMS has enabled co-acquisition of EELS and EDS at 1000 spectra per second. Examples of co-acquired data will be shown.

This workshop will be useful for researchers who have not used EELS before, but also, especially, for users of older generation systems who will appreciate the improvements in the new systems and software. Also, if you have been told “EDS is all you need”, then come and hear an account of why you would be better with EELS.
Thursday, June 2, 2016

12:00-12:30 Lunch – 5th floor
12:30-13:15 Workshop

Workshop 7

**PROTOCHIPS IN SITU ELECTRON MICROSCOPY SOLUTIONS: CAPABILITIES AND APPLICATIONS**

Moderator: **Mathias O. Mosig, EMEA Protochips Inc.**

New innovations are transforming the Transmission Electron Microscope (TEM) from a simple high-resolution image acquisition tool into a nanoscale materials research and development laboratory. Researchers can now better understand material behavior by analyzing samples in real-world gas or liquid environments, at high temperature and with ultra-low noise electrochemical and electrical biasing techniques. With the new in situ tools from Protochips, materials research occurs in highly controlled environments at high resolution without sacrificing the analytical capabilities of the TEM such as EDS. Applications for these tools include heterogeneous catalyst reactions, nanostructure nucleation and growth, battery and fuel cell materials, high temperature nanoparticle behavior, and semiconductor devices. Protochips will present their latest innovations around the Protochips Atmosphere™ 200 Gas Environmental Cell, the newly released Protochips Poseidon™ Select flowing liquid and electrochemistry cell, and the newly released Protochips Fusion™ heating and electrical biasing system.

Workshop 8

**ADVANCES IN SUPER-RESOLUTION IMAGING: DELTAVISION OMX SR MICROSCOPE**

**Danyel Biotech for GE Healthcare**

Moderator: **Przemyslaw Fleszar, GE Healthcare Life Sciences**

The new and growing portfolio for GE Life Sciences Cell Imaging tools, including DeltaVison OMX and DeltaVision OMX SR open up advanced applications in high and super resolution live cell fluorescence and transmitted light imaging, using widefield deconvolution, localization microscopy, and structured illumination microscopy (SIM) super resolution modalities. These cutting edge hardware platforms use class leading technologies in concert with sensitive and powerful image analysis platform give the user the power to address biological questions in cell biology that previously lay out of reach, covering microscopic scales from tissues, cell colonies and single cells, through to molecular organization of individual cells in live samples, using multi-channel fluorescence detection and transmitted light methods. During the workshop we will discuss the principles and benefits of SIM technology. We will also cover wide range of applications, presenting examples of cell biology, microbiology, virology and live cell super resolution imaging.
This workshop will discuss the increasingly popular Serial Block Face SEM technique, as deployed with the Gatan 3View system.

Advanced research, for example in Neuroscience, increasingly requires full knowledge of 3D structures and connections over large volumes but with spatial resolution at FEG-SEM levels. Fields of view of order 1x1x1mm³ can be achieved via a SEM hosted microtome, within realistic timescales. Such volumes are not attainable with FIB-SEMs. The block face image datasets are inherently aligned and acquisition is highly automated, unlike TEM serial sectioning where sections may be lost, distorted and misaligned and the whole process is labour intensive.

The Gatan 3View system is a based on a solid stage which replaces the SEM’s own stage, assuring reproducibility and fine control, rather than a lightweight module on a standard SEM stage. Z resolution is controlled by slice thickness and there is no need (or benefit) for multiple voltage images on one block face. The Gatan 3View hardware will be discussed and experimental workflows described.

Applications of Serial Block Face SEM in neuroscience, cell biology, botany, and drug delivery will be mentioned. The technique is also used in polymers and Material Science; example of work on soft metals will be presented.
MEASURING PHYSICAL AND ELECTRONIC PROPERTIES AT THE NANOSCALE

David A. Muller
Kavli Institute at Cornell for Nanoscale Science, Cornell University, Ithaca, NY, USA

Electron energy loss spectroscopy (EELS) in a new generation of aberration-corrected electron microscopes provides direct images of the local physical and electronic structure inside a material at the atomic scale. The sensitivity and resolution can extend to imaging single dopant atoms or vacancies in their native environments. Comparable advances in detector technology are now poised to enable a similar revolution in the measurement of structure and fields in materials. Here we describe a high speed, high dynamic range imaging hybrid pixel array detector (EMPAD - electron microscope pixel array detector) for use in electron microscope applications, especially as a universal detector for scanning transmission electron microscopy. The in-pixel circuitry provides a 1,000,000:1 dynamic range within a single frame, allowing the direct electron beam to be imaged while still maintaining single electron sensitivity. A 1.1 kHz framing rate enables rapid data collection while scanning. The scattering is recorded on an absolute scale, so that information such as local sample thickness can be directly determined. By capturing the entire unsaturated diffraction pattern in scanning mode, the detector can simultaneously capture bright field, dark field, and phase contrast information, as well as being able to analyze the full scattering distribution, allowing true center-of-mass imaging for electric and magnetic field measurements, and opening the way for new multichannel imaging modes.
SUPER-RESOLUTION AND LIGHT SHEET-BASED FLUORESCENCE MICROSCOPY

Ernst Hans Karl Stelzer

Buchmann Institute for Molecular Life Sciences, Goethe Universität Frankfurt am Main, Frankfurt am Main, Germany

In light sheet–based fluorescence microscopy (LSFM), optical sectioning in the excitation process minimizes fluorophore bleaching and phototoxic effects. Since biological specimens survive long-term three-dimensional imaging at high spatiotemporal resolution, light sheet-based microscopes (LSM) have become an indispensable tool in developmental, three-dimensional cell and plant biology. LSFM is based on two main optical paths. The detection path consists of a microscope objective, a spectral filter, a tube lens and a camera. The excitation path is perpendicular to the detection path and directs a light sheet into the side of the specimen. The thin light sheet and the focal plane of the detection objective overlap. LSFM provides at least three important degrees of freedom, which are usually not available in an epifluorescence microscope: a) the axial and b) lateral locations of the light sheet and c) the axial location of the focal plane. Further, probably less important, degrees of freedom are the tilt and the incline of the light sheet. LSFM takes full advantage of modern cameras, massively parallelizing the data acquisition process and recording ten to one hundred images per second with a high dynamic range. LSFM does not rely on traditional features that are required for ergonomic reasons. A powerful multiple-sensors-based image processing pipeline is, therefore, an inherent feature. Traditional fluorescence microscopy enforces specimen preparation schemes that rely on hard and flat surfaces. LSFM places the specimen in the center and arranges the optics around it. Specimens can be prepared in new ways, their three-dimensional integrity is maintained, and they can be used in experiments hitherto regarded as impossible. Fluorescence microscopy has several basic limitations. First, the excitation light is absorbed not only by fluorophores but also by many endogenous organic compounds, which are degraded much like fluorophores and thus unavailable for vital metabolic processes. Second, the number of fluorophores in any volume element at any given time is finite, and fluorophores can degrade upon excitation. As a consequence, the number of photons that are retrieved from a fluorophore-labeled specimen is limited. Finally, life on Earth is adapted to the solar flux, which is less than 1.4 kW/m². This might not be a hard limit, but it indicates that irradiance should not exceed 1 nW/µm² = 100 mW/cm² when dynamic biological processes are observed. When imaging living biological samples, these challenges must be addressed. LSFM is perhaps the best technology we have so far, which makes a sincere and honest effort to address these challenges: 1) it provides optical sectioning, 2) a true axial resolution, 3) reduces fluorophore bleaching and 4) phototoxicity at almost any scale, 5) allows one to record millions of pixels in parallel and 6) dramatically improves the viability of the specimen. Stelzer, E. H.K. (2015). Light-sheet fluorescence microscopy for quantitative biology. Nature Methods, 12(1), 23–27.

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HIGH RESOLUTION CRYO-EM OF PROTEASOME COMPLEXES AS A NEW TOOL FOR THERAPEUTIC DRUG DEVELOPMENT

Paula da Fonseca  
Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK

Over the last few years the field of biological structural electron microscopy has seen an enormous transformation, primarily triggered by the availability of improved electron microscopes and direct electron detectors. It is now possible to use electron cryo-microscopy (cryo-EM) and single particle analysis to determine the structure of proteins to resolutions that used to be achievable only by crystallography or NMR methods. The structural information attainable by such methodologies can in principle be used to infer into the detailed molecular mechanisms of proteins and protein complexes. We explored their application to study protein/ligand interactions using the human 20S proteasome core.

The proteasome is a highly regulated protease complex fundamental for cell homeostasis and controlled cell cycle progression. The proteolytic active sites of the proteasome are enclosed within its 20S core. In eukaryotes, the 20S core is a 750 kDa complex formed by 7 individual α and 7 individual β subunits, arranged in a barrel shaped two-fold symmetric α7β7β7α7 assembly. While the 20S proteasome core is a well-established target for cancer therapy, its inhibition is being explored for an increasing range of further therapeutic usages. We used cryo-EM and single particle analysis to determine the structure of the human 20S proteasome core bound to a substrate analogue inhibitor molecule, at a resolution of around 3.5Å. The resulting map allowed the building of protein coordinates as well as defining the location and conformation of the inhibitor at the different active sites. These results serve as proof of principle that cryo-EM is emerging as a realistic approach for more general structural studies of protein/ligand interactions. This has the potential benefits of extending such studies to complexes unsuitable for other methods of structure determination and allowing closer to physiological conditions to be used. Within this context, we extended our studies to assist in the development of new highly specific inhibitors targeting the Plasmodium falciparum proteasome. Plasmodium falciparum is the parasite responsible for the most severe form of malaria, against which artemisinin is currently the forefront medication. The spreading of artemisinin resistant parasites, first identified in the Southeast Asia, represents therefore a major threat to human health and to the current programs aiming at controlling and eventually eradicating malaria. We determined the structure of the Plasmodium falciparum 20S proteasome core bound to a new specific inhibitor, at a resolution of around 3.6Å. This inhibitor was developed by our collaborator Matt Bogyo, Stanford University, based on the profiling of substrate cleavage sites specific to the parasite proteasome. Our structure, and its comparison with that of the human 20S proteasome core, revealed the molecular basis for the inhibitor specificity for the parasite complex. The structure obtained has further guided the improvement of this ligand into a more effective anti-malaria drug prototype, with demonstrated low toxicity to in vivo model hosts.
Scandium is the element of choice for creating stable \( \text{L}_{12} \) precipitates in aluminum, with \( \text{Al}_3\text{Sc} \) having the same gamma-prime \( \text{L}_{12} \) structure as \( \text{Ni}_3\text{Al} \) in nickel-base superalloys and the same strengthening effect by blocking dislocations. We present various strategies for partially replacing Sc with less expensive transition metals (TM = Zr, Ti, Hf), where the resulting core-shell \( \text{Al}_3(\text{Sc}, \text{TM}) \) nano-precipitates display better coarsening resistance due to the lower TM diffusivity in Al. Scandium can also partially be replaced with even numbered heavy rare-earth elements (RE = Er, Yb). The resulting \( \text{Al}_3(\text{Sc}, \text{TM}, \text{RE}) \) precipitates have a larger lattice parameter mismatch with the matrix, thus enhancing the alloys’ creep resistance. We discuss the nucleation effect of Si upon the trialuminides, and describe industrial applications for these alloys, taking advantage of the unique combination of coarsening and creep resistance up to 400 °C, excellent thermal and electrical conductivity, high corrosion and oxidation resistance, and high ductility, combined with good castability and cold- and hot-working. The important roles of atom-probe tomography (APT) in understanding the coarsening and creep resistance is strongly emphasized.
PUSHING BACK THE FRONTIERS OF ELECTRON MICROSCOPY: THEORY AS A GUIDE

Peter Rez
Department of Physics, Arizona State University, Tempe, Arizona, USA

Electron loss spectroscopy as an adjunct to imaging has added elemental and chemical analysis to the high resolution imaging capabilities of electron microscopy. The development of monochromator and spectrometers that give meV energy resolution has revolutionized spectroscopy in the electron microscope. Now we can probe vibrations with peaks in the IR region\(^1\), optical absorption from defects, as well as the UV and soft X-ray excitations that have been the traditional area of interest in electron loss spectroscopy. Theoretical analysis showed that CH, NH, NH\(_2\) and OH vibrations would give a strong signal\(^2\) and that they could be excited from distances of up to 100 nm. It was predicted by Cohen et al\(^3\) that it would be possible to use an aloof beam to minimize specimen damage. This has been used in our recent demonstration of “damage-free” spectroscopy of guanine fish scales\(^4\). Furthermore, theory predicts that there should also be a high resolution signal, possibly even atomic resolution\(^2,5\), and more detailed analysis is defining the necessary microscope operating conditions.

It is not just electron spectroscopy where theory has shown the way forward. Simple theoretical analysis showed that it would be possible to image beam sensitive biological specimens with low radiation exposure using annular dark field STEM, if the detector inner cut off were small enough\(^5\). This has been experimentally realized in the cryo STEM work of Wolf et al\(^6\). Further development of the theory has shown that it will be possible to map out the contents of small prokaryotic cells at about 2 nm spatial resolution in 3D using cryo-STEM tomography.

HIGH SPEED BIOLOGICAL IMAGING AT AND BEYOND THE DIFFRACTION LIMIT

Hari Shroff
National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD, USA

I will discuss our efforts to improve structured illumination microscopy (SIM) and light-sheet microscopy. SIM doubles the spatial resolution of light microscopy, requiring lower light intensities and acquisition times than other super-resolution techniques. I will present SIM implementations that enable resolution doubling in live volumes 10-20x thicker than possible with conventional SIM, as well as hardware modifications that enable effectively ‘instant’ SIM imaging at rates 10-100x faster than other SIM.

The second half of the talk will focus on the development of inverted selective plane illumination microscopy (iSPIM), and subsequent application to the noninvasive study of neurodevelopment in nematodes. I will discuss progress that quadruples the axial resolution of iSPIM by utilizing a second specimen view, thus enabling imaging with isotropic spatial resolution (dual-view iSPIM, or diSPIM). Applications of this technology will be presented, including computational methods for untwisting worm embryos.
50 YEARS OF THE ISRAEL SOCIETY OF MICROSCOPY – A RETROSPECTIVE

Martin Kessel
Institute for Medical Research Israel-Canada (IMRIC), The Hebrew University of Jerusalem, Jerusalem, Israel
Lab. of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

In celebration of the 50th Anniversary of the founding of the Israel Society of Microscopy (formerly the Israel Society of Electron Microscopy) we will take a chronological look at the people and events that have shaped the ISM since its inception in 1964.

The history of the Society can be viewed through the scientific achievements of its members and can be divided into four periods: 1. The Founding Fathers 2. The Intermediate Generation 3. The Consolidation Generation 4. The Modern Generation.

We will highlight the unique achievement of the Society which hosted the 6th European Congress on Electron Microscopy in Jerusalem in 1976.

We will pay tribute to Society members no longer with us but whose seminal scientific contributions endure. We will recognize a pinnacle achievement in the award of the 2011 Nobel Prize in Chemistry to Prof. Dan Schechtman of the Technion-Israel Institute of Technology.

We will pay tribute to the Officers of the Society from its inception until the present day.
HOW ADVANCED MICROSCOPY DEVELOPED IN THE PAST 50 YEARS TO ANALYSE THE MOLECULAR MACHINERY IN CELLS

Hans Tanke, Roman Koning, Frank Faas, Christina Avramut, Bram Koster
Molecular Cell Biology, Leiden University Medical Center, Leiden, Netherlands

The introduction of immunofluorescence by Coons in early 1940 and fluorescence microscopy with incident light illumination by Ploem in 1967 has set the scene for decades, in which Abbe’s law defined the achievable spatial resolution. At present, advanced microscopic techniques have allowed us to observe molecular machines at work in intact living cells at nm resolution, bypassing the diffraction limit whereas light sheath illumination (instead of epi-illumination) has become a superior alternative for many applications. Green fluorescent proteins have replaced good old fluorescein and rhodamine dyes, and optogenetic tools allow us to introduce function with light. Microscope systems have become “filter less” with the introduction of optoacoustic devices and “white lasers” are used as excitation light sources. This presentation will look back at 50 years of advanced microscopy and show some of the highlights in the field of automated cell analysis, chromosome analysis by FISH karyotyping, and rare event detection.

How is advanced microscopy positioned in an “omics world”? The overwhelming amount of information that has become available after sequencing the human genome, and subsequent developments in “omics-type” of disciplines, have learned us a lot about the molecular composition of cells. How regulatory mechanisms form the basis of cell growth, differentiation and death in normal tissue, but also in case of disease, is not yet clear in many cases. Notably, a protein can only exercise its function if it is present at the right time, in the right amount, properly activated and in the presence of other proteins, at a particular position in the cells. Thus, spatio-temporal information is crucial which is not provided by “omics technologies” only.

In this context combined use of light- and high resolution microscopy (CLEM) as well as cryo-tomography and 3D EM is a powerful combination. For instance, for the study of the production of Von Willebrand factor and Weibel Palade bodies (1), the barrier function of the glomerular endothelial surface layer in the kidney and the growth and differentiation of Streptomyces, the main commercial producing micro-organism of antibiotics. Also, a technique will be presented to generate and visualise ultra-large high resolution microscopy maps (2).


EDUCATION AND OUTREACH AT THE ROYAL MICROSCOPICAL SOCIETY: DEVELOPING AND IMPLEMENTING A PROGRAMME FOR ALL - FROM SCHOOLS EDUCATION TO CPD FOR PROFESSIONAL MICROSCOPISTS

Susan Anderson
School of Medicine, University of Nottingham and Royal Microscopical Society, Nottingham, UK

As the Israel Society for Microscopy celebrates its Golden Jubilee, it looks forward to supporting the next generation of microscopists. This talk will focus on the development of a full programme of education and outreach for the Royal Microscopical Society. Since the establishment of the Education and Outreach Section there have been major developments in these areas with the aims of: (1) promoting engagement of young people in science education through the medium of microscopy (2) supporting continued professional development of professional microscopists and (3) public awareness and engagement concerning the value and applications of microscopy.

One key way of achieving these aims have been to develop a Microscope Activity Kit, containing curriculum mapped activities, along with samples and microscopes to engage primary school children and their teachers. These are loaned free to Schools for a term and have now reached almost 50,000 children with excellent feedback. Smaller projects in Ireland have also engaged several thousand young people. A second strand has been the development of a diploma qualification for microscopists. This is based on a portfolio of things including a major project, technical essay, course attendance and presentation/outreach/writing skills development. Public engagement is achieved via contributing to a variety of science festivals and exhibitions.

Our journey over the last number of years has been exciting and productive and there is much to do in the future. Our current focus is on internationalising the MAK, working in partnership with other Societies and Universities as well as measuring and evidencing the educational impact of these activities for young people. In terms of second level education, we are developing interactive web resources and workshops focussing on the wide range of careers associated with microscopy. Finally, we seek to accredit the DipRMS.
Invited Lecture
ENERGY-FILTERED AND LOW-VOLTAGE CHROMATIC ABERRATION-CORRECTED HIGH-RESOLUTION TEM

Lothar Houben\textsuperscript{1,2}, Juri Barthel\textsuperscript{3}, Martina Luysberg\textsuperscript{2}, Peter Hartel\textsuperscript{4}, Rafal E. Dunin-Borkowski\textsuperscript{2}

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\textsuperscript{4}, CEOS GmbH, Heidelberg, Germany

The advent of chromatic aberration-correction in the transmission electron microscope (TEM) offers new prospects for high-resolution imaging at low voltages and energy-selective imaging. Recent improvements in the setup of the achroplanatic CEOS CCOR corrector allow for sub-ångstrom resolution at an acceleration voltage of 50 kV and enhanced optical stability over the timespan of several minutes provides for reliable energy-filtered transmission electron microscopy (EFTEM) spectrum image data at atomic resolution. Experimental examples of low-voltage high-resolution and energy-filtered images of complex oxides, thin layered materials and nanoparticles obtained with Jülich’s chromatic aberration corrected microscope “PICO” will be presented to demonstrate the unique optical properties of the CCOR.

Atomic-scale transmission electron microscopy pushed towards low electron energy by virtue of chromatic aberration correction opens a new horizon for direct imaging of atomic details of nanostructures at reduced radiation damage. Here it is used to analyse the structure and defects in atomic sheets of 2D materials and catalytic hybrid nanostructures.

EFTEM showing atomic detail becomes practicable because of the negligible chromatic focus spread after chromatic aberration correction. The achroplanatic CEOS CCOR corrector allows to record elemental maps on a large field of view with large energy windows, which is essential for the dose-efficient acquisition of atomic resolution images formed by the weak inelastic core-loss scattering. Aspects of atomic resolution EFTEM will be discussed. The quantification of EFTEM maps towards atomic resolution chemical composition maps is in general complicated by the preservation of elastic contrast emerging from elastic scattering. Thin specimen and careful choice of contrast transfer settings yield directly useful qualitative elemental maps on the atomic scale.
PERFORMANCE AND FIRST RESULTS OF THE CC/CS-CORRECTED 20-80KV SALVE MICROSCOPE

Ute Kaiser¹, Johannes Biskupek¹, Zhongbo Lee¹, Tibor Lehnert¹, Felix Börrnert¹, Harald Rose¹, Peter Hartel², Martin Linck², Heiko Müller², Stephan Ehlemann², Max. Haider², Marcel Niestadt³

¹Central Facility of Electron Microscopy, Ulm University, Ulm, Germany
²CEOS, Corrected Electron Optical Systems GmbH, Heidelberg, Germany
³FEI Company, Niestadt Marcel, Eindhoven, Netherlands

Spherical aberration correction has become inevitable in materials science for atomic-resolution imaging of conventional objects by transmission electron microscopy at medium accelerating voltages (80-300kV). However, low-Z objects and radiation-sensitive low-dimensional objects require lower accelerating voltages because the threshold for knock-on damage is below 80kV for these materials. Unfortunately, the resolution of spherical aberration-corrected transmission electron microscopy at an accelerating voltage less than 80 kV with a standard Schottky type or field-emission electron source is strongly limited by the chromatic aberration of the objective lens.

Here we report on our approach to achieve atomic resolution at voltages in the range between 20-80kV in transmission electron microscopy [1, 2]. We employ for our investigations the newly-developed aberration corrector that corrects for both, spherical and chromatic aberration of the objective lens. First experimental results of low-dimensional objects will be presented.

The excellent performance of the SALVE (Sub-Angstrom Low-Voltage Electron microscopy) microscope enables atomic-resolution imaging and high-resolution energy-filtered (EF)-TEM with large energy windows even at 20kV accelerating voltage. Moreover, we discuss sample preparation approaches [3,4] and show first results on low-dimensional objects. Using, as an example, high-resolution imaging of graphene and MoS₂, we compare quantitatively the contrast between experiment and calculation. Obviously, no damping of the contrast transfer function by chromatic aberration has to be considered in the image calculation routine, although image spread due to thermal magnetic field noise [5] has been taken into account as a new source of contrast transfer damping. The envelope in calculations results from image-spread and residual defocus [6, 7]. The calculations are performed in dependence of the dose [7]. Figure 1 shows an experimental HRTEM image of single-layer graphene at 30kV. The usable aperture of about 65mrad shows that an information limit of 107 pm has been achieved. The comparison of the line profiles through the calculated and experimental images demonstrates that a good match has been obtained. [8]

References

[8] The authors greatly acknowledge funding from the German Research Foundation (DFG) and the Ministry of Science, Research and the Arts (MWK) of the federal state Baden-Württemberg, Germany in the frame of the SALVE project.
Fig.1 (left): 30 kV Cc/Cs-corrected HRTEM raw image of graphene with inserts: top left: diffractogram of the whole area, middle right: magnified image of the white rectangular, (right column): Comparison between calculated (top) and averaged experimental (middle) images, the corresponding lines are shown as profiles below.
PROSPECTS FOR ELECTRON BEAM ABERRATION CORRECTION USING SCULPTED PHASE MASKS

Roy Shiloh, Roei Remez, Ady Arie
Electrical Engineering - Physical Electronics, Tel Aviv University, Tel Aviv, Israel

Electron lenses suffer greatly from optical aberrations, the solution, though near-perfect, is very costly and cumbersome. We are working towards cheap solutions for probe correction using light-optics techniques, utilizing aberration-correcting phase masks[1].

Electron beams are extensively used in lithography, microscopy, material studies and electronic chip inspection. Unlike the standard in lenses for light, electron lenses suffer greatly from spherical aberration, among others. Electric and magnetic corrector elements are commercially available; however, disregarding practical difficulties, the cost of a corrected microscope almost doubles (from 1.5M€ to 2.7M€!). Using the shaping techniques we propose, a corrector element could be fabricated for ~100€. These elements cannot be dynamically tuned, however, they do not produce stray fields and thus are advantageous to field-sensitive measurements, and potentially offer complex wavefront correction schemes which are hard or impossible to achieve using commercial correctors.

Some of the known aberrations from every-day light optics are spherical aberration and astigmatism. Another aberration is the trefoil or threefold-astigmatism aberration, less known in modern-day light-optics but well known in electron microscopy. Employing knowledge from light optics, we fabricated and measured electron phase-holograms that shaped the electron probe (e.g. for SEM, STEM, and lithography applications) and imprinted it with these aberrations [1]. It is thus a simple matter to revert an aberrated beam and at least partially remove such aberrations.

In this talk, I will present the general idea of probe shaping, review the different aberrations we induced and present prospects for future work.

Fig.1. Measured example phase masks imparting (a) spherical aberration, and (b) two-fold astigmatism, to the electron beam.
Fig. 2: Threefold astigmatism: (a) fabricated phase-mask, dashed line marking extents of the intensity cross-section in the inset. (b) Measurements and (c) simulation of a focal series extending up to the focal point. Contrast and brightness altered for visibility.

An efficient chamber detector for detection of secondary electrons (SE) was designed and tested in Raith’s ionLINE ion lithography tool. The design was based on 3D simulations of electric fields generated by the voltages and the electrode structures of all the elements affecting SE. A realistic physical model for the SE was used in the 3D model to calculate and optimize the SE collection efficiency (CE).

A noise “cascade model” was developed to identify the influence on the SNR of each signal transformation stage from the primary beam to the PMT output.

This model was used to derive the CE from the experimental images SNR, found to closely match the simulated values.

The concept of ”Acceptance Map” was employed for relating the initial directions of the SE to their hit location in the chamber, such as pole piece or detector. It was found that the FIB objective lens attracts most of the vertically emitted SE (at small angles to the normal to the sample) such as SE from Vias or trenches, preventing them from reaching the detector.

Introducing a weak magnetic field under the FIB pole piece, these electrons can be directed towards the detector. Simulations of the electron motion under the combined electric and magnetic fields enables to optimize the collection efficiency by the suitable choice of both fields. Such add-on units, that consists of two small permanent magnets were developed and implemented in the ionLINE tool showing promising preliminary results.
SCALABLE PROTEOMIC IMAGING OF INTACT BIOLOGICAL SYSTEMS

Kwanghun Chung
Institute for Medical Engineering and Science (IMES), MIT, Cambridge, Massachusetts, USA

Combined measurement of diverse molecular and anatomical traits that span multiple levels, from molecules to cells to an entire system, remains a major challenge in biology. In this talk, I will introduce a series of technologies including CLARITY, SWITCH, and stochastic electrotransport that enable proteomic imaging for scalable, integrated, high-dimensional phenotyping of both animal tissues and human clinical samples. The method, termed SWITCH, uniformly secures tissue architecture, native biomolecules, and antigenicity across an entire system by synchronizing the tissue preservation reaction. The heat- and chemical-resistant nature of the resulting framework permits virtually unlimited rounds of relabeling of a single tissue with precise co-registration of multiple datasets. Furthermore, SWITCH synchronizes labeling reactions to improve probe penetration depth and uniformity of staining. With SWITCH, we demonstrated combinatorial protein expression profiling and high-dimensional quantitative analysis of the human cortex. Such integrated high-dimensional information may accelerate our understanding of biological systems at multiple levels.
Non-invasive fluorescence fundus imaging is an important tool for small animal in vivo retinal imaging in a wide array of translational vision applications, including the tracking of fluorescently tagged cells and blood vessels over time, as well as functional fluorescence imaging where calcium probes are used for monitoring retinal neuronal activity; these various applications require systems capable of imaging fine retinal structures in vivo. High resolution funduscopy solutions are primarily based on scanning laser ophthalmoscopes (SLO), with or without adaptive optics, or on wide-field imaging through low-NA objectives and topical endoscopes; two-photon in vivo imaging of the mouse retina was demonstrated only very recently using a very complex custom adaptive optics system.

In this study we use a model-based approach to analyze the requirements from an in vivo mouse retinal imaging system employing commercially available water-dipping objectives, and find that the mouse’s eye strongly constrains the range of imaging optics that can be used in this application. Our novel two-photon imaging solution is based on these design criteria, and uses widely accessible components to yield fundus images of optically sectioned, well-resolved fluorescent microstructures down to the cellular scale. Depth-scanning of the imaging plane is provided by an electronically tunable lens (ETL) integrated into the optical path with a fixed offset plano-concave lens, in order to avoid the perils of moving the objective. The system is found to provide major advantages in comparison to wide field imaging systems based on low NA objectives, and to allow long-term repeated imaging, in a simple, widely accessible design. Moreover, the system enables functional calcium imaging of repeated retinal responses to light stimulation using the genetically encoded indicator, GCaMP6s.

We constructed an intuitive simplified paraxial model for our optical system using published measurements of the mouse eye. Using this model we were able to analyze the position of the expected focal plane inside the eye when using multiple commercially available objectives (and their combinations with appropriate offset lenses). Following this analysis we chose to use long-focal length 10x objectives from Zeiss (0.45NA, WD=1.8 mm) and from Nikon (0.3NA, WD=3.5 mm) which were found to enable retinal imaging, with the latter providing a much wider working range leading to a superior ease of use. In order to obtain a model-based estimate of the lateral resolution in the retina, we developed a detailed ray tracing-based model that was implemented in the Zemax software, and calculated the lateral resolution of the focused beam on the retina to be ~1 µm.

To characterize the system’s axial scanning using this selected configuration, we repeatedly refocused the objective lens onto a single plane of vasculature in the living retina using a motorized z-stage for multiple ETL control values. These measurements provide a strong validation for the model’s predictions, allowing to translate changes in the optical parameters (objective position and ETL focal distance) to actual imaging depth.

Next, we acquired individual, optically sectioned planes of the retina through the mouse’s dilated pupil (Fig. 1A) by remotely scanning the retina with the ETL while keeping the objective static, and reconstructed a 3D image from these separate planes (Fig. 1B). Then, we tested whether the system can provide cellular-resolved images by intra-vitreally injecting an adeno-associated virus (AAV) expressing the genetically encoded calcium indicator GCaMP3. Transduced cells were clearly visualized in microscopic images, and by acquiring a subsequent fluorescence angiogram their location relative to the retinal vasculature could be determined and visualized (Fig. 1C, vasculature shown in false colors). By evaluating cross-sectional widths for the doughnut-shaped cell images (Fig. 1C inset, bottom), we estimated the system’s lateral imaging resolution to have an
upper bound of ~3.5 µm. Therefore, we anticipate that the actual lateral imaging resolution will be somewhere in between this value and the predicted 1 µm value obtained from our optical model.

Figure 1. Imaging performance and characteristics. A) Fluorescent angiograms acquired with different ETL focal lengths (marked retinal depth labels are calculated from the model). B) 3D reconstruction of different retinal planes. C) False color image of retinal ganglion cells expressing GCaMP3 (green) and blood vessels (red, averages over 120 and 50 frames, respectively). Insets: Blowup of area with multiple cells (top) and surface plot of average cell image (bottom, scale bar=5 µm).

Finally, we examined the system’s ability to perform functional calcium imaging in vivo by recording the calcium dynamics from multiple retinal ganglion cells (RGCs) that express GCaMP6s in response to full-field flashes of light in the blue spectrum (Figure 2).
Figure 2. **In vivo functional calcium imaging.** Bottom: System schematic for the functional imaging experiments. Short pulses of blue light (10–20 ms) were flashed onto the retina as repeated scans of the FOV were acquired. Top: Representative calcium traces (gray) from two cells (marked in the average image on left) in response to the flashes of blue light (blue dashed lines) at 15-s intervals. Image scale bars = 20 µm.

In summary, we demonstrated the *in vivo* acquisition of optically sectioned two-photon laser scanning microscopic mouse fundus images using an intuitive optical model-based design, which can be easily integrated into essentially any existing microscope, and enable remote axial scanning, potentially up to multi-kHz rates. The system was found to provide exquisitely detailed 3D micro-angiograms and cellular-resolved images, whose axial location can be translated to “real-world” coordinates using the model. Furthermore, here we demonstrate for the first time the ability to carry out artifact-free two-photon functional imaging of neuronal retinal activity in the mouse in response to natural or artificial-optogenetic visual stimuli in the visible spectrum.
A SIMPLE K-OMEGA ADAPTIVE METHOD TO REGISTER DYNAMIC MICROSCOPY IMAGE SEQUENCES

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Intravital microscopy is a powerful tool, providing the ability to characterize live organism physiology in the natural context of cellular and sub cellular resolution. However the necessity to perform studies on a live organism might associate with severe motion artifacts that should be corrected. Most of the software solutions are dedicated to solve this problem can do it with different levels of success, but they fault in the case when motion artifacts accompanied with a strong structural changes inside the object occur during image acquisition. Here we demonstrate the use of global Kappa-Omega approach to stabilize image without supervision and assignment of a reference image.

As an experimental intravital setup we used previously developed Transcranial Optical Vascular Imaging (TOVI) approach [1], where we use a dynamic fluorescence contrast enhanced microscopic images of cerebral blood vessels accompanied with moderate jerks during image acquisition as well as randomly applied larger jerks after post acquisition fig 1.a. Adaptive approach consists in applying the accurate cone shape Kappa-Omega filter on a 3D Fourier transform matrix [2] (that contains mix of all frequencies including spatial and temporal) fig.1b.

Results are presented in a fig. 1c,d in a temporal color-coded manner that clearly demonstrates the efficiency of the adaptive approach by suppressing motion artifacts while saving structural changes during acquisition 1d.
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Figure 1.

- a) Represented an image stack x,y of TOVI images over time t.
- b) Time-space Fourier filter represented in the k omega space taking into account the symmetric properties of the Fourier transform.
- c) A temporal color-coded image of cerebral blood vessels through the intact cranium before applying an adaptive filter. Color coded bar is a result of temporal color coding (FIJI/ImageJ) which is applied along the time sequence to distinguish the time evolution structures from the image motions when stacking the 800 images.
- d) A temporal color-coded image after applying an adaptive K-Omega filter (same region of interest). White scale bar is 500um.

Developed adaptive approach might be useful when alternative techniques fail to be successful to correct moderate motion artifacts associated with structural changes in the image sequence when no reference image can be assigned. Our proposed adaptive approach would be also a useful tool to combine with standard registration method such as correlation methods based on Fourier transforms.

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References
Invited Lecture

USING TWO PHOTON MICROSCOPY FOR BRAIN RESEARCH

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In this talk I will briefly elucidate the principles of two photon microscopy and the advantages in using this type of microscopy for deep imaging in living tissues. I will describe the work from my lab where we use two photon microscopy in combination of genetically encoded calcium indicators to chronically record from the cortex while the animal is performing a behavioral task. The advantages over other techniques and the limitations will be discussed.
UNDERSTANDING INTRACELLULAR ORGANIZATION IN CELLS AS REVEALED BY 3D ELECTRON MICROSCOPY

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The ways in which cell architecture is modelled to meet cell function is a poorly understood facet of cell biology. However, the compartmentalization of mutually exclusive reactions in different regions of cells by membrane-enclosed organelles or by self-assembling macromolecular complexes is a basic mechanism of life. Fluorescent tags can display proteins confined to compartments in living cells, but provides no glimpse of the underlying ultrastructure. The electron microscope has the resolution to display cellular ultrastructure. Transmission electron microscopy (TEM) tomography of thin slices (~100-300nm depth) can reveal complex subcellular cellular ultrastructure. Serial Block Face Scanning Electron Microscopy (SBFSEM) can provide ultrastructural resolution throughout a much longer (μm to mm) sample depth, enabling quantitative analysis of ultrastructural features throughout the length of most complex cells.

With these approaches we have studied the cytoarchitecture of a cell with highly specialised organisation, the cochlear inner hair cell (IHC), using multiple hierarchies of 3D electron microscopy analyses. We have shown that synaptic terminal distribution on the IHC surface correlates with cell shape, and the distribution of a highly organised network of membranes and mitochondria encompassing the infranuclear region of the cell. Structural linkages between organelles that underlie this organisation were identified by high resolution imaging. Together these techniques have the potential for clarifying functionally specialised cytoarchitecture of other cell types. Strategies employed to improve data quality will be discussed.
INVESTIGATIONS OF EUKARYOTIC TRANSLATION MACHINERIES THROUGH SINGLE PARTICLE CRYO-EM

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Ribosomes are giant ribonucleoprotein machineries residing within all living cells that translate the genetic code unto proteins, thus play an important role in cellular maintenance and viability. The key function and abundance of these machineries make them an effective target for various therapeutic approaches. Over the past decades, thorough structural and biochemical investigations of bacterial ribosomes revealed some fascinating aspects of the prokaryotic translation apparatus as well as the various mechanisms by which antibacterial drugs target the ribosome. Nevertheless, due to the more complex and variable nature of the eukaryotic ribosome, the molecular details underlying its unique characteristics remain largely obscure. The recent technological progress in high resolution structure determination by single particle cryo-EM has opened new opportunities in the field of ribosome research. Using this technology in our recent structural investigations of eukaryotic ribosomes has enabled us to create a sub-atomic level structure which highlights several differential characteristics of highly distinct eukaryotic species. This structure and its resulting insights may be useful not only in shedding light on the underlying mechanisms of eukaryotic translation but also could be of great importance in development of specific selective therapeutics.
THE BACTERIOPHAGE LIKE INFECTION OF THE LARGE EUKARYOTIC INFECTING VIRUS PBCV-1

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Paramecium Bursaria Chlorella Virus-1 (PBCV-1) is the prototype member of Phycodnaviridae family and shares common features with other group members of the Nucleo Cytoplasmic Large DNA Viruses (NCLDV) clade. The virus is an icosahedral shaped and it harbors ~331 Kbp of dsDNA genome enclosed by one internal membrane. Except for Mimivirus and Vaccinia Virus all DNA viruses replicate their genomes in the nucleus of their hosts. The rationale is that the nucleus is a suitable platform to carryout viral transactions such as DNA replication and transcription of viral genes. In the case of PBCV-1 it was postulated that viral genomes have to be transported to the nucleus of the Chlorella cell in order to enable viral DNA replication and this is due to the facts that no recognizable RNA polymerase gene was identified in the virus genome and that several viral genes possess introns that have to be cleaved by the spliceosome machinery. Trafficking of large genomes in the highly crowded cytoplasmic milieu is associated with substantial barriers including intracellular membranous compartments and cytoplasmic nucleases. In addition, the large chloroplast of the Chlorella cells act as a substantial barrier for the movement of large molecular assemblies. In our studies we investigated the infection cycle of the large dsDNA virus PBCV-1 in details. By combining advanced high-resolution fluorescence and electron microscopy techniques we have shown that in contrast to other members of the NCLDV, PBCV-1 infection is initiated with a unique mechanism reminiscent that of bacteriophages. Using Scanning Transmission Electron Microscopy (STEM) tomography we showed that soon after adsorption to the cell wall, PBCV-1 generates a hole in the cell wall through which a narrow membranous tunnel is formed by the fusion of the plasma and viral membranes, and is used for viral genome delivery into the cytoplasm. We carried out high resolution florescence imaging studies using Stochastic Optical Reconstruction Microscopy (STORM) in order to track viral genomes. These studies revealed that soon after being ejected into the cytoplasm viral DNA is transported to the nucleus. The STORM studies were further supported by immuno-labeling experiments of high pressure frozen and freeze substituted (HPF-FS) Chlorella infected cells and Electron Microscopy In Situ Hybridization (EMISH) assays that provides specific detection and localization of viral DNA. Viral DNA replication most likely occurs in the nucleus of the cells. Our results strongly support this notion as we were able to detect viral DNA egress from nuclei of infected cells in thin TEM sections of HPF-FS Chlorella infected cells that were labeled with DNA antibodies. The infection proceeds with the generation of elaborated "viral organelles", termed, viral factories that are generated in the cytoplasm of the host. These organelles are the sites where viral transactions such as DNA replication, transcription of viral genes, membrane biogenesis, packaging of viral DNA and assembly of new progeny, take place. The viral factories generated by PBCV-1 are profoundly different from those produced by other group members of the NCLDV, Vaccinia virus, African Swine Fever Virus (ASFV) and Mimivirus. We used several techniques that provide 3D information with nanometer resolution, specifically, STEM tomography and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), in order to investigate the 3D organization and function of the factories generated by PBCV-1. These experiments were corroborated by florescence microscopy studies. Our studies revealed the distinct rosette-like architecture of the factories, with viral genomes at the outer periphery, host cisternae at the inner periphery, and single bilayer membrane sheets used as capsid templates in the central region. The cisternae bud out from outer nuclear membranes and translocate to the periphery of the factories and even penetrate deep in to the core of the factory were they are rendered into single open membrane sheets. Virus assembly takes place at the periphery of the factory, and at the end of the process, viral DNA is packed through a narrow aperture in a well-defined singular vertex. This mechanism of membrane biogenesis demonstrated for PBCV-1 is similar to other group members of the NCLDV such as Mimivirus, ASFV and Vaccinia virus. Overall, our results point towards the notion that the eukaryote-infecting PBCV-1 infection initiates and proceeds through a bacteriophage-like infection.
A 3D surface representation of a STEM tomogram showing the rosette-like crescent structure of the viral factories generated by PBCV-1. The crescents are composed of two distinct layers, an external angular capsid (yellow) and an underlying internal membrane (light blue). The core of the factory consists of open membrane sheets that act as templates for the assembly of the capsids.
Cells employ protrusive leading edges to navigate and promote their migration in diverse physiological environments. Classical models of leading edge protrusion rely on a treadmilling dendritic actin network that undergoes continuous assembly nucleated by the Arp2/3 complex, forming ruffling lamellipodia. Although the dendritic nucleation model has been rigorously evaluated in several computational studies. Experimental evidence demonstrating a critical role for Arp2/3 in the generation of protrusive actin structures and cell motility has been far from clear. Most components of the pathway have been probed for their relevance by RNA interference or dominant-negative constructs. However, given that the Arp2/3 complex nucleates actin at nanomolar concentrations, even a dramatic knockdown could still leave behind a level sufficient to fully or partially support Arp2/3-complex dependent functions.

Our recent work characterizes newly developed fibroblasts cell lines completely lacking functional Arp2/3 complex. We tested the impact Arp2/3-complex function on these genetically matched cells with and without Arp2/3 complex using single cell spreading assays, wound healing assays, long-time single cell motility tracking, chemotaxis assays, and fluorescence imaging with confocal or structured illumination microscopy [1,2]. In the absence of functional Arp2/3 complex, the fibroblasts were unable to extend lamellipodia but generated dynamic leading edges composed primarily of filopodia-like protrusions (FLPs), with formin proteins (mDia1 and mDia2) concentrated near their tips. These mutant fibroblasts maintained an ability to move but exhibited a strong defect in persistent directional migration in both wound healing and chemotaxis assays [1,2]. Here, we will highlight our advances in determining the molecular-level organization of the actin networks of these cells through an integrated approach that employs electron cryo-tomography of whole mammalian cells in conjunction with correlative light microscopy.

References:
[3] This work is supported by NIGMS grants P01 GM066311 and P01 GM098412.
ADVANCES IN COMPUTATIONAL IMAGING FOR QUANTITATIVE 3D FLUORESCENCE MICROSCOPY

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Many biological and biomedical applications increasingly require the ability to visualize living specimens at higher resolution and in new ways that provide information not available in the past. Computational imaging plays an important role in the advances achieved in high resolution fluorescence microscopy [1]. A major challenge in high resolution microscopy for most biomedical applications is the inherent depth variability of the imaging process, i.e., as the imaging depth increases within a sample the three-dimensional (3D) point spread function (PSF) changes [2]. This is due to the refractive index (RI) mismatch in the imaging layers [2, 3], which introduces spherical aberration (SA) in microscopy images. Although computational optical sectioning microscopy (COSM) [4] has extended the fluorescence light-microscope to a 3D imaging tool, imaging of optically thick specimens is limited due to depth-induced aberration that worsens when focusing deeper into a sample and varies spatially with sample RI variability. In traditional COSM only one 3D PSF is used in the imaging reconstruction process (aka deconvolution) [5], but when the PSF variability is significant, the use of multiple depth-variant or space-variant PSFs is necessary to reduce undesirable computation artifacts [6]. Computational complexity generally increases with the number of 3-D PSFs used in the restoration and thus, reducing the sensitivity of the 3D PSF to SA could decrease the number of PSFs needed to achieve the desired image quality.

The widespread use of COSM has motivated development of new computational methodologies to improve the restored image quality by accounting for PSF variability while reducing computational cost. In this talk I will discuss two complimentary approaches that we have been developing to address depth-induced aberrations. The first approach involves the development of new computational algorithms for 3D data processing, based on either a depth-variant [7] or a space-variant [8, 9] image formation model for a traditional microscope. The latter, accounts for sample RI variability. The second approach relies on an instrument modification for PSF engineering through wavefront encoding, which renders the microscope less sensitive to depth aberration [10] and allows the use of model-based restoration methods previously developed for COSM.

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Acknowledgement:
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Proteins carry out most of the functions of the cell. Therefore, dissecting how proteins are organized is fundamental to our understanding of biological processes. We present a microscopy setup that we have been developing to quantify protein concentrations and distributions in living yeast cells at high-throughput. The live cell imaging is performed using a spinning disk confocal microscope. It enables the imaging of a 384 well-plate with three channels and in 3D in under an hour. The resulting images are processed automatically, enabling the analysis of thousands of images. As each image typically contains hundreds of cells, this setup uniquely allows investigating phenotypic variability. We have been developing ImageJ plugins to allow cell segmentation from brightfield images and the extraction of fluorescence properties from individual cells. We present three applications of these plugins. In a first use, we examined cell-to-cell variability in protein concentration and the impact that protein degradation can have on this variability. In a second implementation, we examined the concentration dependence of protein aggregation. In a third project, we explored how protein concentration and stoichiometry impact the assembly of multivalent interacting proteins.
THREE-DIMENSIONAL RECONSTRUCTION FROM CRYO-ELECTRON MICROSCOPE IMAGES OF SINGLE PARTICLES IN FOURIER SPACE USING A NEW PROGRAM, ICR3D

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Cryo-electron microscopy (cryo-EM) coupled to single particle analysis is an important technique for determining the structure of isolated proteins and protein complexes. A key step in the procedure is three-dimensional reconstruction in which individual two-dimensional molecular images in different orientations are combined to produce the final three-dimensional reconstruction. Here we describe a new computer program, icr3d, which carries out this step by merging the input data in Fourier space. This is achieved by calculating a weighted average of the input data for each Fourier component of the final three-dimensional map. Weighting factors are used to take account of the contrast transfer function of the input data and the interpolation onto the required three-dimensional grid. The latter step uses a sinc function scaled to the fractional spatial occupancy of the target structure within the reconstructed volume. The program has been used in recent high resolution cryo-EM studies (da Fonseca and Morris, 2015, Li et al, 2016) and is freely available for download as part of the tigris package for image analysis of cryo-EM data at sourceforge.net.

References


Invited Lecture
SINGLE MOLECULE APPROACHES FOR STUDYING GENE EXPRESSION IN INTACT MAMMALIAN TISSUES

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Mammalian tissues are composed of heterogeneous cells, interacting in highly structured microenvironments to achieve physiologic goals. To understand how cells operate in tissues we apply single molecule Fluorescence in-situ Hybridization (smFISH), a technique enabling visualization and quantification of individual mRNA molecules of any gene of interest in the intact tissue. I will describe applications of these approaches to measure not only amounts of mRNA per cell but also transcription and mRNA degradation rates, as well as intra-cellular localization patterns. Using this approach we have uncovered bursty transcription of genes in the intact mouse liver and identified cellular strategies to dampen the associated single-cell variability in mRNA copy numbers.
Invited Lecture

USING HIGH RESOLUTION ELECTRON MICROSCOPY FOR STRUCTURE-PERFORMANCE CORRELATION IN ORGANIC AND HYBRID SOLAR CELLS

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Organic and hybrid photovoltaics devices have emerged as promising, solution processed thin film solar cells. In such devices, the active layer is composed of blend of an electron donor and electron acceptor species mixed on the nano-scale and termed bulk heterojunction (BHJ). When light is absorbed by the donor, an electron is promoted to an excited state generating an exciton (e-h pair). Excitons at the donor:acceptor interface dissociate into free carriers by electron transfer to the acceptor. The carriers then migrate in the electric field and along the respective phases, electrons on the acceptor and holes on the donor, to the electrodes. Therefore, the performance of such devices strongly depends on the BHJ morphology which is complex and includes ordered, disordered and mixed domains. The aim of this presentation is to show how electron microscopy techniques are used to characterize the nanostructure morphology of organic and hybrid BHJs and allow a structure-performance correlation. Few examples will be demonstrated including: the use of HRSEM EDS to calculate the mass of an inorganic acceptor in the organic donor; EFTEM to determine the size and distribution of all-organic donor and acceptor domains; and HAADF STEM to follow the distribution of organometallic complexes in the BHJ.
EFFECT OF POLYELECTROLYTE STIFFNESS AND SOLUTION PH ON THE NANOSTRUCTURE OF COMPLEXES FORMED BY CATIONIC AMPHIPHILES AND NEGATIVELY CHARGED POLYELECTROLYTES

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The interaction between amphiphiles and polyelectrolytes has been widely investigated in recent years due to their potential application in industry and medicine, with special focus on gene therapy. The binding of polyelectrolytes to oppositely charged amphiphiles is dominated by electrostatic interactions, but hydrophobic forces and the molecules nature also play an important role; thus influencing the final nano-complex structure.

In this work we used direct-imaging cryo-TEM and SAXS to study the nanostructure of complexes formed in different double-tailed amphiphile/polyelectrolyte systems. The cationic lipid di-oleoyl trimethylammonium propane, DOTAP, and the oppositely charged polyelectrolytes, sodium poly(acrylic acid), NaPAA, and sodium poly(styrene sulfonate), NaPSS, form multilamellar complexes in water. Due to the different molecular stiffness of the two polyelectrolytes, the different morphology of the complexes is distinct. Also, because of different ionization behavior of the two polyelectrolytes, pH affects differently the complexation of the polyelectrolytes with didodecyldimethylammonium bromide (DDAB), a cationic surfactant. PAA demonstrates a pH-dependent behavior, because the carboxylate it has a pKa of 4.2. In contrast, the sulfonate groups, attached to the PSS backbone, disassociate in the entire pH range, hence the complexes formed between the surfactant and the polyelectrolyte are insensitive to pH variations.

In addition, SAXS results demonstrated that the periodic distance in DDAB/NaPSS complexes is almost twice as much as the periodic spacing of DDAB/NaPAA complexes (~6 nm as compared to ~3.3 nm). That is most probably due to the differences in the polyelectrolyte conformation at the surfactant-water interface. Because polystyrene sulfonate is much more hydrophilic than poly(acrylic acid), fully deprotonated at all pH, those charged groups that do not interact with the positively charged headgroups of the surfactant, interact with the water molecules. PAA, with a more hydrophobic backbone, adsorbs more tightly to the surfactant lamellar surface, where it may adopt a flatter conformation, while the more hydrophilic PSS chains interact better with water, and thus may adopt a more coiled conformation, which leads to a thicker layer, and, hence, a larger periodic distance. These differences were not observed in the DOTAP/NaPAA and DOTAP/NaPSS systems, due to a more similar interaction of both polyelectrolytes with the DOTAP head group.
**Figure 1.** Cryo-TEM images of: DDAB and NaPAA (A-C); DDAB and NaPSS (D-F); CR=1 in all. Bars correspond to 50 nm.
ONE-DIMENSIONAL SELF-ASSEMBLY OF DIACETYLENIC PHOSPHOLIPID INTO TUBULAR STRUCTURES

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Since they were first introduced by Schnur and colleagues over 3 decades ago\textsuperscript{1}, lipid nanotubes are being explored due to their principal roles in biology and medicine, as well as their potential in diverse applications. A specific class of synthetic lipids comprising diacetylene moieties in their tails, diacetylenic phospholipids, were shown to present distinct self-assembly, and form multilamellar nanotubes in alcohol-water solutions.

The first tube-forming amphiphilic lipid - 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine designated DC\textsubscript{8,9}PC, have highly ordered and kinked alkyl chains which has been found to induce chiral self-assembly, regardless whether the constituent molecules are chiral\textsuperscript{2}. Previous work focused on the properties and possible applications of the tubes, yet fundamental questions regarding their formation mechanisms were not addressed.

Using advanced electron microscopy techniques as the main research tools, we provide the first structural insight into the mechanism of formation of multilamellar DC\textsubscript{8,9}PC nanotubes. We show that these lipid nanotubes form through fast reorganization of the lipid membrane that involves unfolding, curling, bending and fusion. This new path provides an alternative pathway for the formation of peptide nanotubes, known to proceed from ribbon elements\textsuperscript{3, 4}.

In addition to direct-imaging cryo-TEM we applied 3D cryo-TEM analysis using cryo-electron tomography (cryo-ET), a powerful method for 3-dimensional reconstruction of structures in solution\textsuperscript{5}. Cryo-ET was applied for resolving overlapping morphologies and understanding complex membrane-tubular intermediate structures identified through the self-assembly pathway.

**Fig 1.** Left - Cryo-TEM image of DC\textsubscript{8,9}PC membranes in 70% EtOH/H\textsubscript{2}O solution captured during fast cooling to room temperature. Scale bar=200nm. Right - Segmented fragment from a tomogram showing membranes healing to form a multilamellar tubule wall.

**References:**
Invited Lecture
STRONGER CEMENT

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Nanotubes (NT) are considered as promising nano-reinforcement fillers in cement-based materials. The main challenge towards achieving a significant enhancement in cement properties is an effective dispersion of the agglomerated NT. In the talk I will demonstrate how efficient NT (carbon or WS$_2$) dispersion in cement results in substantial flexural and compressive strength enhancements at NT concentration below 0.15 wt%. The reinforcement by WS$_2$ NTs (see figure) remains significant after a variety of curing processes, suggesting a genuine nanoscale reinforcing effect. Finally, by employing a comprehensive fractography we show that the WS$_2$ NTs inhibit crack propagation by bridging with a pullout failure mechanism.

SEM image of WS$_2$ NT-based cement composite showing a WS$_2$NT covered by a layer of Calcium-Silicate-Hydrate (C-S-H) phase.
Invited Lecture
NON-PLANAR NANOSTRUCTURES AT ATOMIC SCALE: FROM ATOMIC STRUCTURE TO PHOTONICS IN THE (S)TEM

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Technology at the nanoscale has become one of the main challenges in science as new physical effects appear and can be modulated at will. Materials for spintronics, electronics, optoelectronics, chemical sensing, and new generations of functionalized materials are taking advantage of the low dimensionality, improving their properties and opening a new range of applications. As developments in materials science are pushing to the size limits of physics and chemistry, there is a critical need for understanding the origin of these unique physical properties (optical and electronic) and relate them to the changes originated at the atomic scale, e.g.: linked to changes in (electronic) structure of the material.

During the seminar, I will show how combining advanced electron microscopy imaging with electron spectroscopy, as well as cathodoluminescence in an aberration corrected STEM will allow us to probe the elemental composition and electronic structure simultaneously with the optical properties in unprecedented spatial detail.

The talk will focus on several examples in advanced nanomaterials for optical and plasmonic applications: quantum structures self-assembled in a nanowire as well as metal multiwall nanoboxes and nanoframes for 3D plasmonics. In this way the latest results obtained by my group on direct correlation between optical properties at sub-nanometer scale and structure at atomic scale will be presented.
ELECTRON BEAM INDUCED CURRENT STUDY OF HALIDE PEROVSKITE-BASED SOLAR CELLS

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The rapid improvement in halide perovskite (HaP)-based photovoltaic (PV) device efficiency and their prospective use in LED and laser application has resulted in an accelerated world-wide effort for materials characterization. The halide perovskites are ABX$_3$ compounds, with A a monovalent cation (methylammonium, formamidinium or Cs are those found suitable till now), B is a divalent cation (Pb or, possibly Sn) and X is a halide anion. Using a mixture of organic cations and halides, 20% PV conversion efficiency device has been demonstrated in state of the art devices.

Electron microscopy and associated analytical methods are the most common tools to gain information regarding film morphology, chemical composition and electronic properties at high spatial resolution. We use electron beam induced current (EBIC) to learn about the mechanism of HaP-based PV cell action. During EBIC measurement, the high energy electron beam locally excites carriers while measuring the resulting electro-voltaic current. This allows mapping of the collection efficiency across the device cross section. The collection efficiency profile can then be used to determine electronic properties of the device, such as operating mechanism, carrier diffusion lengths and even doping density. Here the use of EBIC for three types of HaP-based PV devices will be demonstrated. Furthermore adding a light as a second source of excitation and biasing the sample allows a more in-depth study of the electronic properties of the materials involved.

With increasing exposure to the electron beam the EBIC signal shape and intensity gradually change, affecting the apparent electronic properties. By understanding the degradation processes in the material/device, we show that the sensitivity of the method towards beam-induced degradation can be used to add to our understanding of the device function. Comparing the EBIC signal degradation of methylammonium lead iodide (MAPbI$_3$) with that of MAPbBr$_3$ PV cells, we uncover quasi-neutral regions in the former. In addition, comparing the degradation of Cs-based devices with MA-based ones helps correlate the induced damage with core level ionization of the ions.
DISCERNING INTERFACE ATOMISTIC STRUCTURE BY PHASE CONTRAST IN STEM

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The stability of metal films on oxide surfaces is important for the performance of devices such as solid oxide fuel cells (SOFCs) and thermal barrier coatings (TBCs) [1, 2, 3]. Ni-YSZ serves as an anode material in SOFCs. During SOFC operation, the metal-ceramic interface is subjected to high temperatures and a reducing atmosphere, which can lead to coarsening of the Ni nanoparticles, which decreases the number of three-phase boundaries. The three-phase boundaries (Ni/YSZ/fuel-gas) are essential for catalytic activity which controls the electrical properties. A better understanding of the equilibrated Ni-YSZ interfacial structure and energy can lead to improved adhesion and long-term stability of SOFCs [4, 5].

In this work, solid-state dewetting of continuous Ni films deposited on the (111) surface of yttrium stabilized zirconia (YSZ) was used to produce equilibrated Ni particles, and after determination of the solid-solid interface energy [6], the structure of the same interface was determined using aberration corrected transmission electron microscopy. The ~150nm thick Ni films were annealed at 1350°C (0.94 Tm) in Ar+H2 (99.9999%) at a partial pressure of oxygen of 10^{-20} atm for 6 hours. Transmission electron microscopy of equilibrated particles which was conducted to analyze the structure at the interface revealed that despite the 31% lattice mismatch between Ni and YSZ, the interface is semi-coherent and a two dimensional network of misfit dislocations was identified [7].

References:
A detailed analysis on the quality and microstructure of various metal/semiconductor superlattices employing HR(S)/TEM (high-resolution (scanning)/transmission electron microscopy) imaging and energy dispersive x-ray spectroscopy (EDX) mapping on as-deposited and annealed samples is presented.

Epitaxial metal/semiconductor superlattices are known to be promising candidates for compounds in electronic, photonic, and plasmonic devices, but are also of interest for applications as hard coatings, and in thermoelectric materials [1]. The crystalline quality of the superlattices, in terms of their defect density, phase purity, interface roughness, and stoichiometry of the individual layers, plays a crucial role with respect to the physical properties and thus the applicability of such superlattice stacks. It was recently shown that metal/semiconductor superlattices based on (Al,Sc)N as the semiconductor component can be grown epitaxially with low-defect densities by magnetron sputtering on [001]MgO substrates [2].

Phase formation and thermal stability studies of as-deposited and long-time annealed cubic TiN/(Al,Sc)N superlattices employing a combination of HR(S)/TEM and EDX mapping revealed intermixing of the TiN and (Al,Sc)N layers by interdiffusion of the metal atoms with increased annealing time [3].

Improved (Ti,W)N/(Al,Sc)N [4] and (Hf,Zr)N/ScN [5] superlattices were grown by magnetron sputtering and analyzed with various TEM methods, and their microstructural evolution as well as thermal stability becomes presented here. Figure 1 demonstrates thermal stability of Zr,Hf_{1-x}N/ScN by comparing an as-deposited with a 120 hrs annealed superlattice stack via EDX mapping.

All experiments were conducted at Linköping’s image- and probe-corrected and monochromated FEI Titan³ 60-300 microscope equipped with a Gatan Quantum ERS GIF, high-brightness XFEG source, and Super-X EDX detector, operated at 300 kV [6].
Invited Lecture
CORRELATIVE LIGHT AND ELECTRON MICROSCOPY IN CELL BIOLOGY

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In recent years correlative microscopy, combining the power and advantages of light and electron microscopy, has become an important tool for biomedical research.

Light microscopy has the advantage of easily searching large areas, even volumes, for the cells of interest, e.g., a special cell type in tissue, astrocytes in brain\textsuperscript{2} or for cells that have been modified either by transfections or by RNAi in a large population of non-modified cells. Also on thin sections, the low magnification of light microscopy and therefore ease of searching large areas are very beneficial to speed-up the analysis of rare events\textsuperscript{3}. The predominant disadvantage of this technique, however, is that only the fluorescently labelled structures can be imaged in relation to each other.

Electron microscopy reveals the cellular ultrastructure a high resolution and individual organelles, even large protein polymers like cytoskeletal filaments or ribosomes can unequivocally identified. Proteins of interest can be labelled with colloidal gold. Searching for a few gold particles within a few cells of a large tissue, however, is very cumbersome and can be extremely time consuming.

Seen the advantages of light and electron microscopy suggests that the optimal approach is to combine both techniques for cell biology research. Localisation of rare cellular events are followed and identified by (fluorescence) light microscopy, the high resolution data and fine localisation to cellular substructures are done by electron microscopy.

In this presentation we will describe the approach we have chosen to follow the cell(s) of interest from sampling the tissue until the analysis by electron microscopy\textsuperscript{4}.

We have developed a novel correlative imaging and analysis technique that combines optical cryo-fluorescence imaging with high resolution morphological (cryo-SEM), spectroscopic (cryo-EDS) and electron density (BSE) characterization in the scanning electron microscope.

For the correlative imaging, live specimens of foraminifera, unicellular marine protozoans with calcitic shells, were cultured in calcein-containing sea water. The specimens were prepared for cryo-SEM imaging by high pressure freezing and freeze fracture. Each foraminifer exposed in the fracture was first imaged in cryo-SEM at low magnification to obtain an overview image of the fracture surface, and then systematically scanned in SE and BSE mode at medium magnification. These images were stitched together to provide a detailed view of the cytoplasm contents. Specific areas of interest were further imaged at higher magnification. The vitrified specimens were then transferred in a cryo-Correlative Light Electron Microscopy (cryo-CLEM) stage to the confocal microscope, and fluorescence images were taken. Maximum intensity projections were used for alignment to the SEM images. Adjustments to the alignment were made using the shell outline and the intracellular symbionts as anchors in the SE mode collage and the fluorescence image (Fig. 1).

Using this correlative approach, we studied the foraminifer cell cytoplasm under close to physiological conditions, without dissolution of the shell, and analyzed the elemental compositions of different phases within their cellular context. We also observed Mg$^{2+}$-rich dense mineral particles both in the cytosol and in sea water vacuoles (Fig. 2). We suggest these may be related to the mechanism of removal of excess Mg$^{2+}$ from sea water. Imaging the interior of shelled organisms is difficult by traditional light microscopy due to scattering by the shell itself. The new method provides a high resolution view of the cytoplasm, symbionts, and transport vesicles where ions may be concentrated or removed. Ion-sensitive fluorescent dyes and cryo-EDS mapping are used to identify the ions. This technique may well have widespread applications to many other shelled single-cell mineralized organisms in addition to foraminifera, such as coccolithophorids, diatoms, radiolarians, ciliates and acantharia etc., as well as for documenting aspects of mineral transport pathways in multicellular organisms.
Figure 1: Correlative cryo-SEM/fluorescence microscopy. A) Low magnification cryo-SEM image in SE mode of an *A. lessoni* foraminifer; B) The same specimen imaged in BSE mode. The shell mineral is seen with high contrast. This image was obtained by stitching together tens of higher magnification images that were taken with partial overlap to facilitate reconstruction. C) Fluorescence image of the same specimen. Symbionts are red and calcein staining is green. D) Overlay of the fluorescence image on the electron microscope image. The shell and the symbionts are used for alignment.

Figure 2: Mg rich particles in a specimen of *A. lobifera*. Correlative microscopy. Above: BSE micrograph and overlay of fluorescence on SE micrograph. The white arrows mark the position of the Mg rich particles, which are electron dense, but are only weakly fluorescent. Below: Cryo-EDS mapping, Ca map and Mg map: In the Ca map, the shell is clearly visible, in the Mg map the signal from the particles is very strong.
CORRELATIVE FIB/SEM AND DYNAMIC MICROSCOPY REVEAL MACROPINOSOMES ARE KEY PLAYERS IN *SHIGELLA* INVASION

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Intracellular pathogens include all viruses, many bacteria and parasites capable of invading and surviving within host cells. Key to survival is the subversion of host cell pathways by the pathogen for the purpose of propagation and evading the immune system. The intracellular bacterium *Shigella flexneri*, the causative agent of bacillary dysentery, invades host cells in a vacuole that is subsequently ruptured to allow growth of the pathogen within the host cytoplasm. We applied dynamic imaging and advanced large volume correlative light electron microscopy (CLEM) to study the highly transient events of *S. flexneri*'s early invasion into host epithelial cells and elucidate some of its fundamental features. The results obtained provide the basis for a new model of the early steps of *S. flexneri* invasion, establishing a different view of the enigmatic process of cytoplasmic access by invasive bacterial pathogens.
A major challenge for standard electron microscopy has been the identification of rare cellular events or specific cells of interest in a complex tissue. Immuno-EM offers one solution but technical challenges and limitations frequently render this approach insufficient. Correlative Light and Electron Microscopy (CLEM) procedures have been developed to unite optical methods that provide an overview of protein expression in cells, with ultrastructural methods that offer nanometer-scale resolution. These procedures have proven successful for cells grown in a cultured monolayer, however, few solutions exist to adapt them to polarized multicellular samples.

The principal challenge in analyzing multicellular samples is identification of the Volume of Interest (VOI) and subsequent correlation of this region between light and electron microscopy images. In addition, small model organisms (including C. elegans, Drosophila, zebrafish, planaria, and tardigrades) impose additional challenges during sample preparation due to the need for proper 3D orientation of the block for sectioning. In order to recognize the structures of interest within this complex anatomy, the electron microscopist needs to have a deep familiarity with each organism’s anatomy at the ultrastructural level, which is rarely the case.

We recently developed a workflow to optimize EM sample preparation of small multicellular organisms while allowing fluorescent protein localization in ultrathin resin sections, and we have demonstrated its utility in C. elegans and Drosophila. Briefly, rapid high-pressure freezing is used to retain native fluorescence in the samples. Next, a two-step flat embedding procedure simplifies the generation of 3D maps, which are further used to orient the sample within the block and to target sectioning precisely to the VOI. This method uses a combination of anatomical cues and engineered landmarks, recognizable in both LM and EM, to orient the sample. The resulting serial sections are transferred to silicon wafer support, using a modified Array Tomography (AT) approach. The ability to retain fluorescence in the embedded sample facilitates rapid recognition of the VOI by light microscopy and serves to localize that region for subsequent SEM. Correlation within the 3D volume and reconstruction of 2D data is thus more precise and direct. These methods can be combined with recent advances in super-resolution light microscopy to accurately map protein localization to ultrastructural features. As thousands of strains expressing fluorescently-tagged proteins or cells of interest have already been generated by these research communities, this approach has the potential to be widely adopted and solve a long-standing problem in connecting traditional fluorescence microscopy experiments with ultrastructural information.
Figure 1: A. En block fluorescence of C. elegans pharyngeal muscles GFP after high pressure freezing and fast freeze substitution. B. AT sections on silicon wafer. C. A LM image of transmission and GFP fluorescence on wafer. D CLEM image of a section showing fluorescent, EM and the superposition of both.
Invited Lecture

NANO OPTICS WITH FAST ELECTRONS

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How light behaves and interacts with matter at the nanometer scale is a fascinating subject. Indeed, at this scale, both the electromagnetic field and the electron wave functions may be subject to confinement. This is why the optical properties of nano-objects will in general depend drastically on their shape, size and local environment. This is the case for surface plasmons on metallic nanoparticles, which can be viewed as classical electromagnetic standing waves, or for the excitons in quantum emitters (such as Quantum Dots), where the confinement now affects the excitons wavefunction.

The typical sizes at which confinement becomes crucial range from few angströms (for excitons) to tens or hundred of nanometers (for plasmons). It is thus important to have tools able to probe optical and structural properties at these scales. Of course, regular optical microscopies and spectroscopies are not able to deliver such spatial resolution. Recently, electron spectroscopies such as Electron Energy Loss Spectroscopy (EELS) and Cathodoluminescence (CL) used in a Scanning Electron Microscope (STEM) have shown to address this issue.

In this presentation, I will thus present how recent technical and conceptual developments in EELS and CL have allowed to explore various aspects of nano-optics (plasmonics, photonics, quantum optics) at the scale relevant for plasmons and quantum emitters: few nanometers, few meV, few nanoseconds.

Figure 1: False color multispectral CL mapping of GaN quantum wells in an AlN shell, showing the identification of light emission from individual quantum confined structures.
MEASURING CHARGE DISTRIBUTION IN NANOSCALE MAGNESIUM ALUMINATE SPINEL BY ELECTRON ENERGY-LOSS SPECTROSCOPY AND ELECTRON HOLOGRAPHY

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Segregation of dopants and defects in ionic materials result in the formation of a space charge zone (SCZ), which is studied extensively for its critical role on functional properties [1]. Although significant theoretical advances have been achieved, the experimental evidence in nanocrystalline ionic materials is indirect. Therefore, we investigated the distribution of defects on the formation of a SCZ in a model system of nanosized MgO·nAl₂O₃ (MAS, n= 0.95 and 1.07). The SCZ was investigated experimentally by electron energy-loss spectroscopy (EELS) and off-axis electron holography (OAEH).

EEL spectra were collected along directions perpendicular to grain boundaries (GB’s), from which the magnesium-to-aluminum relative cation concentrations were calculated, as presented in Fig.1. We found that regardless of annealing processes, the vicinity of GB’s of MgO·0.95Al₂O₃ has excess Mg⁺² cations while the vicinity of GB’s of MgO·1.07Al₂O₃ has excess of Al⁺³ cations. Additionally, the cation distribution shows strong dependency on the grain size. For non-stoichiometric MAS, cation concentration is proportional to the defect concentration, because deviation from stoichiometry results in adjacent defects that compensate for the electric charge [2, 3, 4]. In both materials, the cation distribution is inhomogeneous for grains smaller than 40 nm. For larger grains, the defect concentration approaches the bulk value at the center of the grain. Furthermore, excess of Mg (Al) cations at the vicinity of the GB decreased with increase of grain size. Maier et al. [1] calculated that for grain size at the scale of the Debye length (estimated at 9nm for non-stoichiometric MAS studied here [7]), the GC is no longer electrically neutral, instead influenced by accumulation or depletion of charge at the boundaries.

Due to the lack of accurate values for defect formation energy [5, 6], we applied OAEH to measure directly the electrostatic charge distribution in nano-sized MAS. We show that charge distribution and the buildup of electrostatic potential between GB and core are linked to the spatial distribution of defects rather than the overall composition of MAS (Fig. 2). At the vicinity of GB’s, excess Mg⁺² or Al⁺³ cations accumulate depending on the composition, the magnitude of which increases with decreasing grain size. Indeed, the potential distributions show the relation between the grain size and the Debye length, in agreement with theoretical models [1].

Such results underscore the importance of comprehensive nanometer scale characterization of the chemistry and electrostatic potential in ionic complex oxides. Our experimental study enables to verify current models, and more important, develop and test new theoretical models, thus providing a comprehensive understanding of defects in complex oxides.
References:
0D METALLIC BEHAVIOR AT THE LaLuO$_3$ / SrTiO$_3$ INTERFACE

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One of the observed and reported phenomena in heterogeneous interfaces of perovskite oxides is the presence of a two-dimensional electron gas. In this study, the imperfect interface that is formed between pulsed laser deposited (PLD) LaLuO$_3$ (LLO) films of 30nm and 250 nm on SrTiO$_3$ (STO) were studied.

From cross-section transmission electron microscopy (TEM, FEI Titan 80-300 FEG-S with CS correction) imaging of the LLO/STO system for the 240 nm thick LLO layer (Fig. 1) it can be observed that the LLO film grows with a columnar structure. When looking at the interface of a single column of LLO and the STO substrate, it can be seen that both the substrate and film are crystalline and that they form an abrupt interface with no interfacial layer.

Pole figure textures using x-ray diffraction (XRD, Rigaku Smart Lab) allowed for an extensive analysis of the structure and orientation with better statistics. The samples were measured at to include the \{240\} and \{042\} diffractions of LLO while also including the \{210\} diffractions of STO (fig.2). The orientation relations are \{100\}$_{STO}$ || \{110\}$_{LLO}$ ; \{101\}$_{STO}$ || \{001\}$_{LLO}$ and \{100\}$_{STO}$ || \{001\}$_{LLO}$ ; \{130\}$_{LLO}$ for the part of the film adjacent to the interface and the top of a thick film, respectively.
It can be observed in images obtained by scanning tunneling microscopy (STM, Omicron AFM/STM system) that the intensity of the Z axis trace of either side of the interface is different (fig 3). On the left hand side of the interface the STO shows a higher intensity and appears as a smooth surface. Due to its relatively high density of states the STM tip remains in electrical contact with the surface. On the right hand side of the interface the LLO presents a lower intensity of a rough surface.

Localized I-V measurements on the STO and LLO sides and at their interface were performed by scanning tunneling spectroscopy (STS) (fig 4). Wince the I-V characteristics of the three types of positions: STO, LLO and interface, were extremely different, the derivative of the tunneling current around 0V was used to map the cross section of the interface (fig. 5b) while simultaneously collecting the z trace of the same position (fig. 5a). When superimposing the map of the metallic behavior over the STM scan (fig. 5c) it can be seen that the metallic behavior is found on the LLO side of the interface. This indicates that an accumulation of charge carriers is present on the LLO side not as a 2DEG but as 0D segments of metallic like behavior.
First principle calculations (not shown here) predicted an increase in local potential at the interface, but the discontinuity of the increased carrier concentration can explain why in many cases a 2DEG layer cannot be measured in many oxide combinations although its existence has been predicted by calculations. We hypothesize that the discontinuous nature of the carrier density was created at this interface due to the presence of defects at the imperfect LLO/STO interface.
Semiconductor nanowires (NWs) are one of the most promising building blocks for near future nano-electronics. The fabrication of nanowires is categorized into two main groups: bottom up approach, where the wires are grown by vapor-liquid-solid (VLS) chemistry, and the top down approach where the wires are patterned using standard microelectronic techniques. In this talk I will describe our recent studies of dopant profiles, electrical junctions and electronic states in single crystalline and poly crystalline Si NWs [1-3].

SOLVING CHALLENGING CRYSTALLOGRAPHIC PROBLEMS WITH AUTOMATED ELECTRON DIFFRACTION TOMOGRAPHY

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Many materials, ranging from minerals or catalysts to framework compounds and pharmaceuticals are not suitable for growing large crystals prohibiting single crystal X-ray analysis. Yet, introduction of nano crystallinity and special crystallographic features like disorder, defects, pseudo symmetry or stress/strain effects creates new or allows optimizing existing physical properties. With increasing complexity, special structural features and decreasing size of crystalline domains, X-ray powder diffraction becomes more and more difficult for structural characterization, which is fundamental for understanding material properties. High-resolution transmission electron microscopy (HR-(S)TEM), visualizing structural features directly at the atomic scale, introduces often beam damage whereas electron diffraction is less destructive. For a complete structure solution, delivering atomic positions in sub Ångstrom accuracy, needs three-dimensional experimental data with high completeness. Data collection from oriented nano crystals limits the amount of measurable reflections significant and thus, delivers mostly heavy atom positions but hardly lighter atoms. Dynamical scattering effects, strongly enhanced in oriented zones, reduces using electron beam precession. Automated electron diffraction tomography [1] uses diffraction patterns from non-oriented nano crystals taken during a fixed tilt sequence. In such a way electron diffraction data collection of most of the independent reflections is possible with significantly reduced dynamical effects. Additionally, in order to integrate reflections fully the electron beam is precessed. The resulting diffraction patterns are processed with ADT3D/eADT, which, after some geometrical corrections, reconstructs the three-dimensional reciprocal space. From the 3D volume, cell parameters can be determined using clustering-routines. Subsequently, reflections indexing and intensities integration is performed. The three-dimensional reciprocal space reconstruction provides the chance to inspect the volume by eye and detect crystallographic specialties such as disorder, twinning or additional individuals.

For ADT data sets standard kinematic approach (intensities proportional to \(F_{hkl}^2\)) delivers ab-initio the complete structural model solved by direct methods or simulated annealing. Based on ADT and ADT/PED data the solution of a wide range of crystal structures from nano particles down to 30nm was possible [2]. Large cell porous minerals, zeolites (doped and calcinated), highly beam-sensitive metal-organic frameworks, organic-inorganic hybrids, small organic molecules as well as quasicrystal approximants [3] have been successfully solved. Recent structure solutions cover high-pressure samples like the novel boron oxynitride (BON) including a direct refinement of the twinned structure [4].

References:
SHAPE AND PHASE CONTROL OF NANOCRystALLINE $\pi$-SnS AND $\pi$-SnSe – NEW CUBIC PHASES IN THE TIN CHALCOGENIDE SYSTEM

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We report on the synthesis of the newly discovered cubic phase of tin sulfide, $\pi$-SnS, and compare its properties to the well-known phase of tin sulfide, $\alpha$-SnS. This work follows our recent report in which we used Precession Electron Diffraction Tomography (PEDT) technique for obtaining a full structure solution for the $\pi$-SnS phase ($P2_13$, $a_0=11.7$ Å), a previously unknown simple cubic binary phase of tin mono-sulfide.$^1$ $\pi$-SnS polymorph was first identified as tetrahedral nanoparticles present in minute amounts among the reaction products. Selected area electron diffraction (SAED) patterns taken along major zone axes did not conform with $\alpha$-SnS, the conventional orthorhombic phase of tin sulfide. Yet, all SAED patterns could be fully indexed if a large cubic crystal structure is considered. Due to the small amount of material present in the powder, PEDT technique was the only technique available for full structure solution of $\pi$-SnS.

As the research progressed, we were able to improve our synthetic protocol to allow synthesis of both $\alpha$-SnS and $\pi$-SnS separately by controlling reaction parameters.$^2$ It was found that temperature plays a key role in determining the crystallographic structure of the nanoparticles. At higher temperatures (above 200$^\circ$C) the orthorhombic polymorph of $\alpha$-SnS appeared, while in milder temperatures (150$^\circ$C) the new $\pi$-SnS phase was dominant, thus phase control in the synthetic procedure has been demonstrated. Furthermore, shape control was achieved by varying other synthesis parameters, such as the reaction medium and surfactant additives, resulting in cubic, rhombic dodecahedral and tetrahedral shapes of the $\pi$-SnS nanoparticles (Figure 1). Shape determination of the cubic and tetrahedral nanoparticles was trivial and did not require further attention. It was found that the cubic shaped nanoparticles are formed by exposing (100) facets while the tetrahedral shape is obtained by exposing (111) facets. Regardless, shape determination of the rhombic dodecahedral nanoparticles required thoughtful consideration. In order to realize what is the exact geometrical shape of the particles, a series of HRTEM micrographs were taken with respect to the main crystallographic axes of the nanocrystal. The analysis was based on the relation between the atomic structure of the crystal, its shape and orthogonal projection. Scanning transmission electron microscopy (STEM) was used in order to support the HRTEM data; since the contrast in STEM is mass-thickness, we were able to assign the crystallographic facets coinciding with the nanoparticle surfaces (Figure 2). We conclude that the rhombic dodecahedral shape is obtained by exposing {110} facets, 12 in number.

Recent collaborative work together with the group of K. Nair (UNAM, Mexico) showed that the new $\pi$-phase of SnS exists also in thin film form. The films were synthesized in UNAM using chemical bath deposition, and forwarded to BGU for structural analysis. This work was recently submitted for publication jointly with the Nair group.$^3$

Electron energy-loss spectroscopy (EELS) low loss measurements have been carried out in order to monitor possible dielectric function differences between $\alpha$-SnS and $\pi$-SnS particles. This was done by use of Kramers–Kronig analysis in the Digital Micrograph (Gatan) software, which provides an indication for both the real ($\varepsilon_1$) and imaginary ($\varepsilon_2$) parts of the dielectric function. The results can be seen in Figure 3. A notable difference between the two phases was observed. This signifies that appreciable differences in refractive index and electrical properties between the two nanoparticle types are expected.
We have extended our research to explore the possibility of the existence of analogues structure of $\pi$-SnS in the Sn-Se system. SnS and SnSe share much in common, chemically and structurally. Hence, it is natural to question if the selenide analogue of the $\pi$-SnS prototype also existed in the form of $\pi$-SnSe. In a modified synthesis protocol we have used selenourea as the selenide precursor and investigated the synthesis products. We found that in a similar fashion to the SnS case, the orthorhombic phase was dominant at higher temperatures. At low temperature regime the XRD diffractogram could not be indexed to the orthorhombic phase of SnSe which indicate a different crystal structure. TEM examination of the particles revealed that they appear to be cube shaped. HRTEM of those particles along major zone axes showed net symmetry patterns which could only be fully indexed if a large cubic crystal structure is considered. By adopting the structural model of $\pi$-SnS and replacing S atom by Se we established a new model which could account for both XRD results and the HRTEM micrographs. Refining the structural model against the experimental results verified the proposed model in high certainty thus, validating the existence of new binary compound $\pi$-SnSe.

Characterizing the properties of the new $\pi$-SnSe phase is currently one of our top priorities. It is in our intention to use EELS in order to evaluate the dielectric function of both $\alpha$-SnSe and $\pi$-SnSe. We speculate that the complex dielectric function might vary similarly to the $\alpha$-SnS and $\pi$-SnS case.

References:

Figure 1. (Left) The four crystal morphologies presented in this work (a) Cubic, exposing (100) facets (b) Rhombic dodecahedral, exposing (110) facets (c) Truncated tetrahedral, exposing combination of (110) and (111) facets (d) Tetrahedral, exposing (111) facets. The dodecahedral, truncated tetrahedral and tetrahedral shapes were obtained using tin-ethylxanthate single precursor with different ODA:OLA ratios. Cubes were obtained using the dual precursor method and were synthesized at 150°C.

Figure 2. (right) π-SnS rhombic dodecahedra shaped nanoparticles viewed at different orientations. (a-c) HRTEM micrographs of select nanoparticles. (d-f) Corresponding FFT micrographs, respectively. (g) STEM micrograph of the nanoparticle seen in (c). (h) Line profile of the selected area labeled “1” in (g). (i) Line profile of the selected area labeled “2” in (g).

Figure 3. The real (a) and imaginary (b) parts of the dielectric function of both π-SnS and α-SnS. Low loss EELS measurements taken from each nanoparticle type were processed using Kramer-Kronig analysis in order to evaluate the dielectric function.
CSPOT – A COMPUTER PROGRAM FOR SIMULATION, INDEXING AND ANALYSIS OF TEM
DIFFRACTION PATTERNS

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Electron diffraction in the transmission electron microscopy (TEM) is a well-established and widely used
technique in laboratories all over the world. In order to extract relevant crystallographic information, obtained
diffraction patterns may require a significant amount of post-acquisition analysis. In order to meet this
challenge, a computer program called CSpot (Fig. 1) was developed [1]. CSpot deals with three types of TEM
diffraction patterns: spot, Kikuchi and ring (polycrystalline) diffraction patterns. The ability of the software
to work with different types of diffraction patterns gives a user the possibility to cover a wide range of analyses
and extract various information. CSpot allows for manipulation of simulated diffraction patterns in a real-time
and interactive manner by changing and visualizing crystal orientation (e.g., pole figures) or adjusting
simulation parameters. The main feature of the program is the ability to perform automatic indexing and
crystal orientation determination using spot and Kikuchi diffraction patterns. When working with ring
diffraction patterns, the user can present them in integral form, being analogues of X-Ray diffraction patterns
and analyzed in a similar manner. CSpot operates on the information regarding microscope operating
conditions (e.g., accelerating voltage, camera length, detector pixel size) and crystallographic structures. The
software supports all space group symmetries and can work with Crystallography Open Database [2]. The
software has built-in crystallographic calculators. CSpot is mainly dedicated to be a laboratory tool, helping
in the analysis and interpretation of data. However, it can be also used as teaching aid. CSpot runs under
Windows family operating system (XP or higher).

Figure 1. CSpot user interface

References
Strong inter-linkage exists between the physical/chemical properties-chemical composition and crystal structure of the materials. In order to gain improved properties - composition and/or structure should be changed. Such researches are normally done either via theoretical route (i.e. prediction) or experimental (i.e. “trial and error”). Experimental route is time and resource consuming. Prediction (basing on energy landscapes) is not always possible, especially when system of an interest exhibits complex electronic structure. A-T-Al systems (where A-actinide/lanthanide and T-transition metals) are perfect examples of such systems since they contain f-electron elements. In these systems, AT$_2$Al$_2$0 alloys were intensively studied with a purpose to find aluminides with possible heavy fermion properties. Our study concentrated on the ThT$_2$Al$_2$0 system (where T-3d transition metal) in order to formulate a general “rule of thumb” which will allow to estimate the symmetry of the Al-rich ternary structure forming in the A-T-Al systems. Such prediction will shorten the research time spent on search for the heavy fermion materials with interesting magnetic and electrical properties. We have proved experimentally and theoretically that ternary aluminides structure’s symmetry changes abruptly as a function of atomic number of T (i.e. $Z_T$). At T=Mn, $Z_T$=25, the symmetry decreases from cubic (for ThT$_2$Al$_2$0 with $Z_T$25) to orthorhombic. This change inevitably imposes modification of magnetic and electrical properties. At $Z_T$=28 (i.e. T=Ni) three new structures were formed. Despite the prolonged heat treatment, equilibrium was not attended. Applying novel Electron Diffraction Tomography method (known as EDT) for structure characterization of these phases - geometry of all phases was proposed. EDT datasets were collected manually by tilting the crystal around an arbitrary axis in a tilt step of 1°, reducing the dynamical effects significantly. In order to increase the completeness of data, collection was done in precession illumination (with precession angle of approximately 2°). Solution of atomic structure of the major Th$_2$Ni$_{10}$Al$_{15}$ phase was performed. Th$_2$Ni$_{10}$Al$_{15}$ phase was found to be orthorhombic, I$mmm$, with lattice parameters a=3.992Å, b=11.172 Å and c=17.343 Å. Although Th, as heavy scatterer, did smeared the Fourier difference map so that finding Al atom positions was not an easy task, coordinated of all 54 atoms in the unit cell were determined. It should be noted that despite the drastic compositional difference between the new Th$_2$Ni$_{10}$Al$_{15}$ and studied in this research AT$_2$Al$_2$0 stoichiometry, atomic structure of the Th$_2$Ni$_{10}$Al$_{15}$ phase can be related to the orthorhombic phase discussed above since they both belong to a family of so called layered structures.
Invited Lecture
VARIABLE TEMPERATURE STM STUDIES OF INDIVIDUAL MAGNETITE NANOCRYSTALS

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Magnetite (Fe₃O₄) nanocrystals have very interesting electronic properties. These properties were explored in two types of variable temperature STM experiments. In the first one, the high spin-polarized conductance of magnetite was used to measure the magnetization switching dynamics using a spin-filtering substrate and by tuning the temperature close to the magnetization blocking temperature of the nanocrystals. The temporal analysis of the magnetization switching events enabled us to extract various parameters relating to the nanocrystals. In the second experiment, the current/voltage characteristics measured as a function of temperature over selected single magnetite nanocrystals revealed a sharp jump in the density of states and bandgap magnitude at about 100K, which is a manifestation of the well-known Verwey metal-insulator transition, studied in bulk magnetite crystals since 1939. The nanocrystal experiments reveal new information regarding the existence of this transition in such small systems.

Sample configurations for the two types of experiments mentioned above.
Nanostructures are one of the most extensively researched systems in nanoscience. Various materials are investigated due to their size dependent optical properties or due to their enhanced functionality as catalysts. Although they have been extensively researched for a few decades now, even today new fabrication routes are still explored to improve properties and to gain precise control of their structure. While reports on the optical properties of single particles are available, the quantitative characterization of atomic order on a single particle level and the growth mechanism that resulted in that specific rearrangement, are still generally missing. The majority of characterization procedures are performed on ensembles that average properties and may hinder the understanding of fundamental aspects in the colloidal synthesis.

Atomic resolution analysis, which has emerged with aberration corrected instruments, has mainly provided analysis of few particles per sample. It is now, due to the Cc correction that offers superior resolutions in low voltages that the atomic ordering can be achieved on a routine basis to deliver new statistical data. New hardware facilitates efficient acquisition of chemical data by EDS without causing deformation due to beam damage. We use these state-of-the-art instrumentation to understand growth processes and to correlate the atomic structure with properties.

Here, I will present several systems such as 2D nanosheets of ternary compounds such as CdSe$_{1-x}$S$_x$, doped Bi$_2$Se$_3$ and seeded rods of CdSe@CdS with bimetallic tips.
NANOPOROUS SINGLE CRYSTALS OF GOLD

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Single crystals in nature often demonstrate fascinating intricate porous morphologies rather than classical faceted surfaces. We are growing such crystals, drawing inspiration from biogenic porous single crystals. Here we show that nanoporous single crystals of gold can be grown with no need for any elaborate fabrication steps. These crystals are found to grow following solidification of a eutectic composition melt that forms as a result of the dewetting of nanometric thin films. We also present a kinetic model that shows how this nano-porous single-crystalline structure can be obtained, and which allows the potential size of the porous single crystal to be predicted. Retaining their single-crystalline nature is due to the fact that the full crystallization process is faster than the average period between two subsequent nucleation events. Our findings clearly demonstrate that it is possible to form single crystalline nano porous metal crystals in a controlled manner. We also show that nanoporous single crystal prepared by eutectic composition demonstrate superior thermal stability as compared to their counterpart nanoporous gold prepared by dealloying, which is essential for catalysis.

Invited Lecture

DIMENSIONALITY MATTERS: DIMENSIONALITY EFFECTS ON OPTOELECTRONIC BEHAVIOR OF SEMICONDUCTOR NANOCRYSTALS

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Studying the transition of properties of nanostructures as they develop from the zero-dimensional to the one-dimensional regime is significant for unravelling the modifications that occur in the electronic structure of the particle as its length to width aspect ratio is increased. Such understanding can lead to better design and control of the particle properties, with relevance for a wide range of technological applications. The control of shape and morphology of nanoparticles in colloidal synthesis, which allows forming structures of similar composition but of different dimensionalities and shapes, open the way for probing such dimensionality effects. We will present several effects involving the 0D to 1D transition in colloidal nano heterostructures of different morphologies including “sphere in a sphere”, “sphere in a rod” and “rod in a rod”. In addition, a recently discovered new architecture of “nanorod couples” will be introduced.

Both ensemble and single particle based measurements were used to decipher these effects providing complementary viewpoints. The first dimensionality related aspect involves the modification of emission and absorption polarizations, as the dimensionality of the particles and of their cores changes. The second aspect relates to the function of these nanocrystals as donors in energy transfer processes to multiple dye molecules bound on their surfaces and functioning as acceptors (see schematic of the FRET process in the figure below). We will show how the dimensionality of the particles’ core and shell affects the donor’s time dependent survival probability, as well as the behavior of FRET to multiple acceptors on single particle level. The opportunity to tailor the systems dimensionality with multiple acceptors on the surface results in enhanced FRET efficiencies with relevance for optical, sensing and energy funneling applications.
Invited Lecture
ELECTRON MICROSCOPY OF MEMBRANE PROTEINS IN EUKARYOTIC CELLS IN LIQUID

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Studying membrane proteins at the nanoscale in intact eukaryotic cells in their native liquid environment has become possible recently using a new approach involving scanning transmission electron microscopy (STEM), termed Liquid STEM [1, 2]. Cells in liquid are placed in a microfluidic chamber enclosing the sample in the vacuum of the electron microscope, and are then imaged with STEM. It is not always necessary to enclose the cells in the microfluidic chamber. For many studies, it is sufficient to obtain information from the thin outer regions of the cells, and those can be imaged with high resolution using environmental scanning electron microscopy (ESEM) with STEM detector [3]. Liquid STEM was used to explore the formation of the epidermal growth factor HER2 at the single-molecule level in intact SKBR3 breast cancer cells in liquid state [4]. HER2 is a membrane protein and plays an important role in breast cancer aggressiveness and progression. Data analysis based on calculating the pair correlation function from individual HER2 positions revealed remarkable differences in functionality between different cellular regions, and between cells with possible relevance for studying cancer metastasis and drug response.

Fig. 1. Electron microscopy of HER2 in whole cancer cells in liquid. (Top) HER2 proteins labeled with nanoparticles within the plasma membrane of a cell are imaged with a scanning electron beam in transmission mode. The cells remain under a thin layer of water. (Bottom) The nanoparticles are visible as bright spots in the images. Individual labels, pairs, and larger groups are found. Two examples are highlighted, a nanoparticle label attached to a HER2 monomer, and two labels attached to a dimer. Colored molecular models are shown as well.

References

RESOLVING NEW ULTRASTRUCTURAL FEATURES OF CYTOKINETIC ABDICATION WITH SOFT-X-RAY CRYO-TOMOGRAPHY

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Mammalian cytokinetic abscission is mediated by the ESCRT membrane fission machinery. While much has been clarified on the topology and kinetics of abscission through high-resolution microscopy, key questions regarding its mechanism remain open. Here we apply cryogenic Soft-X-ray tomography to elucidate new ultrastructural details in the intercellular membrane bridge connecting cells undergoing abscission. In particular, we resolve defined ring-like structures inside the midbody dark zone that have been inaccessible to EM, and identify membrane extrusions at the abscission sites. In cells at late stages of abscission we resolve a complex array of helical spirals, extending the structural information obtained by EM. Our results highlight the advantages of Soft-X-ray tomography and emphasize the importance of using complementary approaches for characterizing cellular structures. Notably, by providing new structural data from intact cells we present a realistic view on the topology of abscission and suggest new mechanistic models for ESCRT mediated abscission.
In the last decades, multiple methods were developed in an effort to break the classical imaging resolution by Ernst Abbe. Early techniques overcome the optical resolution limit by encoding spatial information into the other degrees of freedom. While such techniques increase the effective numerical aperture, they are still limited by the diffraction. Modern super-resolution approaches employ non-linear labels. Recently, a linear optics approach, which does not require switchable state fluorophores, but is not limited by the aperture size, was developed. This approach employs subwavelength nanoparticles undergoing Brownian motion next to the object, to passively scan the sample. The method, being based on passive scanning, suffers from long measurement times and other significant limitations; for example, only nanoparticles located closer than the illumination wavelength to the sample contribute to the signal.

We present and experimentally achieve super-resolution by active scanning of the sample with optically trapped nanoparticles. Our method, where the optical tweezing is for the first time employed to break the diffraction limit, overcomes the outlined limitations of the passive scanning [1]. As the first proof of concept we successfully resolved features down to 100 nm, well-beyond the Abbe-limit.

In our setup, see Fig. 1, a confocal microscope is combined with holographic optical tweezers (HOT), which trap individual 100 nm size nanoparticles and move them across the sample. The HOT is capable to control multiple beams in parallel, in three dimensions. Furthermore, the scan geometry is adjustable in real time, to best fit the dimensions and the size of the object at hand. With such a scanning, the particle-sample distances are smaller than the optical wavelength, and the acquisition time for the present super-resolution method is considerably reduced. Thus, the beam damage to the sensitive samples is minimized. HOT systems are widely used in parallel with optical microscopy for particle manipulation, so that our super-resolution technique does not necessarily require a construction of a specialized new setup, making it affordable and widely applicable.
Fig. 1. Experimental system. Imaging is done from the upper side using a confocal microscope. OT is done from below using a 1064 nm laser, an SLM and an inverted objective.

Two types of samples were used to test the setup. In the first type of samples, gold nanolines were formed on fused silica cover slips by e-beam lithography. The samples consisted of horizontal and vertical lines with varying line widths between 100 and 250 nm, and varying spaces, 80 to 480 nm. Fig. 2 presents an atomic force microscopy (AFM) scan of a typical sample of this type. The second type of samples is randomly deposited nanowires, 6 mm long and 50 nm wide.

Fig. 2. An AFM image of a typical gold nanolines sample. (a) A wide area AFM scan. (b) A height line profile of the zoomed area [marked by a red rectangle in section (a)], taken along the nanolines normal (shown by a horizontal line in the inset).

We demonstrated that an active scanning of the sample by HOT-trapped nanospheres allows features down to the size of the scanning particles to be resolved. Our technique does not require sample staining, which is a great advantage compared to most other SR technologies, where sample labeling by switchable fluorescent markers challenges preparation of bio-samples. The proposed biocompatible and noninvasive SR imaging thus opens new directions for future applicative engineering.

Fig. 3. An enhancement of resolution, as obtained for a nanowire, Section (b) show the conventional confocal image of the samples. The regions where the SR resolution scanning was carried out appear in the yellow rectangle as dark blobs in (a). Note the dramatic enhancement of resolution, where the nanowire appears much thinner in the SR-scanned region. The corresponding intensity profile is shown in (c), where the intensity profiles were averaged over the dashed lines in (a,b).

Invited Lecture

LABEL-FREE NONLINEAR PHOTOACOUTIC NANOSCOPY AND SPECTROSCOPY

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Super-resolution microscopy techniques have opened new opportunities to explore sub-cellular structures and dynamics not resolvable in conventional far-field microscopy. However, relying on staining with exogenous fluorescent markers, these techniques can sometimes introduce undesired artifacts to the image, mainly due to large tagging agent sizes and insufficient or variable labeling densities. By contrast, the use of endogenous pigments allows imaging of the intrinsic structures of biological samples with unaltered molecular constituents. Here, we present label-free photoacoustic (PA) nanoscopy, which is exquisitely sensitive to optical absorption, with 88 nm resolution. At each scanning position, multiple PA signals are successively excited with increasing laser pulse energy. Owing to optical saturation or nonlinear thermal expansion, the PA amplitude depends on the incident optical fluence non-linearly. The high-order dependence, quantified by polynomial fitting, provides super-resolution imaging with optical sectioning. In addition, we use a photoacoustic spectrometer to show how the nonlinear PA spectrum of different molecules (e.g., oxygenated and deoxygenated hemoglobin) has both wavelength and concentration dependence.
Invited Lecture

“GREAT EXPECTATIONS” OR CHALLENGES FOR TRANSMISSION ELECTRON MICROSCOPY IN THE IDENTIFICATION OF THE DIVERSE POPULATION OF EXTRACELLULAR VESICLES

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Extracellular vesicle (EV) is the common name of a heterogeneous group of lipid bilayer enclosed vesicles which can be released by practically all cells. These vesicles have been proved to have crucial roles in intercellular communication, signaling processes from inflammation to antigen presentation or tumor development and also in the transfer of genetic information. Especially the EV related biomarker and therapeutic applications made this research field attractive not only for basic but clinical research, too.

Although the story of extracellular vesicles started about 70 years ago we had to wait for the first transmission electron microscopic /TEM/ evidence of these mysterious little particles till 1981. Since then several great techniques and methods have been used or emerged to investigate and indentify EVs such as flow cytometry, atomic force microscopy, mass spectrometry, tunable resistive pulse sensing (TRPS), nanoparticle tracking analysis (NTA), microfluidic device etc., however, none of them is able to cover the whole size range of these vesicles /from 30nm till about 5µm/ as well as to demonstrate of their origin, the cells or tissue, moreover to distinguish the vesicular and non-vesicular particles. Not surprisely, electron microscopy has become the golden standard of EV research, however it does not mean that TEM results would be generally accepted without any debate. The basic problem is the preparation of the extracellular vesicle populations. Numerous protocols have been applied by the different laboratories and the results published can be misleading. What can be accepted and which results should be refused, how can we avoid pitfalls during the vesicle preparation and whether the classical TEM can provide reliable results or instead everybody should use the fancy and expensive ultracyro microscopy?

Extracellular vesicles released by 5/4 cells
LIVE IMAGING OF APOPTOTIC CELL CLEARANCE IN DROSOPHILA EMBRYO

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Normal embryonic development is accompanied by elimination of unwanted or aberrant cells through apoptosis and subsequent phagocytosis of apoptotic cells. “Professional” phagocytes, macrophages and immature dendritic cells, and “non-professional” tissue-resident neighboring cells accurately and efficiently remove apoptotic cells during development. Apoptotic cell clearance is a highly dynamic process, which proceeds in four main steps: (1) recruitment of phagocytes to apoptotic cells, (2) recognition, (3) engulfment and (4) degradation of apoptotic cells inside phagocytes. However, very few studies in the field come from live in vivo observations of the process leaving many questions open including time courses of different stages in phagocytosis and comparison between “professional” and “non-professional” phagocytes.

In our research, we use the live Drosophila embryo as a model to elucidate the molecular basis and cell biology of apoptotic cell clearance during development. Two types of phagocytes are studied in parallel: “non-professional” glia in the developing central nervous system (CNS) and “professional” macrophages outside the CNS. By monitoring the embryos with Zeiss LSM 700 confocal microscope and Imaris (Bitplane) software, we demonstrate that tissue-resident clearance by neighboring glia plays a major role in the removal of apoptotic neurons during embryonic CNS development. A detailed investigation of two phagocytic receptors Six-Microns-Under (SIMU) and Draper reveals that SIMU is required for recognition and engulfment of apoptotic cells, whereas Draper is mostly involved in degradation of apoptotic cells by glia and macrophages. Moreover, we aim at understanding how phagocytes acquire their phagocytic ability during development. Our results show that distinct developmental programs are responsible for establishment of embryonic glia and macrophages as potent phagocytes of apoptotic particles.

Since phagocytosis of apoptotic cells is highly conserved throughout animal evolution, investigating its mechanisms in the Drosophila model, which permits comprehensive and dynamic in vivo studies, shall provide new insights into this process with prospective translation into studies in higher organisms.
REGULATION AND A POSSIBLE PHYSIOLOGICAL ROLE FOR THE BI-DIRECTIONAL MOTILITY OF THE BIOLOGICAL KINESIN--5 MOTOR PROTEIN, CIN8

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The homotetrameric bipolar kinesin--5 motor proteins perform essential functions in mitotic spindle dynamics by crosslinking and sliding apart antiparallel microtubules (MTs). Plus-end-directed motility of kinesin-5 motors is required for sliding apart of interpolar spindle MTs and providing the outwardly-directed force to separate the spindle pole bodies during spindle assembly and anaphase spindle elongation dowering mitosis. However in previous work at our lab we demonstrated that the kinesin-5 from Saccharomyces cerevisiae (baking yeast), Cin8 is minus-end-directed on the single-molecule level and can switch directionality under a number of conditions (Duselder et al., 2015; Gerson-Gurwitz et al., 2011). Recently, two additional yeast kinesin-5 motors were reported to be bi-directional, the Saccharomyces cerevisiae kinesin-5 Kip1 (Fridman et al., 2013) and the Schizosaccharomyces pombe Cut7 (Edamatsu, 2014). These findings indicate that minus-end-directed motility in kinesins-5 motors may be more common than previously believed, and that the directionality switch of yeast kinesin-5 motors may be physiologically important for unique functions required during yeast mitosis. In previous work we have also demonstrated that Cin8 is differentially phosphorylated during mitosis at three cyclin-dependent kinase 1 (Cdk1) sites located in its motor domain. This phosphorylation regulates Cin8 localization during anaphase (Avunie-Masala et al., 2011), but its mechanism remains unclear.

Here we examined the in vitro motile properties and in vivo localization of Cin8 by TIRF microscopy and live-cell imaging. We found that addition of negative charge in a phospho-mimic Cin8 mutant weakens the MT--motor interaction and regulates the motile properties and directionality of Cin8. We also found that in vitro under high ionic strength conditions, Cin8 not only moves to- but also clusters at the minus--end of the MTs. This clustering causes Cin8 to reverse its directionality from fast minus- to slow plus--end directed motility. Clustering of Cin8 at the minus--end of the MTs serves as a primary site for capturing and antiparallel sliding of MTs. Based on these results, we propose a revised model for activity of Cin8 during closed mitosis in yeast and propose a possible physiological role for its minus--end directed motility.
The eukaryotic cell cycle is regulated by ubiquitin mediated degradation of cell cycle regulators. Degradation takes place at phase transitions like entry into and exit from mitosis as well as entry into S-phase. Cell cycle specific ubiquitin ligases identify degradation sequences called degrons in their target proteins. Fusion of such a degron to a fluorescent protein (or any other reporter) recapitulates the degradation event. In most cases degradation is either turned on or off. When expressed from a constitutive promoter cells either express the reporter, when degradation is off, or do not express it when degradation is on. It is thus possible to follow individual cells throughout the cell cycle or sort entire cell populations. Most reporters described so far can distinguish between cells in G1 and G0 to cells in the S-G2-M phases, which proved very useful for many different studies. We have recently developed a new reporter that yields a much higher temporal resolution. This reporter is based on the Cdc6 protein that plays a role in licensing of replication. Cdc6 and its fusion protein undergo two waves of degradation during the cell cycle – the first upon cell division and the second upon entry into S-phase. Unlike other proteins degraded in G1 Cdc6 becomes stabilized not at the end but in mid G1. This is thus the first reporter that enables to distinguish between early and late G1 opening many exciting new research possibilities. Moreover the cellular localization of Cdc6 and its fusion protein changes from nuclear to cytoplasmic, further improving our ability to measure the length of the various sub-phases.
Invited Lecture
OPPORTUNITIES AND LIMITATIONS OF ELECTRON MICROSCOPY IN LIQUID ENVIRONMENTS FOR IN SITU STUDIES OF CRYSTAL GROWTH AND DISSOLUTION

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Studying materials and processes in liquid environments by electron microscopy has recently become a major topic of interest across a wide range of disciplines with the arrival of high-performance liquid cell TEM holders and atmospheric scanning electron microscopy. As an example studying the dynamics of crystal growth and dissolution with nanometer or even atomic level resolution clearly describes a “promised land” of Materials Science catching the underlying mechanism in the act while giving the freedom of mixing materials and solutions and recording imaging data. Furthermore progress in holder design and detector technology has made it possible to perform element analysis using energy dispersive X-ray spectroscopy in liquid cell TEM. However, it has also become clear that care has to be taken when interpreting images and videos recorded from materials in liquid media since the interactions between electron beam and sample can be complex and significant and hence the validity of data depends on the thoroughness by which a clear distinction is made between “genuine” processes and those partially or solely triggered or caused by the electron beam. On this background my presentation explores potentials of liquid cell TEM and ASEM and their current limitations for understanding processes e.g. in biomineralization and crystal growth focusing in particular on the kinetics of precious metal nanoparticle, carbonate and sulphate formation and dissolution in the presence and absence of additives. First data will be presented on Mg carbonate precipitation obtained by a novel heating liquid cell TEM holder allowing for the controled realisation of temperatures between 25°C and 100 °C.
DIRECT OBSERVATIONS OF INTERACTIONS BETWEEN WATER AND SINGLE
WS₂NANOTUBES–IN SITU SEM AND AFM STUDIES

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The use of different nanostructures as fillers in polymer composite materials attracts an ever-growing interest. While the impact of nanoparticles on composite properties is well studied, the relations between individual filler nanoparticles and the host matrix are yet to be fully understood. The elucidation of these interactions on the bulk composite properties requires the study of the wetting, adhesion to the matrix, surface energy, morphology etc. of a single nanoparticle.

It was reported that the addition of these inorganic nanoparticles leads to improved mechanical properties [1], thermal stability [2] and electrical properties [3] of the polymer composites. Although those nanoparticles alter the properties of the composite, the nature of the nanotube-matrix interactions is still vague. The goal of our study is to investigate the interactions of single WS₂ nanotubes with different matrices and liquids.

In this work, a new technique to measure the interactions of individual nanotubes with different liquids and polymers is presented. This technique is based on pullouts of nanotubes from water films and other liquids using environmental scanning electron microscope (ESEM) and atomic force microscope (AFM) [4] systems. Using these techniques the effect of WS₂ nanotube morphology and structure on the interaction strength between different liquids and the nanotubes are assessed. From these experiments, as well as from theoretical simulations, it follows that the morphology of the WS₂ nanotube has a significant effect on the interaction of the NT and the surrounding media.

The interaction energy/cross-section area of the nanotubes falls-off dramatically with the diameter of the nanotubes (30-70 nm) and then levels-off. These differences are currently attributed to the capillary interaction of the small hollow core nanotubes and the water molecules. MD simulations show that the highly confined space results in large interaction energy between the water molecules and the inner core of the nanotubes. This interaction leads to imbibition of the water molecules into the nanotube’s hollow core. These effects and the impact of the nanotubes surface chemistry on the interaction energy are presented and discussed.

SYNTHESIS AND CHARACTERIZATION OF NANO-SCALE BUILDING BLOCKS WITH ELECTRONIC AND STRUCTURAL HETEROGENEITY BY POST-SYNTHESIS MODIFICATIONS

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The intentional introduction of heterogeneity at the nanoscale plays a key role in the design of functional nanostructure building blocks. The heterogeneity is frequently manifested in structure, shape, composition, and electronic structure modulation of the nanostructure regions. I will present our research that resulted in two methodologies for post-synthesis modification and symmetry breaking of semiconducting nanostructures using nanowires as the basic building blocks covering two aspects of post-synthesis modification of nanowires:

(I) Ex-situ doping of silicon nanowires.

(II) Self-processing synthesis of coinage metal-semiconductor hybrid structures.

In my talk I will present the new methodologies and the characterization challenges that emerge and that were addressed by several advanced analytical techniques including atom-probe tomography, electron holography, in-situ scanning transmission electron microscopy in liquid and scanning tunneling spectroscopy. The combination of these advanced analytical techniques resulted in significant insights regarding the structure, electronic properties and mechanisms involved in the synthesis of the new type of structures.
Wetting properties of surfaces at high spatial and temporal resolution is an emerging investigation field involving theoretical aspects as well as biotechnological and materials science applications. Wettability research using Environmental Scanning Electron Microscope (ESEM) is here reviewed based on two approaches: (a) reflected secondary electrons for analysis of bulk sample surfaces down to sub-micron spatial resolution (b) transmitted electrons for analysis of low dimensional structures and thin films at nano-scale. We focus on the transmitted-mode using the wet scanning transmission electron microscope (wet-STEM) detector in ESEM.

The quantitative droplet shape and contact angle were provided by fitting Monte-Carlo simulation for transmitted electrons to the wet-STEM experimental results. The dynamics of the initial stages of both film-wise and drop-wise condensation was in-situ studied over self-supported thin liquid films. The role of pinning centers on nanodroplet dynamics was explored as well as the droplet growth and coalescence processes. The corresponding results on delayed coalescence lifetime are compared with other studies on nanodroplets using in-situ transmission electron microscopy (TEM). The results are discussed in relation to possible deviation of nano-scale dynamics from bulk properties in addition to possible e-beam charging effects. These are expected to have an impact on biological processes and nano-scale fluidic applications.

Super-resolution microscopy by single-molecule photoactivation or photoswitching and position determination (localization microscopy) has the potential to fundamentally revolutionize our understanding of how cellular function is encoded at the molecular level. Among all powerful high-resolution imaging techniques introduced in recent years, localization microscopy excels at delivering single-molecule information about the distribution and, adequate controls presupposed, even absolute numbers of proteins present in subcellular compartments. This provides insights into biological systems at a level we are used to think about and model biological interactions. We briefly introduce basic requirements of localization microscopy, its potential use for quantitative molecular imaging, and discuss present obstacles and ways to bypass them. Finally, we demonstrate the advantageous use of dSTORM for quantitative imaging of synaptic proteins, the study of plasma membrane organization, and the molecular architecture of multiprotein complexes.
USING TEMPORAL CORRELATIONS OF FLUORESCENCE INTENSITY TO IMPROVE SINGLE MOLECULE LOCALIZATION MICROSCOPY

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Single molecule localization microscopy (SMLM) enable the localization of individual molecules within intact cells with a resolution of ~20nm. However, SMLM requires longer acquisition time compared to diffraction limited microscopy and is often challenging under low signal to background (SBR) conditions. Here, we utilized temporal intensity fluctuations of fluorophores to efficiently reject background in acquired SMLM movies of various tagged proteins in fixed and live T cells. Our approach could be used to automatically enhance SMLM imaging under detrimental imaging conditions.
SINGLE-SHOT PTYCHOGRAPHY, OVERCOMING THE SCANNING-LIMITED TEMPORAL RESOLUTION AND ALSO DEMONSTRATE SPARSITY-BASED SUBWAVELENGTH PTYCHOGRAPHIC MICROSCOPY APPROACH, OVERCOMING THE ABBE SPATIAL RESOLUTION LIMIT

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Ptychography is a very powerful coherent diffractive imaging (CDI) technique that has recently gained remarkable momentum in optical microscopy. In ptychography, a complex-valued object is scanned in a step-wise fashion through a localized coherent beam. In each step, the far-field diffraction intensity is measured. Critically, the illumination spot in each step overlaps substantially with neighboring spot. The set of diffraction patterns is used for reconstructing the complex object.

Here we address two major limitations in ptychography: i) the need for scanning, which poses a major limitation of the data acquisition time, and ii) diffraction-limit spatial resolution. First, we propose and experimentally demonstrate single-shot ptychography, where all diffraction patterns from array of partially overlapping illuminating spots are recorded in a single exposure. Whereas scanning limits the acquisition time of ptychographic microscopes to the range of ~0.1secs, single-shot ptychography allows for ultrafast ptychographic microscopy. Second, we demonstrate sub-wavelength ptychography, utilizing the fact that the sought information (the complex field of the object) can be represented compactly (sparsely) in real space or in a suitable mathematical basis. As an example, we present ~3.5 times resolution enhancement (beyond the Abbe resolution limit) of a biological specimen.

For single-shot ptychography, a coherent monochromatic plane wave illuminates a square array of N×N pinholes positioned at the input face of a 4f system (Fig. 1a). The object and CCD are located at distance d≠0 before the Fourier plane and at the output plane of the 4f system, respectively. Lens L2 transfers the field after the object to k-space domain at the CCD plane. The detected intensity pattern consists of clearly distinguished N^2 diffraction patterns, where each pattern can be associated with a beam illuminating the object at a specific given spot. Figure 1 presents experimental single-shot ptychography. The spatially coherent light illuminates an N^2=49 square array pinholes. The measured intensity pattern is displayed in Fig. 1c. Figures 1d and 1e show our single-shot ptychographically reconstructed amplitude and phase, respectively, using ePIE algorithm[1].
Next, we move to subwavelength ptychography by employing sparsity, i.e. prior information that the image can be represented compactly in a known physical or mathematical basis. Previously, we demonstrated experimentally sparsity-based sub-wavelength CDI of relatively simple objects [2]. Implementation of the same approach in ptychography yields sub-wavelength resolution of “real world” objects because the number of measurements in ptychography is much larger than the number of degrees of freedom of the sought image. Figure 2 presents a numerical example. Figure 2b shows the diffraction limited ptychographic reconstruction while the sparsity-based reconstruction, that searches for the best estimate of the object that is consistent with the set of measurements and is sparse in known mathematical basis, is shown in Fig. 2c. Figure 2d shows a lineout of the original image, its diffraction limited image and the sparsity-based reconstruction. Clearly, the sparsity-based reconstruction yields subwavelength resolution.
References

VISUALIZING ESCRT MEDIATED MAMMALIAN CELL ABSCISSION AT NANOSCALE RESOLUTION

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Conserved from archaea to mammals, ESCRT filamentous system executes membrane fission in a variety of processes in cells including viral budding, formation of multivesicular bodies, plasma membrane repair, nuclear envelope assembly and cytokinetic abscission. Yet, many of the mechanistic steps that lead to ESCRT-driven membrane fission in cells have not been resolved. We tailor diverse high-resolution microscopy techniques to study ESCRT driven membrane constriction and fission during cytokinetic abscission of mammalian cells. With this approach we aim to unlock the mechanistic principals of ESCRT mediated membrane fission in physiological process utilizing the ESCRT machinery for its function.
VISUALIZING ESCRT MEDIATED ABSCISSION DURING EARLY DEVELOPMENT IN LIVE ZEBRAFISH EMBRYOS

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During cytokinesis, the membrane constricts and the cytosol is equally separated into two daughter cells. At the end of this step the cells remain connected by an intercellular bridge composed of membranes and microtubules. Completion of cytokinesis, termed abscission, is mediated by membrane fission driven by the ESCRT protein complex. While much is known on ESCRT mediated abscission through cell culture experiments, little is known about this process during embryonic development and in the whole organism. Zebrafish (Danio rerio) early embryonic stages are diverse and can be used to investigate cytokinesis and abscission in different contexts: for example comparing cytokinesis in different cell sizes and during synchronous (early blastula) or asynchronous (late blastula) divisions. To study abscission and ESCRT involvement in abscission during embryonic development, we established an assay to visualize ESCRT mediated abscission in live zebrafish embryos. Using this assay we visualize ESCRT proteins together with tubulin GFP for long periods in live developing embryos. Using this assay we investigate the spatiotemporal organization of ESCRT proteins in intercellular bridges of cells undergoing cytokinesis at different division cycles. We find that CEP55 localize to the microtubules fibers during early cytokinesis whereas TSG101 localizes to the intercellular bridge in late cytokinesis. Comparing the spatial characteristics of the intercellular bridge in cells at different division cycle, obtaining different cell size, we find that prior to abscission the intercellular bridge constricts to a typical diameter of ~ 1.25 μm regardless of cell size, these finding suggest a regulation on bridge diameter prior to abscission onset and ESCRT recruitment. Taken together our data indicate that Zebrafish embryonic developmental system is an ideal model system to investigate ESCRT mediated abscission during embryonic development. Our preliminary results already highlight new features of abscission that could not be studied using a tissue culture model.
Understanding the ultrastructure of intact biological tissues at different scales (from whole tissue organization to cellular and subcellular compartments) can lead to far-reaching mechanistic insights. By using conventional imaging methods (such as SEM, TEM and microCT), a compromise between the imaging resolution and the sample size has to be made. Serial FIB milling and block face SEM imaging (FIB-SEM) enable high resolution imaging of tissues at up to 5 nm resolution, with the ability to detect large volumes (dozens of micrometers). Conventional FIB-SEM imaging requires intense sample processing that may damage or modify the specimens during preparation (fixation, dehydration, staining etc). This procedure is also time consuming (1 week). The recently developed cryo-FIB-SEM technique (Schertel et al, 2013) allows 3D imaging of high pressure frozen biological samples in conditions that are very close to their native state, avoiding any chemical procedures. Cryo-FIB-SEM workflow is extremely fast, requiring less than an hour from organism sacrifice, to the first cryo-FIB-SEM results.

By utilizing the cryo-FIB-SEM technique, we show the ultrastructure of cellular, sub-cellular and extracellular compartments of two highly studied biological model systems; the sea urchin embryo and the zebrafish larva. The large volume of imaging reveals intra and extra cellular compartments in the tissue in their biological context. By combining simultaneous detection of secondary and backscattered electrons, we locate and characterize mineralized elements embedded in the tissues, and show their interactions with their environment. By correlating the backscattered electron signal with secondary electron gray level data, we characterize different features and organelles inside the tissue. Cryo-FIB-SEM technique is advantageous for 3D imaging of biological systems in which tissue dehydration and processing may cause morphological changes. In addition, cryo preservation is highly beneficial in cases where sensitive or transient moieties are present inside the tissue. In the future, cryo-FIB-SEM could be combined in a correlated manner with other imaging or analytical methods, such as fluorescence, cryo-EDX and cryo-STEM.

TRACKING shRNA-MEDIATED INHIBITION OF mRNA TRANSCRIPTIONAL ACTIVITY IN THE NUCLEUS OF SINGLE CELLS

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RNA interference (RNAi) is a highly efficient regulatory process that induces post-transcriptional gene silencing in most eukaryotes. RNAi machinery factors are mostly present and active in the cytoplasm. Several studies have shown the presence of RNAi machinery factors within the cell nucleus, suggesting a nuclear role for the RNAi machinery. In order to determine whether a short hairpin RNA (shRNA)-dependent RNAi mechanism can act in the human nucleus, we tested whether shRNA specifically targeted to an inducible mRNA transcribed from a candidate gene, would affect its transcription within the mammalian cell nucleus. This cell system allows the detection of the active gene during transcription, since the transcribed mRNA is tagged using the MS2 RNA-tagging method. Thereby we are able to visually track and quantify a potential nuclear RNAi effect on the active gene using RNA FISH and live-cell imaging. The expression of the shRNA caused a significant reduction in the cellular mRNAs transcribed, as might be expected, but moreover was accompanied by a dramatic weakening of the transcriptional activity of the inducible gene. The latter demonstrated an effect of the RNAi machinery in the nucleus, and actually at the site of transcription. To examine the mechanism, we checked if the silencing activity was targeting the mRNA (classical RNAi effect), or the gene. The presence of the transcription machinery was observed at the transcription sites during shRNA expression, indicating that transcription could continue but at low levels. We did not observe a change in the levels of RNAi factors in the nucleus following shRNA treatment, nor enrichment of these factors on the active gene. Additionally, no methylation of CpGs sites was detected on the promoter and on the gene region during shRNA expression, showing that the silencing was not occurring on the DNA level. Following these results, and based on recent studies, we examined the involvement of histone methylation by histone methyltransferases (HMTs). Using specific HMT inhibitors, we could observe an increase in the transcriptional activity of the gene, including during shRNA presence. Altogether, our data demonstrate the presence of a nuclear RNAi mechanism, probably involving histone methylation of the gene region.
COMPUTERIZED CELL FLOW-PROPERTIES ANALYZER (CFA) – INSTRUMENT AND METHODOLOGY FOR VISUALIZATION AND CHARACTERIZATION OF RED BLOOD CELL FLOW PROPERTIES

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Red blood cells (RBC) have special flow properties that play a key role in blood flow, namely their deformability, adherence and to the endothelium and aggregability.

**RBC deformability** refers to the ability of the cells to adapt their shape to the dynamically changing flow conditions in order to minimize their resistance to flow. **RBC adherence** to the blood vessel walls (the endothelium) is a potent catalyst of blood vessel occlusion. Normally RBCs do not adhere significantly to EC. However, enhanced RBC adherence has been implicated in pathophysiology relating to RBC abnormalities such as in sickle cell disease, cerebral malaria, diabetes, and thalassemia, and was found to correlate with the occurrence and severity of vaso-occlusion. **RBC aggregability** refers to RBC ability to form multicellular aggregates. Normally, the blood flow is sufficient for dispersion of the aggregates before entering the capillaries, which is essential for adequate tissue perfusion. However, in pathological situations which are associated with low-flow states or altered RBC properties, larger-than-normal and stronger RBC aggregates may form, which might be resistant to disaggregation by the blood flow.

A number of methods and instruments have been proposed for determination of the different RBC flow-properties, but they are not satisfactory, and none of them provides a facile method for determination of all three flow-properties at the same time.

To meet these needs we have designed and constructed a **Computerized Cell Flow-Properties Analyzer (CFA)** that enables the monitoring of blood cells directly visualized in a narrow-gap flow-chamber, under controllable flow rates, resembling those in a small blood vessel. Using original algorithms and software, the CFA analyze the dependence of flow-properties on shear stress (flow rate) and their distribution in large RBC population, as occurs in vivo, and provides a list of parameters for comprehensive characterization of all three RBC flow-properties and their deviation from normal values.

The CFA consists of an adaptable narrow-gap flow chamber (from 10 to 200 mm), placed under a microscope, which is connected to a CCD camera. For characterization of RBC adherence, a slide with confluent EC is placed in the flow chamber. The RBC dynamic organization is continuously monitored by direct visualization of the cells in the flow-chamber, under controllable shear stress resembling that in a small blood vessel at 37°C, and their images are transmitted to a computer for analysis of the desired flow property.
THE LOCATION AND INTERACTIONS OF NXF1/Tap DURING mRNA EXPORT EXAMINED AT HIGH RESOLUTION WITHIN INDIVIDUAL NUCLEAR PORES

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Export of mRNA from the cell nucleus is one of the pillars of the gene expression pathway in eukaryotes. After transcription, mRNA binding proteins (RBPs), some of which are directly involved in the export process, associate with the transcript to create an mRNP. The protein composition of the mRNP changes during export. Key factors such as Nuclear Export Factor 1 (NXF1/Tap/Mex67), Aly, and the DEAD-Box protein Dbp5 are needed to execute the export process. Aly is recruited to the mRNA by the exon junction complex (EJC), and together with UAP56 and THOC5, are responsible for the binding of NXF1/Tap to the mRNA. NXF1/Tap can bind to several Nups at the nuclear pore complex (NPC) and mediate the transport of the mRNA through the pore. We knocked down Nxf1/Tap and followed the dynamics of single mRNPs in living cells. We found that mRNPs could bind but not translocate through the NPC. In contrast, during knockdown of Nup153, mRNPs could not bind nor translocate. This suggested that mRNA binding to the NPC does not require NXF1/Tap, whereas mRNA passage through NPC and release into the cytoplasm does. In accordance, super-resolution microscopy showed that NXF1/Tap was enriched on the cytoplasmic side of the NPC. We next found that NXF1/Tap was continuously present at all NPCs and under all conditions tested, namely, even during transcription inhibition, mRNA export inhibition and RNase treatment. Moreover, a NXF1/Tap mutant that cannot bind mRNA was also present at the NPC under regular conditions. These findings indicated that NXF1/Tap is consistently occupying positions within the NPC. To test this we wanted to be able to differentiate at high-resolution between the functional interactions occurring on the nuclear side of the nuclear pore complex (NPC), the inner channel of the pore, and the cytoplasmic side. To this end we implemented a FLIM-FRET approach (FRET measurements using fluorescence lifetime measurements) that enabled the detection and measurements of specific interactions taking place between NXF1/Tap and Nups in individual NPCs, in intact cells. We demonstrate the discrimination between specific interactions under regular conditions or when mRNA export is blocked, and present a map of NXF1/Tap interactions within the NPC together with the characterization of interactions involved in mRNA export.
MULTI-SCALE CHARACTERIZATION OF PLATELET-MATRIX ADHESION

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Platelets arise as sub-cellular anucleated fragments of megakaryocytes and play a major role in bleeding arrest amongst other functions. They recognize exposed matrix proteins upon vessel injury; and thereafter adhere, activate and aggregate at the site. Given the importance of platelet function in many vital aspects, little is known about the organization and three-dimensional structure of platelet-matrix (focal) adhesions, involved in the critical step of platelet adhesion and spreading. We demonstrate the use of interference reflection microscopy (IRM) and cutting edge cryo-electron tomography (cryo-ET) to elucidate the 3D structure and the morphology of time-dependent spreading of platelet-matrix adhesions, filopodia and lamellipodia on collagen IV and fibrinogen surfaces. Preliminary data suggests a specific organization of the actin cytoskeleton, and early observations of possible adhesion-related particles in filopodial extensions by cryo-ET. Live-platelet imaging by IRM time-lapse movies shows a surface specific mode of spreading, depending on the use of different platelet preparations, platelet-rich plasma (PRP) and purified platelets (PLT).
DETECTION OF ISOLATED METAL ATOMS ON PROTEIN BY CRYO-SCANNING TRANSMISSION ELECTRON MICROSCOPY

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Metal ions play essential roles in many aspects of biological chemistry, including oxygen transport, enzyme catalysis, and maintenance of biopolymer integrity. Cryo-electron microscopy is sensitive to metal ions because of their strong Coulomb potential relative to surrounding light elements. However, the conventional mode of defocus phase contrast in wide-field cryo-TEM is not well suited for the identification of single metal atoms against the protein and water background. In practice, in order to resolve single metal atoms, atomic resolution of the whole protein complex is needed and at lower resolution even small gold nanoparticles can be difficult to identify.

Annular dark-field STEM imaging provides quantitative contrast based on atomic scattering. We show that this alternative modality, recently applied to cryo-tomography, can detect the presence and location of isolated metal ions in frozen-hydrated protein complexes. Image simulations were used to optimize experimental conditions to provide the highest SNR for the metals compared to protein and water. In experiment, we imaged the 24-subunit ferritin complex bound to stoichiometric amounts of Zn or a limited number of Fe atoms. Cryo-STEM micrographs of metal-loaded ferritin were processed by conventional 3D single-particle alignment and averaging. Even in very small datasets (hundreds of particles), clear density peaks for isolated Zn and Fe atoms were observed. The density peaks localize with angstrom precision to the predicted binding sites of Zn and Fe on the ferritin shell. Our results offer a new understanding and a straightforward technique for atomic detection of metals in macromolecular and cellular contexts, as well as to use of synthetic metal tags as specific molecular labels.
IMAGING INTRACELLULAR MINERAL PHASES IN ALGAE USING CRYO-X-RAY TOMOGRAPHY

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A major part of the global carbon cycle is the burial of calcium carbonates in deep sea sediments. This process is primarily biologically driven, with unicellular calcifying algae being dominant players. We aim to elucidate the underlying mechanisms of intracellular calcification in the most important calcifier in modern oceans, the coccolithophore Emiliania huxleyi. This unicellular alga forms intricate arrays of calcium carbonate crystals, called coccoliths, which are made inside a specialized intracellular compartment. The intracellular pathway of calcium ion accumulation from seawater to the coccolith-forming vesicle has remained elusive. This is mostly due to the experimental difficulties in following mineralization processes in vivo. Such process usually involves metastable and short-lived mineral phases that are difficult to follow at the spatial and temporal resolution characteristic for cellular activity. Therefore, the intracellular pathways responsible for the transport of the constituent ions from seawater to the growing coccolith are mostly unknown.

We used synchrotron soft-X-ray tomography at cryogenic conditions in order to map the intracellular calcium in cells of E. huxleyi. The cells were rapidly frozen and maintained at cryogenic conditions to preserve their intracellular organization. Single cells were imaged with the X-ray microscope at a resolution of 50 nm. Two types of data sets were acquired. The first is a tilt-series at the ‘water window’ energy range. At this X-ray energy the best contrast between carbon-rich intracellular membranes and the water-rich cytoplasm is achieved so the data can be used for 3D reconstruction of cells. The second data set was an energy scan around the Ca L-edge. From these data a complete X-ray Absorption Near-Edge Spectroscopy (XANES) spectrum can be extracted for each pixel in the image, providing information on the concentration of calcium inside intracellular organelles and spectroscopic information on the crystallinity of this Ca-rich phase. In the cell tomograms all major organelles were visible, as well as intracellular membrane-bound coccoliths in status nascendi. To our surprise, the cells contained distinct intracellular compartments packed with highly absorbing material, which the spectroscopic data showed to be rich in calcium. The XANES spectra collected from multiple Ca-rich compartments were clearly different from the spectra of coccolith calcite and exhibited characteristics of disordered local environment around the calcium atoms.

These data provide the first insights on the spatial distribution of calcium in coccolithophorid cells. We discovered high amounts of calcium to be concentrated in membrane-bound compartments that are separate from the coccolith producing compartment and we propose that this calcium pool is used for coccolith calcite formation.
IS THE ESCRT COMPONENT VPS4 A MICROTUBULE BINDING PROTEIN?

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The AAA ATPase VPS4 functions at the very last step of ESCRT mediated membrane fission. During these processes, it’s involved in the membrane scission reaction through disassembly of ESCRT-III helical filaments. VPS4 is the only enzyme in the ESCRT machinery and one of the most conserved ESCRT components in evolution. Here, we present a novel function of human VPS4A as a microtubule (MT) binding protein. Using an in vitro microscopic assay, in which rhodamine-labeled MT are incubated with a GFP tagged protein, we characterize VPS4 binding to MT. We show that VPS4A-GFP, overexpressed in HEK293T cell lysates, binds MT and demonstrate that this interaction is specific using recombinant VPS4A-GFP. Additionally, we find that the addition of nucleotides reduce the MT binding ability of VPS4A. These results were further validated using a MT binding protein spin-down assay. This MT binding property has not been attributed, as of yet, to any ESCRT component. Phylogenetic analysis reveals that VPS4 is closely related to the MT severing proteins spastin and katanin, which may hint at a possible similarity in function. A VPS4A deletion mutant, in which a sequence that is based on spastin’s MT binding domain has been removed, still binds strongly to MT, suggesting that VPS4A binds MT through a different domain than spastin. This novel function of VPS4 as a MT binding protein could provide new insights on its role in the mechanism of the ESCRT machinery and on the regulation of many ESCRT-mediated cellular events.
A MODEL SYSTEM FOR THE RECONSTITUTION OF THE CELLULAR ACTIN CORTEX

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The cell cortex is a dense actin network, few hundreds of nanometers thick, which localizes just below the plasma membrane of animal cells and plays a central role in cell shape control. The structural and mechanical properties of the cell cortex depend on its protein composition. Several actin bundling and cross-linking proteins localize to the cortex as well as myosin II motors that provide the cortex with its contractile ability. Myosin motors were shown to affect cortex thickness, structural organization, and mechanical properties. The cortex is nucleated at the membrane surface by actin nucleators that link the cortical network to the membrane surface and it undergoes dynamic remodeling which allows cells to rapidly transform, move, and exert forces in response to internal and extracellular signals.

A difficulty inherent in studies in vivo is that cells have many ways to control their mechanical properties and it can thus be difficult to draw conclusions about the principles of cytoskeletal organization from these experiments. For this reason reconstituted systems have become popular. In principle, these systems allow for full control of the constituents and for studying the effects of specific changes in molecular composition and the impact of the system’s geometry. This renders reconstituted systems optimal for physical analysis.

In this work we investigate the self-organization of actomyosin gels grown on flat supported membranes with the aim to reconstitute an actin cortex under well-defined and controlled conditions. The system is composed of purified proteins that include actin, actin binding proteins that regulate the rate of actin turnover, myosin motors and nucleating molecules that are confined to the membrane surface and physically link the network to it. Specifically, we varied the concentration of myosin motors in wide concentration range to explore the effect of contractility on the cortical actin gel dynamics and structural organization. We show that the addition of myosin motors affects both the thickness of the cortical actin layer as well as the density of the gel across the actin layer. Specifically we find that the thickness decreased with the increase in myosin concentration. The contractile stresses generated by the motors seem to affect density profile of actin, from exponential to a more step-like function.
RESOLVING MIXED MECHANISMS OF MOLECULAR SUB-DIFFUSION AT THE PLASMA MEMBRANE OF LIVE T-CELLS BY SINGLE PARTICLE TRACKING

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The plasma membrane (PM) of a cell can be regarded as a complex fluid in which molecules diffuse and interact. The complexity of the PM can result in anomalous sub-diffusive motion and thus affect molecular function. In spite of the potential significance of molecular mobility to cell function, physical properties of the PM and molecular dynamics within remain poorly understood.

Here, we performed single particle tracking (SPT) of individual membrane proteins that served as nanometer tracers at the PM of live T-cells. The tracers were part of the human immunodeficiency virus (HIV) envelope glycoprotein gp41, whose assembly at the PM plays a critical role in viral budding. By tracking a large number of individual proteins, we observed surprising heterogeneity in their mobility. To better understand the mechanisms that lead to this heterogeneity, we classified the trajectories based on their mobility characteristics, including their instantaneous velocity, area covered by the trajectory and the exponent $\alpha$ in the anomalous diffusion equation $\langle r^2 \rangle = 4Dt^\alpha$ (see figure below). Further statistical tests of the trajectories of the molecular subpopulation showed different dominating mechanisms underlying the heterogeneity in mobility, including molecular trapping, crowding, viscoelasticity of the PM, and their combinations.

Our approach sheds light on the complexity of molecular diffusion of proteins at the PM of live cells, and could serve to further characterize its physical properties and molecular dynamics within.
DECIPHERING THE STRUCTURAL ORGANIZATION OF THE ESCRT COMPLEX DURING CYTOKINETIC ABSCISSION AT NANO SCALE

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The ESCRT machinery (composed of five different subfamilies: 0, I, II, III and VPS4) is designated as a system for membrane remodeling and scission inside cells. Cytosolic ESCRT-III proteins, the driving force for membrane fission, assemble into cortical filaments to induce membrane fission. During cytokinesis, the last step of cell division, the ESCRT machinery mediates constriction and fission of the intercellular membrane bridge that connects two daughter cells. Although the spatiotemporal organization of different ESCRT III proteins on the intercellular bridge was mapped using imaging techniques, many of the mechanistic steps that lead to ESCRT-mediated abscission remains unresolved. For example, it is unknown how ESCRT filament dynamically organizes inside the bridge to mediate abscission. In this study, we employ the super resolution microscopy techniques SIM and STORM to map the distribution of different ESCRT-III components inside the intercellular bridge. By mapping the organization of different ESCRT components on the intercellular bridge, relative to each other we aim to understand how ESCRT filaments modulate their composition during different stages of abscission. The results of this research will provide valuable information on the biophysical mechanism of membrane scission in the cell.
BIOGENIC LIGHT INDUCED TUNABLE PHOTONIC CRYSTALS

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Strikingly brilliant structural colours using 2D or 3D arrays of chitin or cellulose fibers, calcite or guanine crystals were independently evolved by organisms to fulfil a variety of functions. Many of these optical systems are based on intra-cellular arrays of thin guanine crystal platelets.

The silver iridescence of fish scales and skin, as well as the brilliant blue colour of the neon tetra lateral stripe, are both produced by constructive interference of light reflected from ordered arrays of guanine crystals separated one from the other by cytoplasm [1], [2].

Certain families of marine crustacean copepods produce some of the most spectacular colours in nature. They produce different colours covering the whole visible spectrum, and each colour is characterized by well-defined spectral characteristics. By combining correlative reflectance and cryo-electron microscopy image analyses, together with optical time lapse recording and photonic band-gap modelling, we first showed that each colour is created by specific and precise cytoplasm spacings within stacks of uniformly thick guanine crystals [3]. In addition, the same organisms have the remarkable ability to change their reflectance spectrum in response to changes in the light conditions. This change is reversible, and is both intensity and wavelength dependent, forming truly tuneable photonic crystal arrays [4].

The insights gained from the understanding of how biology modulates reflectivity can provide inspiration for the design of artificial optical systems with properties yet hardly achievable, if at all, in artificial materials.

Figure 1. a) Light microscope image of a Sapphirinid copepod. b) Cryo-SEM micrographs of high pressure frozen, freeze fractured Sapphirinid copepod.
INTRACELLULAR DYNAMICS AND LOCALIZATION OF β-CATENIN IN RESPONSE TO WNT SIGNALING DURING THE CELL CYCLE

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The β-catenin protein functions in regulating transcription activity following Wnt signaling. β-catenin recruits transcription factors from the TCF/LEF family to the promoter region of several genes to facilitate gene activation. We are investigating the intracellular dynamics and localization of β-catenin in response to Wnt signaling and during cell cycle progression. We use a cell system that enables the detection of β-catenin in vivo using live-cell imaging based on HEK293 and U2OS cells stably expressing YFP-β-catenin. Under steady state conditions, the YFP-β-catenin protein is expressed mainly in the cell membrane mimicking endogenous β-catenin, and following Wnt activation, significant accumulation in the nucleus and the cytoplasm are observed. Previous studies have shown that β–catenin co-localizes with the centrosome and we wish to further investigate these dynamics using live-cell imaging. Measurements of the β-catenin increase in the membrane, nucleus, cytoplasm and centrosome have revealed similar response dynamics to Wnt. While imaging cells under Wnt activation conditions we observed an interesting phenomenon where β-catenin puncta were detected moving from the cell membrane into the cytoplasm. The movement appeared to be in a directed manner, towards the centrosome area. Since β-catenin puncta movement seemed to be oriented and since microtubules are concentrated at the centrosome, we hypothesized that β-catenin might be traveling on microtubules, and are conducting experiments along these lines. In addition, in order to investigate the influence of the cell cycle on the β-catenin response, we created cells that in live-cell imaging could be identified according to their stage in the cell cycle. Fucci is a reporter gene system containing mCherry-Cdt1 and CFP-Geminin. Cdt1 is a protein that is mainly expressed during G1/S phase while Geminin is a protein that is mainly expressed during G2/M. Experiments performed with the Fucci system show that the β-catenin response to Wnt signaling varies from cell to cell. Namely, cells at the same stage of the cell cycle will respond differently to Wnt signaling. Altogether, we are able to show for the first time the response dynamics of β-catenin to Wnt signaling in real time.
SUPER RESOLUTION MICROSCOPY ELUCIDATES THE INTERACTION BETWEEN ANAEROBIC RESPIRATORY COMPLEX II AND THE FLAGELLAR MOTOR

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*E. coli* is well equipped for living in rapidly changing conditions of its natural ecosystem: it has flagella that enable it to move and navigate in nutrient-poor environments, and when oxygen is limited, it has the ability of switching to anaerobic respiration, using fumarate reduction for making energy. Recently it was discovered that there is a crosstalk between the fumarate reduction system (FRD) and flagellar motility: during fumarate respiration the flagellar motor operates in different, ultra-sensitive mode. Super-resolution microscopy reveals that the two systems communicate via physical interaction between their protein components and sheds light on the molecular mechanism by which FRD controls the output of the flagellar motor. Moreover, super-resolution microscopy enabled quantitative analysis of FRD proteins in relation to the precise location of the flagellar motors.
SEARCH FOR COMPOUNDS STIMULATING MITOCHONDRIAL TURNOVER FOR TREATING TYPE 2 DIABETES

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Normal physiology relies on maintaining mitochondrial mass and function by a continuous balance between mitochondrial biogenesis and disposal, termed ‘mitochondrial turnover’. Impaired mitochondrial turnover is increasingly acknowledged to be central in aging and in the etiology of several age-associated diseases (including Parkinson, Huntington, Alzheimer). Recent evidence indicate that in type-2 diabetes (T2D) mitochondrial turnover is suppressed as well, leading to the accumulation of damaged mitochondria, which results in beta-cell dysfunction and apoptosis.

The tools used so far to follow mitochondrial turnover are expensive and not suitable for a high-throughput screening. Recently, ‘Mitotimer’, a green fluorescent protein which emission shifts to red within about 24 hours after its translation, emerged as a reliable tool to follow mitochondrial turnover. The ratio between red (older mitochondria) and green (new mitochondria) reflects changes in mitochondrial turnover due to mitochondrial synthesis (biogenesis) or clearance (autophagy).

We aimed to establish ‘Mitotimer’ as a unique tool to search for compounds which increase mitochondrial turnover in pancreatic beta-cells, in a high content screen (HCS). The discovery of drugs which improve mitochondrial turnover in beta-cells is potentially a new approach for treating T2D.

HCS assay was developed at the Drug Discovery Unit, INCPM, Weizmann Institute of Science. The assay includes plating ‘Mitotimer’ expressing INS-1 cells in 384-well plates, exposing the cells to 10μM compounds over 18h and image them in two wave lengths (FITC and TRITC) to quantify the mitochondria age reported by ‘Mitotimer’ in individual cells. Image analysis protocol that calculates Red/Green intensity ratio for every cell was written in MetaXpress using Custom Module Editor extension. Due to low z’ values (0.2) it was decided to screen in duplicates.
SILICA DEPOSITION IN SORGHUM SILICA CELLS TAKES PLACE IN VIABLE CELLS

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Silicon oxide minerals are major constituents of the earth’s crust, which forms the immediate environment of plant roots. Silica comprises 1-10% of grass dry weight. Soluble silica in the form of silicic acid is taken up by root channels. The silicic acid is then pulled by transpiration into the leaves, and is polymerized into solid amorphous silica in the lumen of specialized epidermal cells called silica cells, and cell walls of other epidermal cells. The mechanism of silica polymerization in grass silica cells in not completely understood. To study the dynamics of silica deposition in leaf silica cells, we identified three zones in rapidly growing young sorghum leaves, namely: non-silicified zone, actively silicifying zone, and saturated silicified zone. We found that silica cells were viable in the non-silicified zone; and non-viable in the saturated silicified zone. Consistent with the variation in the number of silicified silica cells in the actively silicifying zone, silica cells displayed great variation in their viability (Figure 1). Using Air-scanning electron microscopy, in combination with epifluorescent microscopy we found that silica cells were viable at the time of accumulating silica (Figure 2). We, thus, were able to answer a long standing question in plant-silicon biology by showing that silicification precedes cell death in silica cells. Interestingly, this unique type of bio-mineralization seems to be taking place in the cytoplasm, unbound by any membrane (Figure 3). The viability of silica cells in sorghum mutant that is unable to uptake silicic acid from soil (0.03% dry weight leaf silicon, as compared to 3.5% in the wild-type) was extended compared to the wild type. We exposed the mutant plants to silicic acid by detaching their roots and supplying silicic acid directly to the shoot. Silica cells were silicified only in young leaf tissues, nicely correlated with their viability. Our confocal observations support the assumption that the mineralization starts from the cell periphery, leaving the cytoplasm within it active and well connected to neighboring cells. As the silicification proceeds, the cell content is petrified, leaving unsilicified voids of vacuoles and vesicles. Our findings show that no transpiration of water is needed for the polymerization, and strongly suggest that silicification is biologically controlled in silica cells.
Fig. 2. Silica cells are viable when they are accumulating silica. (A) Viability assay of epidermal peel from wild type sorghum leaf as seen in the epifluorescence microscope unit of the Air-Scanning Electron Microscope (airSEM). Two viable silica cells (fluorescing green, indicated with arrow) and four non-fluorescent dead silica cells (indicated with asterisks) can be seen. (B) Back-scattered electron image of the same epidermal peel taken using airSEM. Note that the viable cells are accumulating silica (marked with arrow). Dead cells can been seen silicified, although a non-silicified dead cell can also be seen in this image (compare (A) and (B)). Scale bar is 100 μm.

Fig. 3. Silicification takes place in the cytoplasm of silica cells. Vacuoles (indicated with asterisks in one of the silica cells in panel (A)) are membrane bound and are not stained by the fluorescent dye. Vacuoles as seen in the silica cells (marked with arrow) of (A) wild type sorghum leaf, and (B) mutant sorghum leaf which does not accumulate silica. (C) Back-scattered electron image of a silicifying silica cell clearly shows that the cytoplasm is silicified but the vacuoles (indicated with asterisks) are not. Scale bar is 10 μm.
INNOVATIVE PROCEDURES IN PARTICLE ANALYSIS: THE CORRELATIVE MICROSCOPY APPROACH

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Particle analysis is a key discipline in safeguards (SG) and nuclear forensic (NF) investigations, as well as in environmental research. Particles-of-interest (POIs), such as “hot” (radioactive) particles, are usually in the micrometric size range, and intermixed within huge populations of other particles, like air-borne dust, soil, industrial exhaust pollutants or estuary sediments. Characterization of individual POIs is accomplished by the Correlative Microscopy approach, which aims at providing complementary information of the same objects by using a variety of microanalytical tools. The first step in the analytical march in particle analysis consists of appropriate sample preparation methods. This step is followed by sequential application of a variety of microanalytical techniques. POIs must be identified, tagged, located, relocated, isolated, relocated and non-destructively analyzed. The final step in the chain (usually mass-spectrometry) may be destructive.

Uranium-containing POIs in environmental samples (ES) are of major interest for SG and NF. In November 2013 the first two authors launched a joint project aimed at particulate-level characterization of uranium-containing existing environmental reference materials (ERMs). A significant part of the project is to develop several innovative procedures, and to improve existing ones, for the toolbox available to the Particle Analyst. Our efforts have been concentrated in four areas:

- **Sample preparation** (an example is shown in Fig. 1):
  - Dispersion of particles (in powder form) on stubs, planchets and thin-film tambourine-like substrates by the shock-wave dispersal device, developed at the IAEA.

- **Fission Track (FT) detection and analysis**:
  - High resolution imaging of very large area “detectors” containing FT “stars” (Fig. 2).
  - Automated segmentation algorithms for identification and location of FT “stars” in the “detectors” (a typical FT “star” is shown in Fig. 3).
  - Laser Micro-Dissection (LMD) for retrieval (“harvesting”) of individual particles from the “catcher”, in which they are embedded (Fig. 4). The particles can then be lifted by micromanipulation and brought to further treatments and analyses.

- **Alpha Track (aT) detection**:
  - Direct particle location using position-sensitive detectors (instrumental auto-radiography).

- **Advanced analytical SEM** (recently installed at the IAEA labs in Seibersdorf):
  - Integration of TOF-SIMS with SEM search and EDS analysis (Fig. 5).
  - FIBing individual particles to expose their inner structure (Fig. 5).

In this presentation, these procedures and preliminary results will be discussed.
CRYOGEL TEMPLATED TITANIA ALIGNED NANOSTRUCTURES PITTED WITH HIERARCHIAL ASSEMBLED NANOPOCKETS FOR GREEN CHEMISTRY APPLICATION

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Here we review the progress and advantages offered by lyophilization process as a new procedure for synthesis of aligned TiO₂ cryogel templates. Titania (TiO₂) 2D nanolayers with excellent photocatalytic activity and high specific surface area have been synthesized by annealing of TiO₂ cryogel obtained by TiOSO₄ and H₂O₂ as precursors lyophilized 55 hrs at temperature 210 K and pressure 5 - 10 mTorr. Material characterization was performed using powder X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), nitrogen adsorption and electron paramagnetic resonance (EPR). Photocatalytic activity was determined by measuring the kinetics of degradation of Methylene Blue (MB) and 4-chlorophenol (4CP). The formation of hydroxyl radical spin adducts was observed by EPR.

The spherical morphology of as-obtained TiO₂ cryogel within the layered structure is shown in Fig 1 a. The multilayered banded structure indicates growth of the spheroidal particle. The growth process occurs within the droplets of confined volume, and involves the 3D radial organization of leaves, build up inside the spheroids via stacked Ti-O sheets held together by weak van der Waals forces (Figs. 1b and 1c).

TEM image of TiO₂ dense cryogel TiO₂ network is shown in Fig.2a. Hierarchal morphologies of nanopockets assembled onto the surface of TiO₂ layers start to appear at a temperature of more than 650°C and strongly influenced by polymorph TiO₂ evolution competing reaction. The controllable lyophilization process involves a significant topotactic relationship between the as-synthesized TiO₂ nanolayers and the desired nanopockets. This relationship suggests that the formation of nanopockets is an extensive spontaneous self-assembled process due to freezing and melting behavior of pore ice and pore water leading to creation of large cavities of 7-10 nm nanopocket-like pores. The nanopockets could be considered as nanounities with well defined crystallographic orientation with respect to the original anatase/rutile TiO₂ structure. The HRTEM study shown collecting of hierarchical ordered square and hexagonal nanopockets onto anatase (Fig. 2 b) and rutile (Fig.2 c) layers, respectively.

Kinetic of degradation of MB and 4CP provides evidence regarding the potential relevance of TiO₂ layers pitted with nanopockets in the functional properties of the resulting TiO₂ nanostructures and its application as photocatalysts in degradation of environmental pollutants. The activity increased with increasing annealing temperature in order 500°C DegussaP25  600°C  850–950°C. Results of measurement of formation of •OH radicals by EPR also confirmed the unusually high activity of our materials. In comparison with the Degussa P25, the sample annealed at 950°C showed significantly higher production of •OH radicals. We could suggest that nanopocket-rich surface of TiO₂ layers might be responsible for generation of hydroxylated species localized in molecular – sized cavities. On the other hand one can expect a strain-induced domain structure in TiO₂ framework due to oxygen vacancy ordering that could be affected the kinetics of degradation of MB and 4CP.
Fig. 1 SEM images of TiO$_2$ cryogenically fractured at different magnifications

Fig. 2 TEM images of TiO$_2$ nanolayers annealed at 950°C pitted with nanoparticles
PHASE-TRANSITION ANALYSIS OF BaTiO$_3$ NANOCRYSTALS

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Ferroelectric materials are of extreme interest for applications, such as the active layer in non-volatile memory devices, biomedical ultrasound imaging and MEMS. A successful integration of miniaturized ferroelectrics, such as nanoparticles is required for advancing future technologies. However, as the crystal dimensions of a ferroelectric is reduced below a certain size, a well-documented 'size effect' makes a non-ferroelectric cubic phase favorable over the tetragonal ferroelectric phase. This size effect encumbers practical realization of ferroelectric nanomaterials in advancing technologies. The origin of the threshold size is still not clearly understood, mainly because of the limited available experimental observation of the phenomenon. We set to investigate the phase transition at crystals of a few tens to a few hundreds of nm in size.

In-situ TEM imaging during the ferroelectric phase change is an ideal method for understanding the macro- and nano-scale changes occurring in the material. Aside from highlighting different crystal phases in the material with diffraction contrast, TEM imaging is sensitive to different poling directions in the crystal. This way one can obtain an insight into the dynamics of the ferroelectric domain formation (e.g. nucleation) and growth mechanism.

BaTiO$_3$ is a seminal ferroelectric material, with a bulk phase transition as low as 130°C and thus is an excellent model system. We used high-resolution TEM equipped with high-angle annular dark-field (HAADF) detector for in-situ direct observation of the collective ion behavior at the domain wall and nucleation sites around the phase transition of BTO nanoparticles. Our work is expected to give a profound understanding of the BaTiO$_3$ collective ionic re-organization around phase changes, leading potentially, at least to successful future integration of nano-ferroelectric particles in next-generation technologies.
VACUUM RABI SPLITTING IN A PLASMONIC CAVITY AT THE SINGLE QUANTUM EMITTER LIMIT

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The strong interaction of individual quantum emitters with resonant cavities is of fundamental interest for understanding light matter interactions. Recent experiments revealed strong coupling between individual plasmonic structures and multiple organic molecules, but so far strong coupling at the limit of a single quantum emitter has not been reported. Here we demonstrate vacuum Rabi splitting, a manifestation of strong coupling, using silver bowtie plasmonic cavities loaded with semiconductor quantum dots (QDs). We have successfully integrated lithographically fabricated silver bowties with one to a few QDs that reside exactly within the plasmonic cavity. Dark field micro-spectroscopy was used in order to characterize the plasmonic behavior of every single bowtie by measuring its scattering spectrum. The very small plasmon mode volumes of the bowties allowed us to demonstrate vacuum Rabi splitting in the limit of an individual quantum emitter. Rabi splitting as high as ~180 meV was observed with a single QD. These observations are verified by polarization-dependent experiments and validated by electromagnetic calculations. Using 50nm SiN suspended films as the substrate enables us upgrading the lithography results and achieving controllably sub 10nm gap and therefore significantly increase the coupling between the QD and the bowties. Transmission Electron Microscopy (TEM) enables us spatially mapping the QDs within the gap and therefore understanding better the light matter interaction in this system.
REALIZING ULTRASENSITIVE LIVE CELL ATOMIC FORCE IMAGING WITH FUNCTIONAL GLASS PROBES

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Since the invention of atomic force microscopy (AFM) in 1986 live cell imaging has gradually progressed inspite of fundamental limitations in generally applied laser beam deflection (LBD) force sensing. This has been achieved by developing algorithmically based protocols to quantitatively delineate the interactions of AFM probes with cell surfaces. A recent effort was the application of a relatively recent algorithm to image fine cellular protrusions or microvilli, a previously unachievable goal [1]. For this advance ultrasoft silicon probes with cantilever force constants of 0.0611N/m were required. A significant next step would be to implement the same ultrasensitive live cell imaging with an important class of large force constant (1-10N/m) functional glass probes for applications such as near-field scanning optical microscopy (NSOM), AFM sensing with patch clamping pipettes [1], scanning electrochemical microscopy etc. In the presentation this next step in live cell imaging is described, with considerable import for scanned probe imaging of live cells.

MAPPING THE ELECTROSTATIC POTENTIAL IN HETEROJUNCTIONS OF PbS/CdS CORE-ARM NANOSTRUCTURES BY OFF-AXIS ELECTRON HOLOGRAPHY

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Semiconducting Hybrid Nanoparticles (HNPs) show synergistic properties in addition to size-dependent effects at the nanoscale. Therefore, it is important to map charge separation across junctions of HNPs, which is responsible for interesting optical and electrical properties.

We synthesized HNPs with a PbS core and CdS arms that exhibit well-defined epitaxial relations. We observe in HNPs that fluorescence emission corresponding to CdS is quenched, which may be due to the charge recombination in the PbS core. To test this assumption, we used off-axis electron holography (OAEH) in the transmission electron microscopy to map the electrostatic potential across the heterojunction\textsuperscript{1,2}. OAEH was applied at an accelerating voltage of 80kV to samples cooled below 100K.

Phase profiles of HNPs differ from those of separate PbS and CdS nanoparticles (typical example in Fig 1). For separate particles, the phase value within the particles is approximately equal followed by a sharp drop at the edges. In HNP, the phase change across the interface is gradual. In particular, the phase values along the CdS arm show a gradual increase towards the PbS core. This comparison indicates band-bending towards the interface. A finite-element 3D solution of the Poisson equation for PbS/CdS HNPs illustrates a type I interface in which charge carriers formed on CdS arms by optical excitation, flow to lower energy levels within the PbS core, thus preventing recombination in the arm. This in turn is manifested as dynamic quenching of the CdS photoluminescence.

References:

Figure 1. (a) Bright field TEM image of PbS/CdS core-arm nanostructure, (b) Reconstructed phase of the electron wave and (c) the corresponding phase values. The arrow in (b) shows the direction of the phase profile from PbS core to the CdS arm. The phase value profiles of an individual PbS cube and CdS arm are also shown in (c) for comparison.
EBSD - EXTENDING THE CAPABILITY OF SEM EDS ANALYSIS AND DELIVERING QUANTITATIVE MICROSTRUCTURE ANALYSIS

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Scanning electron microscopy (SEM) coupled with an Energy Dispersive Spectrometer (EDS) system is a routine analytical technique. EDS enables elemental analysis on the micro and nano scale within the chamber of the SEM. EDS is a powerful and incredibly useful technique, widely used in diverse fields of investigation and research. However, when characterising a sample in the SEM, it should be noted that EDS gives the quantity and distribution of the elements, rather than specific compounds, or oxidation states etc. EDS analysis is significantly enhanced by the addition of EBSD (Electron Backscattered Diffraction). This is a quantitative microstructure characterisation technique, which extends EDS to allow the study a wide range of material properties, and to identify and quantify compounds as opposed to the presence, association and quantity of elements. Thus EBSD is highly complimentary with respect to EDS.

Electron Backscatter Diffraction (EBSD) is a well established technique. It uses electron diffraction patterns to identify and quantify compounds or phases in a sample. Where EDS is used to display element distribution and can quantify composition, EBSD can identify specific compounds. These are characterised using crystallography. Therefore chemical compounds with distinct crystallography can be easily identified, discriminated, quantified and mapped.

In addition, EBSD benefits from higher spatial resolution than EDS, potentially with spatial resolution 100nm, dependant on the material and acquisition conditions.

EBSD can also be conducted in transmission, known as Transmission Kikuchi Diffraction (TKD). In this mode electron transparent TEM samples are analysed with significantly reduced lateral scattering which further improves the spatial resolution, to the order of 10’s of nanometres.

EBSD is simple and convenient to use on a SEM platform and brings a true surface analytical capability to compliment EDS. It extends the sphere of operation of your SEM to encompass work previously assigned to x-ray diffraction (texture, strain, phase) or TEM. The addition of EBSD transforms a SEM into a formidable, wide ranging analytical tool.

The talk introduces the theory and practice of EBSD and TKD, and gives applications examples to demonstrate the diverse range of applications and measures available. These include; phase and compound identification and distribution, grain size, grain boundary characterisation, crystallographic texture, and strain condition to name a few.
TEXTURE ANALYSIS AND CHARACTERIZATION OF ANNEALED ELECTROLESS PLATED Re-Ni FILMS

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Re-based alloy coatings have been produced so far mainly by chemical vapor deposition (CVD) and electroplating. This work focused on the characterization of high rhenium content ultra-thin Re-Ni alloy (~ 40 nm) films electroless plated on a functionalized SiO\(_2\) substrate. Electroless deposition may be preferable in many applications due to the combination of simplicity, low cost, low process temperature (100°C), and selective deposition. Re-Ni films can be applied as protective or barrier layers for the Ultra Large Scale Integration (ULSI), Micro Electro Mechanical Systems (MEMS) and thermocouple (TFTC) sensor technology. Re-Ni coatings were not investigated yet for this application, although Re-W and Re-Mo alloys are already widely used as thermocouples.

As-deposited Re-Ni films are amorphous and not chemically stable in air at room temperature, therefore thermal treatment is required. Heating causes recrystallization of the coating, which influences the electrical and magnetic properties of polycrystalline material. The grains in such films are usually not oriented randomly. Electron Backscattered Diffraction (EBSD) is a powerful tool for measuring film texture and has become a well-established technique for their crystallographic investigation in SEM. In this study the application of EBSD for micro-texture and phase analysis of the annealed Re-Ni coatings was performed. It was shown that after annealing at 500°C for 1h the films have demonstrated the HCP structure with the preferred [001] orientation at the z axis (Figure 1), which allowed their ferromagnetic properties. In addition, the effect of heat treatment on the structure, morphology, electrical properties and corrosion behavior has been investigated for the prepared Re-Ni films.

Figure 1. EBSD image of phase and texture analysis of Re-Ni (40 nm thick) film on SiO\(_2\)/Si substrate after annealing at 500°C for 1h.
CONTROLLING THE SHORT-RANGE ORDER OF AMORPHOUS OXIDES BY NANOMETER SIZE

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Amorphous materials, in contrast to crystalline ones, lack long-range order. Its order decays rapidly with the distance; yet, the local environment for a particular type of atom is quite similar - though not identical. These fine changes in the atomic structure of the materials lead to new and very interesting phenomena which are unique for amorphous materials. Although many aspects of science and technology rely on amorphous materials, much less research is conducted about their structure than on their crystalline counterparts.¹

In nature there are many organisms that use crystallization via an amorphous phase in order to achieve controlled mineralization.² One of the main advantages of this method is that it enables the organism to exert control over the resulting polymorph, which is not necessarily the thermodynamic stable one, by first controlling the short-range order in the amorphous phase.³

In this research we draw inspiration from nature and study the ability to control various structural aspects of amorphous materials via nanometer size effects. We chose atomic layer deposition (ALD) as our material deposition method, since it is a technique that can provide extremely precise, sub-nanometric, thickness control and can deposit conformal and pinhole-free amorphous films of various materials.⁴

It was shown lately in our group that indeed the short-range ordering changes as a function of size in amorphous aluminum-oxide. The results show that the surface of the amorphous alumina possesses a different short-range order than the average in its bulk, so the thinner the amorphous solid is, the more its short-range order resembles that near the surface.⁵

In this research we continue the study on how size affects the short-range order of different amorphous systems and correlate these changes to different properties. We believe that this amazing strategy if adopted for man-made materials could revolutionize many technological applications.

CHEMICAL BATH DEPOSITION AND CHEMICAL EPITAXY OF CADMIUM SULFIDE THIN FILMS ON GaAs SUBSTRATE

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Chemical bath deposition (CBD) from solution offers a simple and cost-effective route for the fabrication of high quality semiconductor thin films, without the need for high deposition temperatures, stringent vacuum or plasma generators. Chemical epitaxy is particularly advantageous for obtaining monocrystalline thin films with well-defined orientation relations with the monocrystalline substrate. Notably, understanding the chemical and physical mechanisms governing chemical epitaxy can allow us to predict and control the orientation of thin films.

Cadmium sulfide (CdS) is a II-VI semiconductor with a wide and direct band gap of 2.42 eV. In this work, we have studied the microstructure and morphology of CdS films chemically deposited on GaAs(100). The deposition of CdS on GaAs was found to be strongly influenced by several parameters, including the order of reagents addition, reagent concentrations, pH, time and temperature. The CdS films grown on GaAs(100) showed two growth orientations of (00.2)\text{CdS}||(111)\text{GaAs} and (00.2)\text{CdS||(11-1)GaAs} ; [110]\text{CdS}||[-110]\text{GaAs} orientation relationship, providing the first reported evidence for chemical epitaxy in CBD CdS on GaAs(100).
PARTIALLY AGGLOMERATED METALLIC THIN FILMS FORMED BY ANNEALING OF SOLID SOLUTION NANOPARTICLES AT LOW HOMOLOGICAL TEMPERATURES

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Partially agglomerated metallic thin films deposited on ceramic substrates represent an important component of the electrode-electrolyte system in various solid-state electrochemistry applications, such as gas sensing or solid oxide fuel cells. In particular, the electrochemical reaction usually occurs at the triple lines where the metal contact, solid electrolyte and gas meet together. Therefore, the total length of these triple lines should be maximized for improving the electrochemical performance of the system.

In this study, we propose a novel method of manipulating the total length of the triple lines in partially agglomerated metal thin films deposited on ceramic substrates. First, the solid solution nanoparticles have been fabricated employing the dewetting phenomenon. Then, a pure metal layer was deposited on the particles followed by annealing at low homological temperature. During this annealing, the material of the film diffuses into the particles, which accelerates the grain boundary grooving and dewetting processes and results in high area density of holes in the film.

The mechanism of this alloy-accelerated dewetting that was studied employing scanning electron microscopy and a combination of focused ion beam with transmission electron microscopy.
USING DUAL BEAM FIB FOR WIRE BONDING MODIFICATION OF MICROELECTRONIC COMPONENTS

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Circuit editing and wire bonding modifications are occasionally required during packaging qualification and failure analysis of Integrated Circuits and Micro Electronic Components. In this work we report on a unique method of utilizing Dual Beam Focused Ion Beam (FIB) for quick and reliable modification of devices assembled in plastic packages. Two examples of cutting, manipulating and repairing gold wire bonds is presented.

**Case 1:**
In order to expose the wire-bonding of the Silicon die, for debugging purposes, of a failing Plastic Ball Grid Array (PBGA) packaged unit, its epoxy cover is normally etched away using chemical acids. During the decapsulation process of a failing unit, one of the gold wire bonds (A in fig.1) has unintentionally been detached from its bonding pad, thus affecting the functionality of the device. Since reconnecting the broken wire bond using a conventional wire bonding machine could damage this device, we performed the bonding process using FIB:

The edge of the FIB manipulator’s probe needle was used as a “patch” to minimize the bending of the wire for its reconnection (B in fig.1). Then the wire bond was slightly bent towards the edge of the patch using the FIB manipulator. The edge of the wire was finally connected to the top of the patch by forming a conductive connection, using the Platinum gas injection source (GIS) (fig.2). Electrical testing confirmed that the device regained its basic functionality and ready for debugging!

Figure 1: Disconnected wire (A) and prober edge attached to pad for extension (B).
Case 2:

Two adjacent wirebonds were found to be incorrectly connected to their pads and required to be switched with each other. Since the above mentioned FIB process is much more reliable and faster than the conventional wirebonding process it was utilized for this modification too. Following decapsulation, the two wire bonds were first cut in a predefined location found to be suitable for the editing, using FIB milling. The wire bonds were then manipulated to the desired location on the Polyimide coated surface of the die (fig.3). Two Platinum micro lines were then deposited on the die surface, establishing electrical connection between each wire and its correct pad (fig.4).

Following the FIB edit the device was tested and the bug found to be fixed by the FIB edit!
Figure 4: Reconnecting the wire bonds to the correct pads.
TRANSMISSION KIKUCHI DIFFRACTION IN THE SCANNING ELECTRON MICROSCOPE FOR IMAGING OF REVERTED AUSTENITE IN CUSTOM 465® STAINLESS STEEL

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Advanced precipitation hardened Custom 465® stainless steel is characterized by a combination of high strength, toughness and good corrosion resistance. It is used in various applications, including aerospace and medical. Here, we carried out metallurgical characterizations of this steel after different thermal treatments. Specimens were characterized by Environmental Scanning Electron Microscopy (ESEM), Electron Backscattered Diffraction (EBSD), X-Ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). η-Ni₃Ti precipitates and reverted austenite were quantified. Their size and distribution at the nano-scale could explain the difference in the steel properties following various aging conditions.

This presentation shall focus on transmission Kikuchi diffraction analyses in the SEM for nano-scale imaging of reverted austenite in Custom 465 stainless steel. The austenite phase was found both inside and at the inter-lamella interfaces. Austenite distribution and locations are demonstrated in the various aging treatments. The amount of reverted austenite was also quantified by XRD and compared to the EBSD.
CATION EXCHANGE COMBINED WITH KIRKENDALL EFFECT IN THE PREPARATION OF SnTe/CdTe AND CdTe/SnTe CORE/SHELL NANOCRYSTALS

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The controlled synthesis of narrow bandgap nanocrystals (NCs) is highly important scientific and technological issue. The lead chalcogenides have showed a good performance in various applications, but practical applications using these materials have been hindered due to the high toxicity of lead. Recently, tin chalcogenides have been considered as the promising alternative for narrow bandgap materials because of its low toxicity and earth-abundance. Among tin chalcogenides, SnTe is a direct bandgap semiconductor (0.18 eV at 300 K), thus it is most promising material with infrared optical activity. Here, we present the facile solution-phase synthesis of SnTe NCs and their corresponding core/shell NCs. We synthesized monodisperse and highly crystalline SnTe NCs by employing a cheap and less toxic precursor and a reducing agent to enhance low reactivity of precursors. Moreover, we developed a synthetic procedure for the synthesis of SnTe-based core/shell NCs by combining the cation exchange and the Kirkendall effect.
DIRECT IMAGING OF CARBON NANOTUBES IN SUPER ACID SOLUTIONS AND LIQUID CRYSTALLINE PHASES

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During recent years it has been demonstrated that carbon nanotubes (CNTs) spontaneously dissolve in chlorosulfonic acid (Davis et al., 2009), and at high concentrations form a liquid crystalline phase (Davis et al., 2004). Actually, chlorosulfonic acid (CSA) is the only solvent for CNT, to form thermodynamically stable solutions and liquid crystalline phases, from which carbon fibers can be spun. Fiber spinning from a liquid crystal state is essential for the high degree of CNT orientation in the fibers, and hence preserving the intrinsic unique properties of individual CNT in the spun fiber (Behabtu et al., 2013).

The transition between the isotropic and the liquid crystalline phases depends strongly on the CNT type, concentration, and solvent strength. Combination of direct cryogenic transmission- and cryogenic scanning-electron microscopy (cryo-TEM and cryo-SEM) of CNT/CSA solutions at different concentrations allowed us, for the first time to follow phase transformation at nanometric level; from diluted solution to the isotropic phase, through the biphasic region, to the pure liquid crystalline phase, used as the "dope" for fiber spinning (See Fig.1). To allow direct imaging of superacid solutions we developed novel cryo-EM specimen preparation and imaging methodologies, suitable for highly acidic systems. Those techniques preserve the native nanostructure in the system, without harming the expensive equipment and the operator (Kleinerman et al., 2015), and were successfully applied to study CNTs (Davis et al., 2009), graphene (Behabtu et al., 2010), and boron nitride nanotubes (Kleinerman et al., in preparation) in CSA in their native state.

The correlation between direct imaging of the "dope" in its liquid state and of fiber, spun from the "dope" allowed us to study the effect of CNT behavior in the solution on final fiber structure and alignment, which are directly related to fiber mechanical and electrical properties. By combination of x-ray analysis, with focused ion beam (FIB) fiber cross-sectioning, and high-resolution electron microscopy for morphological and chemical analysis, we have provided nanometric structural information of the fibers, in terms of CNT alignment, degree of purity and porosity.

Figure 1: Cryo-EM micrographs of CNT/CSA solution, showing liquid crystalline phase development as a function of nanotube concentration (from (a) to (d) CNT concentration is increased). (a) Cryo-TEM image of an isotropic phase. Empty (black arrowhead) and filled with acid (white arrowhead) carbon nanotubes are
recognized. (b) The beginning of aligned phase formation (black arrows) imaged by cryo-TEM. (c) Cryo-SEM of a network of aligned CNT domains (white arrow), coexisting with an isotropic phase. (d) Liquid crystalline domains at "dope" concentration for fiber spinning. Highly ordered, large (tens of microns long) liquid crystalline domains imaged by cryo-SEM.
“TRANSROTATIONAL” MICROCRYSTALS AND NANOSTRUCTURES DISCOVERED BY TRANSMISSION ELECTRON MICROSCOPY IN CRYSTALLIZING AMORPHOUS FILMS

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TEM bend-contour technique for direct lattice orientation analysis [1, 2] developed earlier gave the chance to discover unusual micro crystals growing in thin (20-100 nm) amorphous films with strong (dislocation independent) internal bending of the crystal lattice planes [3]. Initially we observed the new phenomenon for Se (primarily in situ) and Te. Later on it has been revealed for many other substances and materials of different chemical bonding and various preparation conditions (Se-C, Se-Te, Sb$_2$Se$_3$, Sb$_2$S$_3$, Ge-Sb$_2$Se$_3$, Ge-Te, Tl-Se, Cu-Te, a-Fe$_2$O$_3$, Cr$_2$O$_3$, Co-Pd, Re, W, carbides, amorphous metals, ferroelectrics, phase change materials for memory devices). HREM, correlative AFM-TEM were used in due cases.

The main feature of novel micro-, nanostructure is the permanent regular bending/curving of the lattice planes (about axes primarily lying in the film plane) in thin growing crystal. Different geometries are revealed, Fig.1. Thus one can detect in a perfect crystal (“single crystal”) usual translation complicated by relatively small rotation of the unit cell. Anyway more or less significant rotations, up to 300 degrees per 1 micrometer of the crystal length can be attained. Therefore the new term “transrotation” [4] was introduced for such novel crystals/structures. The geometry and gradient of lattice orientations depend upon crystallography of the substance, crystal growth rate (e.g., upon heating), film thickness (the thinner is the film, the stronger is the transrotation) and composition (for binary films with composition gradients).

Earlier hypothetical mechanism of unusual phenomenon based on surface nucleation has been improved and supported by atomistic model of transrotational microcrystals. The last is based on mathematic instruments of conformal transformations. Generally transrotational crystals/structures revealed by TEM can be considered as a new state intermediate between glassy and crystalline ones (similarly to the structure of liquid crystals intermediate between crystalline and liquid). Alternatively transrotation can be regarded as an example of new kind of extended defect in condensed matter. In this sense transrotations (in thin crystals) supplement dislocations (in crystals) and disclinations (in liquid crystals). Microcrystals and nanostructures with “transrotation” during last years have been eventually recognized/studied in various thin films by different authors (e.g. [5-7]).

Fig.1 Schemes of lattice transrotation geometries with corresponding crystal TEM images placed below for thin-film materials: Se, Fe$_2$O$_3$, Ta$_2$O$_5$, C+Se+C, Cu-Te (from left to right). Bar = 1 µm (where not specified).


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SPACE CHARGE REGION AND DIFFUSION LENGTH OF CsPbBr₃ SOLAR CELLS

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High open-circuit voltage solar cells are important in spectral splitting systems to optimize the use of high-energy photon photons and to drive a variety of electrochemical reactions. Hybrid organic-inorganic lead halide perovskites with the generic structural formula AMX₃ (where ‘A’ is a usually an organic monovalent cation, ‘M’ is the divalent metal center and ‘X’ is a halide) have been thoroughly studied in the last few years but still face stability issues. Among the possible solutions replacing the organic moiety by cesium has gained increasing attention [1], [2]. While it has been shown that high-band gap (2 eV) devices made from CsPbBr₃ as an absorber layer can work equally well as, and with better stability than devices based on CH₃NH₃PbBr₃ [3], there are still large gaps in our knowledge regarding how the inorganic halide perovskite photovoltaic devices operate.

In this presentation we discuss what the working mechanisms of CsPbBr₃-based devices are, by comparing the Cs with the organic perovskite in terms of how free carriers are separated, the width of the space charge region and the diffusion length as measured by Electron Induced Beam Current (EBIC) under different conditions in the scanning electron microscope.

EBIC uses the electron beam to act as a light source equivalent (electrovoltaic, instead of photovoltaic effect), generating electron-hole pairs in the junction area. If these pairs separate into free carriers, and are collected at the contacts, we measure a current in real time and a current collection efficiency image can be drawn.

References

SYNTHESIS OF CORE-SHELL MoS₂ FULLERENE-LIKE INCORPORATING GOLD NANOPARTICLE Au@IF-MoS₂

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Nanoparticles and more specifically gold nanoparticles (AuNPs) attracted a great scientific and technological interest in the last few decades. Their popularity is attributed to their optical, electrical and magnetic properties which are unique and cannot be seen in the bulk. However, one of the main problems of AuNPs is their long-term stability. On the other hand, MoS₂ nanoparticles (NPs) and single layers show great chemical stability, and exhibit excellent mechanical and tribological properties¹ as well as being biologically benign². Moreover, it is known that MoS₂ can form conformal coating on topologically complex surfaces. Finally, due to the MoS₂ NP unique optical properties, a hybrid AuNP core and MoS₂ shell would be a unique, stable and interesting hybrid nanomaterial.

In this work we present a synthesis of AuNPs coated by MoS₂ single-layer. i.e. a core-shell nanostructure (Au@MoS₂). These hybrid nanoparticles can also be considered as fullerene-like MoS₂ (IF-MoS₂) NP with an AuNP core. The characterization of the NPs was carried out by transmission electron microscope (TEM), energy-dispersive X-ray spectroscopy (EDS), electron energy loss spectroscopy (EELS), scanning electron microscope (SEM), Raman spectroscopy and UV-Vis absorption. It was found that the AuNPs are 7-13 nm in diameter and are coated by a complete single-layer of MoS₂ (or more) with an IF-MoS₂ structure. The growth mechanism of the nanoparticles has been studied and is discussed hereby.

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THE EFFECT OF CARBON NANOTUBE PARAMETERS ON THEIR PHASE BEHAVIOR IN SUPER-ACID SOLUTIONS

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On the molecular level, carbon nanotubes (CNTs) exhibit excellent mechanical strength, thermal and electrical conductivity properties, combined with low density. This unique-feature combination makes CNTs ideal candidates for processing multifunctional macroscopic fibers. However, translating the unique characteristics of a single molecule into macroscopic material is challenging.¹²

The most significant limitation that prevents wide-scale use of CNTs in applications is the difficulty of dispersing CNTs to form thermodynamically stable solution, free of aggregates or bundles. Recently it was discovered that CNTs spontaneously dissolve in chlorosulfonic acid (CSA), forming a molecular solution, namely, the nanotubes are dispersed as individual molecules, and at higher concentrations form a lyotropic liquid crystalline nematic phase.³ Alignment of CNTs in a solution is essential to achieve the best properties in a CNT-based macroscopic material.⁴ CNT parameters, such as length, diameter, and degree of purity, dictate CNTs solubility in acid, phase transition concentration from isotropic to biphasic, where isotropic and nematic phases coexist, CNTs arrangement in the biphasic, and the fully nematic phase. Therefore, understanding the influence of CNT parameters on the CNT/CSA system phase behavior, and specifically, the formation of liquid crystalline phase, is necessary for designing a successful CNT fluid-phase process, while preserving CNTs’ unique molecular properties.

In this study we characterize CNT/CSA solutions with different CNT parameters, such as length and diameter, by a combination of polarized light microscopy and cryogenic electron microscopy. Results demonstrate that solutions with higher aspect ratio CNTs, phase separate at much lower concentrations (Fig. 1), in agreement with Flory’s rigid-rod theory.⁵ In addition, there is a difference in nematic phase morphology as a function of aspect ratio, by means of CNTs arrangement within the ordered phase.

Figure 1: Cryo-TEM images of (A) short HiPco SWNTs at 250 ppm in isotropic solution (phase separation occurs at 1400 ppm). (B) long Meijo SWNTs at 100 ppm in bi-phasic solution (phase separation occurs at 100 ppm). Bars correspond to 100 nm.

Bibliography


Since its invention centuries ago, the light microscope has become an essential tool in many scientific fields, and is the basic tool for imaging individual cells or structures in the life sciences. The basic motivation for using microscopes remains the same: to resolve features that cannot be seen by a naked eye, and consequently there is a constant strive to improve their resolution to and beyond the diffraction limit barrier. However, when imaging biological specimens, the resolution is usually compromised due to inhomogeneous optical characters of the specimen. This inhomogeneity causes distortions to the light emitted from the specimen, leading to degradation of the image quality. One of the options for improving the resolution is by deconvolution, using the linear dependency between the optical setup impulse response (PSF) and the object light reflectance in the linear imaging model. By evaluating the PSF, the image resolution can be improved by solving a direct or iterative inverse problem. Since the initial PSF strongly influences the final result, it is generally crucial to obtain a good estimate of the PSF. Another recent approach to improve the microscopic images is adaptive optics. Adapted from astronomy and high resolution retinal imaging, adaptive optics is based on measurement and correction of the emitted wave-front error by measuring it from a laser reference beam. However, when the reference beam also passes through phase deforming media or in the cases of rapidly changing- or significant phase errors, the resolution does not reach the diffraction limited value and a further improvement by PSF estimation approach could be advantageous. Taken together, distortion-resilient methods for PSF estimation could provide a powerful addition to the imaging toolbox.

Here, we present PEPSI (PSF estimation by projection of speckle pattern illumination), a new approach for estimating the transverse PSF when imaging through phase deforming media. The method is based on measuring the deformation of a speckle pattern illuminating a fluorescent object. The motivation for using a speckle pattern to measure the deformations arises from their random phase distribution: these random phases yield a pattern whose statistics are not affected by optical aberrations. Therefore, by illuminating the object with the speckle pattern, an objective measure of the phase errors of the imaging path can be obtained, irrespective of the illumination path’s phase aberrations. Since the speckle pattern is uniformly distributed throughout the field of view, PEPSI estimates the average PSF of the entire field of view from a single pattern projection, and is thus suitable for dealing with dynamic phase aberrations (not requiring prior calibrations or acquisition of multiple images). Moreover, in cases where the aberrations are non-isoplanatic, the local PSF for selected areas in the field of view can be obtained by the same analysis on those areas. As a demonstration of the PSF estimation accuracy of PEPSI, we used the obtained PSF estimates to improve the resolution of microscopic images using a common iterative maximum likelihood-based image reconstruction algorithm. The easy integration of PEPSI to a commercial microscope, which only requires an additional diffuser in the microscope illumination path, is also demonstrated by improving a degraded image taken by a commercial epi-illuminated microscope.
THE AAATPase VPS4 SHAPES EARLY STEPS IN CILIA FORMATION AND ALTERS CENTRIOLAR SATELLITES AND THE PERICENTRIOLAR MATERIAL

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Primary cilia are long (up to 9 μm long) narrow (less than 1 μm wide) microtubules based membrane protrusions that serve as key transducers of sensory stimuli. Cilia are dynamically regulated during the cell cycle and their formation involves centrosome remodeling, vesicle docking and coupled axoneme and membrane extension. Recently, the ESCRT-associated AAATPase, VPS4, was identified in *Chlamydamonas* at the transition zone, a region at the ciliary base that acts as a ciliary gate. Several studies showed that mutations affecting proteins located in this zone result in cilia-related diseases (ciliopathies) including retinal degeneration and kidney disease. In this study we investigate the role of VPS4 in primary cilia formation and maintenance. We find that in mammalian cells depletion of VPS4 or over expression of its dominant-negative version (VPS4 DN) impair cilia formation. Similarly, injection of zebrafish embryos with VPS4 DN reduced cilia formation in Kupffer’s vesicle and resulted in developmental delays and death. Using Structured Illumination Microscopy (SIM) we show that VPS4A DN localizes to the centrosome, specifically, to the transition zone. Mapping the composition of different centrosomal proteins by SIM reveals that several key ciliary components including γ-tubulin and Pericentriolar Material 1 (PCM1) exhibit altered organization while others (CEP164, CP110 and B9D2) are not affected. Moreover, using micropatterns, we demonstrate that over-expression of VPS4A DN alters centrosome positioning. Correlative EM analysis shows accumulation of ciliary vesicles at centrosomes in VPS4A DN expressing cells, suggesting a role for VPS4 in early stages of ciliogenesis. Taken together, our study suggests a role for VPS4 in cilia biogenesis and adds a new dimension to recent work that has defined several of the early steps in ciliogenesis.
IMMUNOGOLD LABELING OF PHOSPHATIDYLSERINE BY ANNEXIN V IN CRYO-TEM SPECIMENS

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Immunohistochemistry is a powerful tool, based on the concept that specific antigens can be detected by complementary antibodies. In the last few decades the use of colloidal gold in transmission electron microscopy has grown at an enormous rate, and has become virtually the only method worth considering for ultrastructural studies of cellular antigens. Immunogold labeling takes advantage of the high electron density of gold conjugated to antibodies, which in turn adsorb to a specific antigen. Although the probing molecules are often antibodies, other proteins of a specific affinity can be tagged.

Phosphatidylserine (PS) is a negatively charged phospholipid, found mostly in the inner leaflet of the cell membrane, facing the cytoplasm. Certain cell processes, such as apoptosis and microparticle shedding, involve PS migration to the outer leaflet. This phenomenon can be studied using the cellular protein, annexin V, which has high affinity to PS. Annexin V binding is Ca2+-dependent, which should be taken into account during the procedure.

Liposomes are spheroidal vesicles, composed of one or more lipid bilayers. They allow the easy and fairly realistic mimicking of bio-membranes; they can be built-up by one or just a few well-defined components, and hence allow better characterization of the physical principles underlying the self-organization processes observed in membranes. Immunogold labeling of PS in mixed lipid bilayer will significantly contribute to the study of the nano-scale domain formation mechanism. Until now these domains have been studied by direct imaging only on the micrometer scale, not on the nanoscopic scale. Understanding this phenomenon is important, because they are involved in a variety of cellular functions and biological events.

Thus far, most immunogold labeling has been performed in ways, which eventually lead to room temperature imaging by TEM or SEM. Our work presents immunogold labeling in cryo-TEM. Cryo-TEM preserves the liposomes as close as possible to their native state, thus providing a more reliable view of the nanostructure and its morphology. We attempted to label PS liposomes prepared by sonication and extrusion. Labeling was performed in solution, using biotinilated annexin V and gold-conjugated streptavidin, in a two-step process. We optimized the working conditions leading to extensive labeling of PS. The gold nanoparticles were not randomly spread in the solution, suggesting that the immunogold labeling in cryo-TEM was indeed successful. Apart from the excellent labeling we observed aggregation, which we attributed to the increased concentration of Ca2+ ions in the vicinity of the highly charged liposomes. The successful labeling of liposomes allows the application of the methodology to synthetic mixed-lipid and natural systems, such as extracellular vesicles.
Figure 1: Cryo-TEM image of DOPS vesicles dispersion in PBS buffer (pH 7.4). These vesicles were prepared by sonication at room temperature, and were labeled in solution by biotinilated annexin V and 5 nm gold conjugated Streptavidin. Bar = 100 nm.
MINERAL SCAFFOLDS ENABLE THE MORPHOGENESIS OF BACTERIAL BIOFILMS

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Biofilms, or surface-attached communities of cells encapsulated in an extracellular matrix, represent a common lifestyle for many bacteria. Within a biofilm, bacterial cells often exhibit altered physiology, including enhanced resistance to antibiotics and other environmental stresses. Here we reveal a novel mechanism maintaining Bacillus subtilis and Mycobacterium smegmatis biofilms, identify the active production of calcite scaffolds. We show the distribution of minerals in the biofilm using micro-computed tomography (microCT). The microCT provides high-resolution assessments of density, geometry and microarchitecture of mineralized tissues and calcification. Furthermore, we develop a calcium carbonate quantitative method within the biofilm that teaches us about the durability and rigidity of the bacteria colony and the amount of the calcium carbonate in each location within the colony. Those scaffolds provide resistance to environmental insults, an efficient strategy for carbon dioxide sequestration also increasing overall fitness of the community. Moreover, by using Environment Scanning Electron Microscopy (ESEM) we show that extracellular matrix mutants interfere with crystal growth within biofilms. Our study sheds light on that the formation of mineral plays a cardinal and conserved role in bacterial multi-cellularity.
ULTRASTRUCTURE STUDY OF BIOLOGICAL MICRO-INJECTION SYSTEM

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Cnidarians (jellyfish, coral, sea anemone, hydra) are characterized by their stinging cells that function mainly in prey capture and defense. The stinging cells contain the cnidocysts, capsules equipped with an injection system and act as biological syringe. These capsules contain highly folded thin tubule resembling a needle, and can hold pressure of 150 bar. Upon activation, the tubule unfolds and penetrates its target at a remarkable acceleration of 5·10⁶g to deliver 10 times capsule volume. Recently a large group of parasites, known as Myxozoa was added to the Cnidaria phylum. These endoparasites have a complex life cycle involving two hosts mainly fish and annelid and the infection is initiated by 2 stinging capsules. Myxozoa fish infection can have dramatic negative effects on fish populations inhibiting growth and causing mortality through diseases such as ceratomyxosis and whirling disease. To understand the adaptations of myxozoan capsules, we studied the function and structure of the tubules of two types of myxozoans and compared our finding with the stinging capsules of the jellyfish Rhopilema nomadica. External tubules ultrastructure of myxozoans and jellyfish were observed by scanning electron microscopy (SIGMA-HD, Carl Zeiss, Germany) and provides evidence of different functional modifictions that were developed through evolution. Our findings demonstrate that myxozoan capsules can infect their host by a complex mechanism of injection and anchoring.
After entering a new host cell, the genetic material of the human immunodeficiency virus (HIV) is encased in a multimeric protein shell: the capsid. Encapsulation is thought to facilitate reverse transcription by reducing the available volume for viral enzymes and genetic material, and also to help protect the integrity of the viral genetic material prior to integration in the host genome. However, the capsid is too large to cross the nuclear pore complex, and must disassemble in a process known as uncoating, thus releasing its contents. There is mounting evidence that the timing of uncoating is of critical importance for HIV infectivity, as drugs and capsid protein mutations which affect the capsid’s stability in the cytosol have been found to greatly hinder the infection process.

Reverse transcription, in which a single stranded RNA molecule (ssRNA) is used as a template to synthesize double stranded DNA (dsDNA), is a major step of the retroviral replication cycle which occurs at roughly the same time as HIV uncoating. Since dsDNA is a considerably more rigid molecule than ssDNA, it has been suggested that the resulting confinement forces due to dsDNA pressure on the capsid`s interior could be sufficient and necessary to initiate uncoating.

To explore a possible role for mechanical forces in HIV uncoating, we used Atomic Force Microscopy (AFM) and nanoindentation techniques to study the morphology and mechanical properties of HIV-1 cores in buffered aqueous environment. Our study was focused on several factors known to affect capsid uncoating: capsid protein mutations, a small-molecule inhibitor of HIV-1 infection (PF-74), and the cellular protein Cyclophilin A. In all cases, we found that the capsid’s stiffness was significantly affected, which supports the notion of internal pressure playing a major role in uncoating. This helps shed further light on this important step of retroviral infection.
REVERSE TRANSCRIPTION MECHANICALLY INDUCES HIV-1 UNCOATING

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The RNA genome of human immunodeficiency virus type 1 (HIV-1) is enclosed inside a capsid shell that disassembles within a cell in a process known as uncoating. Although HIV-1 uncoating has been linked to reverse transcription of the viral genome in target cells, the mechanism by which uncoating is triggered is unknown. Using time-lapse atomic force microscopy, we analyzed the structure and physical properties of isolated HIV-1 cores during the course of reverse transcription in vitro. We find that, during reverse transcription the pressure inside the capsid increases, reaching a maximum after 7 hours. High-resolution mechanical mapping reveals the formation of a coiled filamentous structure underneath the capsid surface. Subsequently, this coiled structure disappears, the stiffness of the capsid drops precipitously, and the cores are partially or completely ruptured. We propose that the transcription of the relatively flexible ssRNA into the more rigid dsDNA elevates the internal pressure, which triggers uncoating.
IMPROVEMENT OF THE DECALCIFICATION PROCESS FOR ALL TYPES OF HARD MATERIAL IN THE ROUTINE HISTOLOGY

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The processing of all types of hard material in the routine paraffin process in histology needs an additional decalcification step.

Routine hard material as bone, teeth or nails will be decalcified with inorganic acids normally.

For sensitive hard material e.g. bone marrow biopsies used with an acid decalcification solution should not be used because the nuclear structure, nucleic acids and especially anti-gene structures will be influenced negatively or might be inactivated completely.

Here will be presented how the results for sensitive and routine hard material can be enhanced by selection of the suitable decalcification agent and to use the right decalcification solution produce best result for immune histochemical methods and PCR which are based on the full anti-gene activity.
TAG-FREE LABELING OF CELLULAR PROTEINS IN LIVE CELLS WITH FLUORESCENT ORGANIC DYES

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High-resolution fluorescence imaging, combined with the latest labeling techniques available today – have yet been able to provide the necessary spatiotemporal resolution to record all cellular processes in live cells. Fluorescent proteins (Fl-proteins) such as GFP had a vast impact on biology following their discovery, as they were the first to enable the study of proteins in live cells. However, Fl-proteins have many drawbacks in the super resolution microscopy era. Fluorescent organic dyes (Fl-dyes), which offer improved photophysical capabilities, are a good substitute to Fl-proteins. However today, all available methods for attaching Fl-dyes to proteins in live cells still relies on the addition of a relatively large protein tag, which in turn reinstate one of the major drawbacks of Fl-proteins (their size).

Based on recent advancement in genetic code expansion (AMBER suppression) and bioorthogonal chemistry, we are developing an innovative system to specifically label cellular proteins with Fl-dyes in live cells. This is done by directly, and non-invasively, attaching the small Fl-dye to a selected amino acid site on a given protein.

Reducing the size of the tag to a minimum and utilizing the superb photophysical parameters of Fl-dyes will offer a unique tool to study cellular proteins in live cells at close-to-physiological conditions.

We will present data showing the development and feasibility of the approach, and will demonstrate its applicability and efficiency using our benchmark protein α-tubulin.
A CORRELATIVE STUDY OF OSTEOCLAST ADHESION TO BONE SURFACES

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Osteoclasts are multinucleated cells from hemotopoietic origin, which resorb bone at certain locations in a highly regulated and coordinated manner. This allows bone to preserve its flexibility and integrity throughout the different loads it encounters. During osteoclast adhesion to bone surface, specialized actin based structures, termed the sealing zone rings, are formed at the cell-bone interface and delimit the resorptive lacuna. Observed differences between the formation and dynamics of these sealing zone structures in osteoclasts seeded on artificial or physiological surfaces suggest surface dependency. However, the effect of different bone surface parameters on the sealing zone rings is still not well understood, despite the importance of osteoclast adhesion to its resorption functionality. In order to gain insights regarding the relation between bone physical parameters to the dynamic behavior of sealing zone rings in active osteoclasts, we have developed a specialized correlative method; the cortical bone surfaces are imaged by a scanning electron microscope and atomic force microscope and overlaid with dynamic tracking of sealing zone structures in live osteoclasts, taken with the deltavision optical microscope. Using this approach, we show preferential formation of rings around bone surface protrusion of limited size, as oppose to possible other physiologically relevant structures, such as microcracks. In addition, we show that resorption functionality relates to larger sealing zone rings that adapt pit morphology. We suggest that this correlative method can be used for a wide variety of applications, which involve cell adhesion to non-transparent surfaces that may be sensitive to standard electron microscopy preparation methods.

STOMATAL CELL WALL CRYSTALLINITY: DISTINCTIVE STRUCTURAL PATTERNS IN DIVERSE PHYLOGENETIC GROUPS

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Stomata are small epidermal pores with a complex opening and closing mechanism, which are responsible for the plant gas exchange. Stomata evolved ~400 million years ago - and have remained a key feature of plant anatomy and physiology. Stomata offer a unique research system, where the function has remained largely the same, even though various cell wall features have evolved and changed. We attempted a renewed look at stomatal cell wall structure utilising digitalized polar microscopy and confocal microscopy. We investigated the distribution patterns of cellulose, including microfibril orientation and crystalinity, lignin and phenolic compounds in the stomata of six species of vascular plants. In addition, we applied a numerical mechanical Finite-Element simulation to understand the mechanical anisotropy of the stomatal cell wall. Stomata of the six species chosen for study cover a broad structural, ecophysiological and evolutionary spectrum: two ferns, two angiosperm species with kidney-shaped stomata, and two grass species with dumbbell-shaped stomata.

Surprisingly, we observed three distinct patterns of cellulose crystallinity in stomatal cell walls: the ferns exhibited pattern Type I, angiosperm kidney-shaped stomata exhibited pattern Type II and the grasses presented Type III. Our data demonstrates for the first time the existence of distinct spatial patterns of varying cellulose crystallinity in guard cell walls. Guard cell walls undergo reversible deformations during opening/closing of the pore and thus must be both extremely strong and flexible. Different cellulose crystallinity patterns could influence those properties. Such spacial patterns could imply different biomechanical function, which in its turn could be a consequence of different environmental selection. In addition, there were taxon-specific allocation patterns of phenolic compounds and lignin in the guard cells. In ferns the polar end walls were lignified, in angiosperm kidney-shaped stomata the inner (ventral) cell walls contained phenolic compounds and no lignification occurred, and in grasses the whole stoma contained...
phenolic compounds. According to our numerical bio-mechanical model, the stomatal end walls develop the highest stresses during the opening. It could be an intriguing assumption that crystalline cellulose replaced lignin in stomatal end walls of more evolutionally advanced plants in order to serve a similar wall strengthening function.
Accumulation of cholesterol in the blood vessel walls is a prominent feature of atherosclerosis (1), a major precursor of many cardiovascular diseases. It is well accepted that precipitation of cholesterol crystals in atherosclerotic lesions is a crucial part of the pathological progression (2). Cholesterol monohydrate crystals can increase the inflammatory response and cause expansion of the lesion core leading to arterial thrombosis (2b). What initiates cholesterol crystal formation is, however, still not well-understood (3). Studies in cell culture (4) and in supported lipid bilayers (5) showed that when a bilayer is loaded with high levels of cholesterol, 2 dimensional (2D) crystalline domains of cholesterol are formed and it was suggested to be an initial step in atherosclerosis development (4). We indeed demonstrated that such 2D cholesterol domains can serve as nucleation sites for the formation of 3D cholesterol crystals in vitro (6). The question of whether this process occurs also in biological membranes was then approached.

High resolution imaging is required to directly visualize cholesterol domains and identify the initial stages of cholesterol segregation and crystal formation in cell membranes. For this, we developed a correlative method that combines cryo soft X-ray tomography (cryo-SXT) with stochastic optical reconstruction microscopy (STORM). The correlative approach provides imaging of the domain distribution in cell membranes and identification of 3D cholesterol crystals. This is done by using a specific antibody (MAB 58B1) which labels cholesterol 2D and 3D crystals (7). To the best of our knowledge, this is the first example of correlative STORM and cryo-SXT providing information on cellular systems at resolution of tens of nanometers and in relatively large volumes (tens of micrometers). Our results show that cholesterol crystals can be imaged in a very early stage at their exact locations inside the cell. The cells were observed under cryo-conditions, without dissolving the crystals or using any other dehydration methods, such that the biological system is as close to the natural condition as possible.

In the future, the method will be applied to determine the crystal nucleation location and mechanism for intracellular, interfacial as well as extra-cellular particles. If our hypothesis will be proven correct, it can provide new inspiration on how to inhibit the critical pathological step of cholesterol crystal nucleation in early stages of atherosclerosis.

References:

3D VISUALIZATION OF BIOLOGICAL TISSUES FROM MICROMETER TO NANOMETER SCALE USING CRYO-FIB-SEM

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Understanding the ultrastructure of intact biological tissues at different scales (from whole tissue organization to cellular and subcellular compartments) can lead to far-reaching mechanistic insights. By using conventional imaging methods (such as SEM, TEM and microCT), a compromise between the imaging resolution and the sample size has to be made. Serial FIB milling and block face SEM imaging (FIB-SEM) enable high resolution imaging of tissues at up to 5 nm resolution, with the ability to detect large volumes (dozens of micrometers). Conventional FIB-SEM imaging requires intense sample processing that may damage or modify the specimens during preparation (fixation, dehydration, staining etc). This procedure is also time consuming (1 week). The recently developed cryo-FIB-SEM technique (Schertel et al, 2013) allows 3D imaging of high pressure frozen biological samples in conditions that are very close to their native state, avoiding any chemical procedures. Cryo-FIB-SEM workflow is extremely fast, requiring less than an hour from organism sacrifice, to the first cryo-FIB-SEM results.

By utilizing the cryo-FIB-SEM technique, we show the ultrastructure of cellular, sub-cellular and extracellular compartments of two highly studied biological model systems; the sea urchin embryo and the zebrafish larva. The large volume of imaging reveals intra and extra cellular compartments in the tissue in their biological context. By combining simultaneous detection of secondary and backscattered electrons, we locate and characterize mineralized elements embedded in the tissues, and show their interactions with their environment. By correlating the backscattered electron signal with secondary electron gray level data, we characterize different features and organelles inside the tissue. Cryo-FIB-SEM technique is advantageous for 3D imaging of biological systems in which tissue dehydration and processing may cause morphological changes. In addition, cryo preservation is highly beneficial in cases where sensitive or transient moieties are present inside the tissue. In the future, cryo-FIB-SEM could be combined in a correlative manner with other imaging or analytical methods, such as fluorescence, cryo-EDX and cryo-STEM.

CANCELLED
AN INSIGHT INTO THE UNKNOWN TERRAIN OF SPERM STORAGE IN DROSOPHILA FEMALE USING CORRELATIVE MICROSCOPY

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Females in taxa ranging from insects to mammals have evolved sperm storage mechanisms. Different organisms store different amounts of sperm for different lengths of time in closed or open reservoirs. Given its wealth of genetic and molecular tools and the high conservation of genes between flies and mammals, Drosophila promises to be an important model system for understanding the molecular basis of sperm-female interactions across animal taxa. Drosophila females have two types of sperm storage organs: paired spherical spermathecae and a single elongate, tubular seminal receptacle. Despite the interest in the evolution and function of the seminal receptacle, the structure of the receptacle epithelium has received little attention in Drosophila. We have used a correlative microscopy approach in which we combined confocal for light, X-ray microtomography for three-dimensional view of the whole organ and high resolution in vivo imaging with focused ion beam (FIB). This combination allowed an increased sample throughput gaining insights into the internal structure of the seminal receptacle. Each imaging technique revealed a different level of information about sperm localization within storage, outline of the organ and details about the internal structures. The obtained results open new avenues and allow new questions to be asked about sperm-female interactions post mating.
MOLECULAR INTERACTIONS UNDERLYING SYMMETRY REDUCTION AND NON-HEXICAL PERTURBATIONS IN THE BACTERIAL FLAGELLAR FILAMENT

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The bacterial flagellar filament is a hydrodynamic propeller that converts the motion of the rotary motor into linear thrust. The canonic flagellin monomer (Salmonella typhymurium SJW1655) is comprised, from the outside in, of four domains D0-D3. It assembles into a helical polymer with major helical lines of orders n= 1, -5, +6 and ±11.

Non-hexical perturbations are known to occur in S. typhimurium SJW23 and SJW117, which are straight, right-handed and left-handed filaments, respectively. These perturbations are a product of pairwise symmetry reduction along the left-handed, five start (-5) helical lines. The resulting helical lines can be indexed only as fractional Bessel orders (-2.5, -13.5, 8.5, 3.5 etc), which indicate disruption of the continuity of the helical lines (and a consequent seam).

Previously we attempted to determine the molecular nature and origin of the non-hexical perturbation: (a) by perturbing the plain structure of a model so that a power spectrum similar to that of SJW117/23 is obtained. This is achieved by a ±10° rotation of alternating globular subunit rows along the 5-start lines. (b) by ‘reverse engineering’ the SJW23 flagellin gene (flC23) and restoring the filament’s plain’ helical symmetry (as indicated by changes in the power spectrum—disappearance of the typical layer-line clusters (n= -2.5, -13.5, 8.5)). We concluded that the non-hexical perturbation is a product of unique interactions in the inner part of the D3 density shell (contrary to interactions at the outer tip of D3 in the case of hexical perturbations).

However, without a full three dimensional reconstruction we cannot specify the spatial and molecular interactions leading to the perturbation. The non integer Bessel orders introduce helical symmetry breaking in the form of a seam which prevents the straightforward implementation of symmetry-based Fourier-Bessel and iterative real-space three-dimensional image reconstruction techniques. Methods exist to reconstruct microtubules with seams; however, these are thin walled, relatively straight tubes assembled from relatively simple globular monomers.

We refined real-space iterative helical reconstruction methods suitable for handling the unique properties of non-hexically perturbed bacterial flagellar filaments, which are complex, dense, helical polymers, constructed from multi-domain monomers resulting in large fluctuations in radial mass distribution which allow for complex perturbations. We reconstruct the filament without using a reference (similar to IHR3R) and bring into account the relation between the seam’s angular position and the tube’s radius.

Our findings suggest that domain D2 is the anchor of the non-hexical perturbation. It allows a sharp tilt of D2, D3 and the reduction in symmetry (dimerization): the connectivities between D2 with the inner part of D3 gives rise to helical lines of order n= -2.5 and -13.5. The tilted orientation of D2, D3 can be explained by the hydrophilic nature of a modified α-helix at the interface of D2 with D1. Another modification of a α-helix, at the interface of D2 with the neighboring D1 gives rise to the helical lines of orders n= -2.5. D2/D2 interactions give rise to n=8.5 lines. These interactions allow for the dimerization of monomers.
INTRODUCING A NEW EDS DETECTOR DESIGN CAPABLE OF SUM 10NM ANALYTICAL SPACIAL RESOLUTION AND HIGH EDS SURFACE SENSITIVITY IN A FEG SEM

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The imaging conditions and microscope settings used in the latest ultra-high resolution FEG-SEMs for investigating the morphology and structure of the smallest nano-materials and layers include very low kV, minimum beam current and short working distances. These conditions are incompatible with current conventional EDS detectors due to limits in geometry and sensitivity.

We introduce a new design approach for EDS detectors, which specifically provides elemental characterisation under these imaging conditions.

The detector front end has been re-designed to minimise sample to crystal distance and operating working distance. The electron trap volume has been significantly reduced to stop low kV backscatter electrons to minimise footprint. A windowless approach is used to maximise the sensitivity to low energy X-rays. Improvements in sensitivity of 10x for sub 1000eV X-rays have been achieved over conventional large area SDD’s.

This detector is used with accelerating voltages in the range 500eV to 3kV to maximise spatial resolution (10nm) and surface sensitivity (1nm). Under these conditions the X-ray lines available for characterisation are different from conventional micro-analysis. This unique approach makes use of previously poorly characterised lines including Li K lines, Al – Zn L lines and Sr – Lu M lines. These peaks can easily be detected, but are often overlapped due to the small energy difference in transitions in this part of the spectrum, despite the excellent detector resolution achieved (typically 35eV for Be K). Therefore these lines have been rigorously characterised with this detector with the aim of providing accurate peak deconvolution.

With its capability to provide elemental information with spatial and depth resolution close to that achieved by electron imaging, this new detector helps bridge the characterisation gaps between FEG-SEM and higher resolution TEM and surface science tools such as Auger and XPS.
CHARACTERIZATION OF ALUMINA FIBERS

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Alumina fibers have significant potential for applications due to their high elastic modulus, and thermal and chemical stability for use as the reinforcing phase in high temperature composites. Alpha-alumina (corundum) fibers, grown by a Vapor-Liquid-Solid (VLS) deposition process, were recently produced by Neoker, based in Spain, which is a spin-out company of the University of Santiago de Compostela. In the present study the fibers were characterized in terms of size distribution, fiber surface morphology, chemical composition and planar defect content using different electron microscopy techniques (SEM, TEM, EBSD and EDS).

The fiber length was between \( \sim 1 \ \mu m \) and 2.5 mm, with a broad distribution. SEM micrographs revealed fibers with different morphologies, such as dendritic or faceted fibers. Growth steps at the surfaces were observed. The growth direction was analyzed by TEM, and initial analysis showed that the fiber growth direction is the C-axis direction, and the growth steps at the surface are composed of the (1 0 -1 1) and (-1 0 1 2) planes. EBSD analysis of 23 fibers showed that more than 95\% of the fibers are single crystalline.
MAXIMIZING THE POTENTIAL OF LAYERED COMPOUNDS FOR HYDROGEN PRODUCTION

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The pursuit after transition metal dichalcogenides (TMDs) with thin edge morphologies began with the identification of edge sites as the catalytic location. In addition, first principle calculations showed that doping and alloying of TMDs can be used to modify their electronic properties. Traditional synthetic approaches for producing these materials limit the end product’s morphology or composition control. We used colloidal synthesis, which is rarely used for TMDs, and were able to produce thin edge nanoflowers of Mo(SₓSe₁₋ₓ)₂ alloys as well as Fe-doped MoS₂ and MoSe₂ using low temperature, controllable synthesis. Various analytical methods were used to determine the formation mechanism, composition and structure of the products as well as their electrochemical and photocatalytic performance. We were able to determine that for this specific approach, the formation mechanism is such that enables the production of homogeneous alloys, which is crucial for composition control. By controlling the alloying or dopant degrees, the electronic properties of the TMDs can be optimized for a variety of applications such as photocatalysis, optoelectronics, transistors and many others.
THE INFLUENCE OF SOLUTES ON GRAIN BOUNDARY MOBILITY IN ALUMINA

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The main goal of the present research is to study the influence of impurities, at concentrations below the solubility limit, on the evolving microstructure of alumina. The microstructure of a sintered body strongly depends on the composition of the powder used for the sintering process, where dopants and impurities are known to affect sintering rates and grain growth. In this study, the impurity content was varied by doping alumina with different amounts of CaO, below the solubility limit. The Ca concentration was determined by conducting fully standardized wavelength dispersive spectroscopy (WDS) and the change in grain boundary mobility as a function of the amount of dopant was characterized by quantitative analysis of grain size as a function of annealing time, using scanning electron microscopy. Unlike segregating dopants which reduce grain boundary mobility by solute-drag, CaO increases the rate of grain growth. Possible mechanisms by which CaO increases grain boundary mobility will be discussed.
COMPOUND ELECTROSTATIC-MAGNETIC FINAL LENS SEM IMAGING FOR INCREASED VERSATILITY

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Two types of columns are typically used in ultra-high resolution SEM to achieve excellent low voltage topographic and compositional imaging. Using magnetic immersion or electrostatic lenses for improved resolution allows angle-sensitive detection to tune in-lens contrast for a wide variety of materials. Recently, a newly developed compound electrostatic-magnetic final lens has been developed which combines magnetic and electrostatic techniques into a single versatile high resolution low-voltage SEM which enables topographic and compositional information tuning across a wide variety of materials.

Electrostatic and magnetic immersion columns have a couple of differences which define appropriate application space advantages for high resolution and contrast. Electrostatic columns generally have resolution in the range of 1.6 to 1.8nm at 1kV whereas magnetic immersion systems achieve ~1.4nm (without monochromator) or better (0.7nm with monochromated FEG source) at 1kV. Both systems have dedicated in-lens, in column detection options for acquiring high contrast signals. Generally for normal samples the magnetic immersion will produce the highest resolution at the lowest currents, however, some magnetic samples may present challenges due to the strong magnetic field. So working without the immersion lens activated is possible but leads to a slightly lower resolution for such samples.

This new SEM makes use of a combination of a magnetic final lens in the pole piece, a magnetic immersion lens and an electrostatic lens formed by the potential in the bottom of the column. The combination of these lenses focuses the primary electron beam to a very tight spot. The resolution of this new system is specified as 1.0 nm at 1 kV.

The contrast performance of the system benefits from the in-lens backscatter detector located at a position close to the sample where the detector produces high signal contrast due to its position and enables ultra-low beam current BSE imaging. In addition there are higher detectors in the lens and column to collect other low loss signals which travel further up the column. The possibility to sort electrons according to their energies and/or emission angles through detection is usually done by influencing the SE or BSE trajectories with exposure to an electric or magnetic field. With the new compound final lens both are available and the efficient detectors are capable of acquiring an energy-filtered BSE image by tuning the lens strength for selective detection of high- and low-loss BSEs. This enables precise materials contrast on the smallest particles and energy selection works as an effective charge filter, allowing the acquisition of charge-free images on insulating samples.

This new versatile SEM combines a range of technologies including the compound final lens, BSE filtering, and segmented detection all into one tool. The system delivers the resolution and contrast that allows materials researchers to capture the maximum amount of information from their sample, with the right detail, with the least amount of compromises.
RETRACTABLE PROJECTION LENS FOR STEM IN SEM; CONTINUING THE EVOLUTION OF LOW VOLTAGE STEM WITH THE HELIOS G4 FX

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STEM in SEM has been evolving for a number of years and the latest designs to achieve resolution down to 3Å at 30kV. Moving from the initial holders where the transmitted signal was reflected to the ETD detector once SE and BSE were created on the target below the sample, the introduction and refinement of solid-state STEM has been enhanced by the addition of a new retractable lower objective "projection" lens to concentrate signals. This innovation is allowing access to lower voltages and high resolution STEM and changing the limits of what we believed was possible.

The first inexpensive STEM in SEM were based on sample holders which operated by reflecting transmitted electrons towards the Everhart-Thornley detector for collection. This was accomplished by placing a slanted surface below the TEM grid and provided an inexpensive STEM in SEM solution. This was however low resolution and improvement came with the introduction of solid state detector crystals.

Solid State STEM revolutionized STEM in SEM by providing an easy to connect, sensitive and robust detector platform. The first iterations used the standard 2 segment BSE detector mounted under a TEM grid to collect BF/DF signal. This was quite inefficient and was replaced by an annular STEM diode specially made to collect signals - which included a DF ring placed outside of this area followed by segments in a ring to collect high angle dark-field signals which are scattered further during transmission. With the later diode designs additional DF rings were added to segment four angular DF signals outside of the BF area. Typical resolution values are under 0.8nm at 30KeV beam energy with this setup.

In order to increase the resolution, FEI incorporated an in-lens STEM mode into the DualBeam FIB/SEM. Since this extra lens must sit between the TEM grid and the STEM detector there were challenges for integration. To solve the challenges this lens has been incorporated into a retractable mechanism which is combined with a special TEM-type holder to more easily change the configuration. This modification is quite special and allows higher resolution double tilt mounting/manipulation for both thinning and analysis in SEM mode. With the new design, resolution below 3Å is routinely achievable in the Helios G4 FX which often eliminates the need to move samples to a TEM for critical dimension measurements.

The contrast and resolution on soft materials at extreme low voltages is opening the door to new analysis. Though this technology is only available on the dedicated Helios model, it is bringing performance of STEM in SEM to a completely new level and ideas for how to use and extend this new capability are multiplying.
IMPROVING THERMAL CONDUCTIVITY OF HYDROGENATED MAGNESIUM COMPACTS WITH THE AID OF CARBON NANOPARTICLES

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Magnesium is considered as one of the most attractive materials for hydrogen storage because of its high hydrogen storage capacity and abundance. However, slow hydrogen absorption/desorption kinetics, high hydride formation enthalpy and poor thermal conductivity of the hydride phase are the main drawbacks that prevent its use in hydrogen storage. The issues are interconnected: low thermal conductivity slows down the heat flow that influences the rate of hydrogen absorption/desorption.

In this work we synthesized solid porous compacts of pure Mg and Mg - 2 wt.% multi-wall carbon nanotubes (MWCNTs) composite processed by high energy ball-milling. We studied a correlation between the morphology and the thermal conductivity in the hydrogenated compacts which absorbed up to 80-90% of their maximum theoretical hydrogen storage capacity. It was found that although prolonged ball-milling leads to a partial destruction of the MWCNTs and formation of carbon nanoparticles, it has a positive effect on the thermal conductivity of the pellets. The role of carbonaceous derivatives of the MWCNTs in enhancing thermal conductivity of the pelletized compacts is discussed.

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A METHOD OF RELIABILITY ASSESSMENT OF EFUSE ROM (READ ONLY MEMORY) IN CMOS BASED ASIC MICRO CONTROLLERS

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Electrical Programmable Fuse (eFUSE) is a One-Time-Programmable (OTP) solution for System-on-Chip (SoC) Integration. Programming of an eFUSE ROM block (figure 1a) is normally done by driving relatively high current pulses through its submicron dimension fuse structures (figure 1b). The long term reliability of the eFUSE is obviously of great importance for the normal operation of the controller as well as the integrated component and system it is embedded in.

In this work we present a method of finding the optimized programming (fuse blowing) conditions for the effective and reliable functionality of the fuse block. This approach has already been successfully implemented for Seagull, one of the recent ASIC controllers designed at SanDisk Omer for the iNAND products.

Introduction:
The correct trimming of the voltage, as well as the resulting current and the pulse duration (figure 2) of the blowing pulse is critical for achieving an accurate and reliable code during operation of the product in the field. The problem is that programming pulse voltage and duration, as provided by the IP vendor, might not always be adequate for a specific design and sometimes a fuse that is detected as correctly programmed by the tester during the programming stage fails in the field causing malfunction of the customer’s device!

The conventional approach is to apply a long period high temperature stress (HTOL) on the burnt fuses and check if they retain their logic state. However, this is a time consuming approach which requires a large sample size and might result false alarms due to the statistical nature of this method!

Theoretical background:
In principle, each fuse element is composed of a high resistive poly Si line, covered by a lower resistance thin Silicide layer, through which the majority of the programing current flows. At spec programming conditions this current is supposed to cause an instantaneous peak of heat resulting burn out of the narrow section (“neck”) of the Poly Si line together with the Silicide film (figure 1b).

We found that if the electrical programming conditions are non-optimized the local heat generated is insufficient, so the silicide layer burns while the narrow poly line does not! However, the fuse might appear to be electrically “open” due to its relatively high resistivity, thus considered as a properly programmed fuse! The problem is that the thin silicide layer in this improperly burnt fuse might “reconnect” under high temperature operating conditions of the device in the field, resulting the programmed fuse to switch its state!

Details of proposed method and experimental results:
We propose to investigate the physical condition of the fuses using Scanning Electron Microscopy (SEM) and Focused Ion Beam (FIB) systems. Figure 3 shows a set of fuses that were programmed at various electrical programming conditions.
Since it is difficult to identify the fuse state from the top view a cross section of the fuse by the FIB is performed. X-section of a properly burnt fuse is shown in figure 4, showing the cut of the narrow section of the poly line while in figure 5 it is still connected!

**Summary:**
We present a quick and reliable method to verify the correct programming conditions of OTP eFUSEs. It is proposed to apply this approach, which was successfully implemented on the Seagull controller, during the qualification stage of each ASIC controller.
MICROSTRUCTURE EVOLUTION OF THE HAVAR ALLOY DURING COLD WORK

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Cobalt based alloys exhibit excellent corrosion resistant and mechanical properties. The mechanical properties were insensitively investigated while the hardening mechanism due to CW is not fully understood because of microstructure complexity and mixing of several hardening mechanisms. The present study was focused on the HAVAR Co based alloy, and the microstructure evolution due to cold rolled process at 20\%, 44\% and 85\% reduction of thickness. It was found that annealed state (reference state) is a solid solution with FCC crystallographic structure, equiaxed grains, very low dislocation density and few twins. At low degree of cold rolled process (up to 44\%), the microstructure contains higher dislocation density, large twins that contain sub-twins and stacking faults. The density of these faults increased as a function of cold rolling. At higher degree (85\%), the matrix contains very high dislocation density, twins, sub-twins and fine grains or broken grains which are described as mosaic structure of sub grains. The overall changes in the microstructure are the hardening mechanisms that are responsible from the increased of the yield stress from 400 MPa for the annealed state, up to 1900 MPa for the 85\% cold rolled degree.
THE GENERATION AND REGENERATION OF BIOLOGICAL WAX IN REGARDS TO SURFACE WETTABILITY ON PLANT LEAVES

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Nature’s unique solutions to problematic natural phenomena can serve as a source of bio-inspiration for material science.

The microstructure of plant surfaces; combining cuticle structures and epicuticular wax crystals in a hierarchal structure; serve multiple different functions such as transport barriers, surface wettability, anti-adhesive and self-cleaning, radiation protection, and many more. A systematic approach to the study of such natural structures, their evolution throughout the different stages of plant growth, and their mechanisms of damage repair and self-healing can greatly help our ability to mimic these properties and apply them to solving a variety of technical challenges.

Observation of the leaves, stems, thorns, and flowers of many different plant species has shown a variety of behaviors when in contact with water from extremely low wettability to high wettability. By studying the microstructures of the different parts of these plants, and the changes in the microstructure of their surfaces throughout the plant’s growth and after being damaged, we can learn about the wax crystal growth mechanisms and in so contribute to finding engineering solutions for self-cleaning and self-repairing super hydrophobic surfaces.
TEM STUDIES OF Ni AND Mn FULL CONCENTRATION GRADIENT MATERIALS AS CATHODES FOR Li ION BATTERIES

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In this work, nickel-rich, layered-structure LiNi\textsubscript{0.65}Co\textsubscript{0.08}Mn\textsubscript{0.27}O\textsubscript{2} cathode materials were synthesized and compared with materials of the same overall composition, but with a concentration gradient throughout the particles: the Ni concentration is higher at the center of the particles and lower at surface, while the Mn concentration is higher at the surface and lower at the center. The synthesis parameters of the co-precipitation method were optimized, with two different annealing protocols for the final products. Both generations of gradient materials provided superior capacity and rate capability than their respective non-gradient materials, at normal operating potentials and temperatures, e.g. 30 °C up to 4.3 V vs. Li.

TEM measurements were used for morphological and structural characterization of pristine (uncycled) and cycled electrodes. We explored the evolution of the gradient structure during cycling, comparing energy dispersive X-ray Spectroscopy (EDX) line scans in order to determine whether the particles’ concentration gradients are stable during cycling. Prolonged cycling, even at elevated temperatures, did not change the initial concentration profiles determined by the synthesis.

The layered-to-spinel (rhombohedral layered R3m to cubic spinel phase, Fd3m) transition is commonly observed in layered materials containing Mn & Ni during prolonged cycling, with Ni-rich layered compounds often showing slower spinel formation during cycling. TEM, electron diffraction studies showed that spinel phase formed on constant concentration particles upon electrodes cycling, but no spinel phase was detected in full concentration gradient particles from cycled electrodes.
TRIPLE BEAM MULTI-MODAL ANALYSIS OF LITHIUM ION BATTERIES

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Lithium ion batteries are a leading energy storage technology for electronic portable devices and hybrid electric vehicles. Simultaneous characterization of the structure, chemical composition and elemental distribution in Li-ion battery materials is an important step for understanding the relationship between transport of Li ion and structural effects on the one hand, and battery performance and its degradation on the other, as shown in [1].

We show here a multi-modal chemical analysis of Li-Ion batteries using a triplet of analytical beams combined within one device. A focused ion beam from a Xenon plasma source (P-FIB) was used for the preparation of fresh cross sections [2] and their immediate analysis using the integrated Time of Flight Secondary Ion Mass Spectroscopy (TOF-SIMS). The focused electron beam of a Scanning Electron Microscope (SEM) was used for imaging using various electron signals (SE, BSE) as well as energy dispersive x-ray microanalysis (EDX). In addition, the focused laser beam of a Confocal Raman microscope (CRM) was applied for high resolution Raman analysis. A Raman Integrated Scanning Electron (RISE) microscope [3] supplemented with TOF-SIMS in a single vacuum chamber is highly advantageous as chemical state mapping can be performed without exposing the highly reactive compounds of the Li-ion battery to an oxidizing atmosphere.

Figure 1- SEM Image of a cross section of a Li-ion battery made by plasma FIB, back-scattered electron signal (A). TOF-SIMS analysis –collected spectra (B) and a map of Li$^+$ ion distribution within the cross section (C). EDX analysis of the same area showing element distribution (D). Micro Raman imaging of a smaller area – spectra of different phases (E), distribution of these phases (F), overlay with white-light image from CRM (G).

3D SHAPING OF ELECTRON BEAMS

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A recent formulation for generating light beams with intensity and phase guided along 3D curves is implemented with electron beams. General design and fabrication methodologies, and potential applications are discussed.

Since commercial aberration-corrected electron microscopes were introduced nearly a decade and a half ago, and with the advance of fabrication technology, new venues for the manipulation of electron beams have introduced themselves. It is directly linked to research in computer-generated holograms, with roots nearly 70 years old in light optics. Thus, we have recently implemented the first computer-generated hologram by using sculpted phase masks for electrons [1]. We proceeded to demonstrate that these phase masks, much like in light-optics, have the potential to be used as cheap aberration correcting elements [2]. In a recent paper dealing with light-optics [3] a method for the encoding of nearly-arbitrary 3D curves is described.

In this work, we present preliminary results showing how binary amplitude computer-generated holograms are designed and fabricated. We will discuss several methods of fabrication, and present examples of masks yielding curves such as the knotted trefoil and Archimedean spiral, see Fig. 1. Potential applications for such beams have already been investigated in light optics, such as particle manipulation; in electron-optics, this is also a possibility, and one could envision synthesis of 3D nano-structures using this method.

![Fig. 1: Examples of 3D curves. (a) Measured and (b) simulated (Fresnel propagation) Archimedean Spiral, (c) measured and (d) theoretical curve of knotted trefoil. Images taken at an arbitrary section on the z-axis. Contrast and brightness altered for visibility.](image-url)


The kinetics of grain boundary (GB) motion can be determined experimentally by measuring the average grain size in samples annealed at different temperatures for different durations. In SrTiO$_3$ annealed under an oxidizing atmosphere, the GB mobility was found to decrease with an increase in temperature (in the temperature range of 1350-1425°C), deviating from the expected Arrhenius behavior \[ [i],[ii] \]. While GB mobility can be measured, the mechanism by which a GB moves has not yet been determined at the atomistic level in general polycrystalline systems. The present work focuses on the atomistic mechanism by which GBs migrate, using high resolution transmission electron microscopy (HRTEM) and SrTiO$_3$ as a model system.

Following the terrace ledge kink (TLK) model \[ [iii],[iv] \], GBs were described as stepped planes which move by step-motion along the boundary plane during grain growth. The concept of steps at GBs includes line defects; such that steps can have both a step and dislocation character (so called disconnections \[ [v] \]). The role of disconnections at GBs in GB motion is an extension to the previously described concept of surface steps which play a role in crystal growth \[ [vi] \].

In order to examine the role of disconnections at GBs, general GBs in polycrystalline SrTiO$_3$ and GBs between a single crystal diffusion bonded to polycrystalline SrTiO$_3$ were characterized using aberration corrected TEM and high angle annular dark field scanning electron microscopy (HAADF STEM). TEM and STEM were used to identify steps in order to correlate them to the grain growth mechanism, following the TLK and disconnections theories.

When orienting general GBs in SrTiO$_3$ (annealed under an oxidizing atmosphere) to the “edge-on” condition (in which the boundary plane is parallel to the electron beam direction), both steps and dislocations were visible, creating overall disconnections. The steps were found to be aligned mainly parallel to \{001\} and \{110\} type planes, regardless of the annealing temperature, annealing duration, cooling rate and orientation of the grains creating the boundary (see Figure 1) \[ [vii] \].

The dislocation component of the disconnections can be partially characterized using a geometric approach, and was found to have an edge component mainly parallel to the same crystallographic planes. The atomistic terminations along the boundaries were found to vary.

Motion of the steps parallel to \{001\} and \{110\} type planes was recorded during in-situ HRTEM experiments along surfaces of grains in polycrystalline SrTiO$_3$ annealed under an oxidizing atmosphere (vacuum). The steps consistently appeared along \{100\} and \{110\} planes regardless of the annealing temperature, indicating their significance in the grain growth mechanism, in agreement to the ex-situ results. The motion of such steps also appeared at the surface of shrinking grains.

Thus, the consistent appearance of certain types of steps along interfaces in SrTiO$_3$ annealed under an oxidizing atmosphere was noted. These steps appeared in both in-situ and ex-situ experiments, indicating their role in kinetic process such as grain and crystal growth.
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Figure 1: HRTEM micrograph of an edge-on general grain boundary in polycrystalline SrTiO$_3$ annealed at 1350°C for 10hr oxygen and furnace cooled. Nanometer length-scale steps and dislocations are visible along the boundary. The micrograph was acquired using a Cs of -5.7 μm, and Wiener filtered to remove noise.


HIERARCHICAL CRYSTALLIZATION OF MEDICINES FROM NANOMICELLES

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It is a long time that nature serves as a source of inspiration for the construction of useful materials and systems. Here, we present a simple, cost-effective and environmentally friendly bio-inspired approach for the creation of novel, hierarchical, porous drug polymorphs at nano and meso scales (Fig. 1). Our fabrication process utilizes the milk protein β-casein.

β-Casein is a 24 kDa amphiphilic and unstructured protein that possesses many properties facilitating its functionality in drug delivery systems, including strong self-association tendency into small core-shell micelles and excellent emulsification and stabilization abilities. We previously demonstrated the capability of β-casein micelles to efficiently encapsulate, stabilize in monomeric state, and deliver relatively high concentrations of hydrophobic drugs. Here, however, we suggest a new use of the drug-loaded protein carriers – as reactors for controlled creation of new semi-crystalline polymorphs (spherulites) of poorly water-soluble drugs. Our fabrication method involves first, encapsulation and stabilization of active agents within the hydrophobic core of the protein micelles. This is then followed by drug release and re-crystallization in the form of new polymorphs as a function of time, and in response to changes in environmental conditions. A proof of concept for the creation of the polymorphs is demonstrated with the desired drug celecoxib, a poorly water-soluble molecule used for the treatment of rheumatoid and osteo-arthritis, and a promising anticancer drug.

High-resolution scanning electron microscopy (HR-SEM) was our method of choice to study the particles structure and morphology at meso and nano scales. Cryogenic temperature transmission electron microscopy (cryo-TEM) and light microscopy were complementary tools for the structural analysis, and x-ray diffraction (XRD) was employed to explore the drug physical state. These analyses revealed novel polycrystalline structures with a spherulitic, highly porous morphology. The high surface area, relatively small dimensions and monodispersity of such particles are highly desired properties in drug design that modern technologies seek for. Furthermore, tuning of the environmental conditions allowed a good control over the fine morphological characteristics of the drug particles. E.g., increasing the driving force for crystallization resulted in particles larger in size, and a gradual transformation from fine and highly arranged morphology into rough, open and somehow disordered morphology.
Figure 1. Drug polycrystals with uniform, porous and branched morphology.

References:
MECHANISTIC INSIGHTS INTO CRYSTALLIZATION. PERYLENE DIIMIDE BASED ORGANIC NANOCRYSTALS IN AQUEOUS MEDIA

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The theory describing crystallization mechanism has been going through an important development over the last years. A significant amount of experiments and simulations that can not be interpreted by the dominating ‘Classical Nucleation Theory’ brought to the rise of alternative theories and descriptions; such as the ‘two-step nucleation’ theory\(^1\) and crystallization through attachment of a wide range of species-particles.\(^2\) Spectroscopic and structural data that is able to support and develop these emerging theories is still lacking, especially in the organic field. We are challenged to monitor the variety of phases and species that are transient and elusive, in order to make the crystallization theory more complete.

References

OPTICAL CHARACTERIZATION OF A SINGLE WS$_2$ NANOTUBE

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Nanostructures which retain both: surface plasmons (SP) and excitons; hold a great promise for the nanoscale integration of photonics and electronics in opto-electrical devices. Here nano-imaging was used in order to study the properties of SP and exciton in an individual WS$_2$ nanotube. The surface waves were detected and imaged in real space in the visible light range using a scattering-type scanning near-field optical microscope (s-SNOM) (Fig. 1). The standing wave appears with specific incident light polarization and is induced by interference between the tip-excited wave and its reflection from the nanotube. This interaction leads to periodic modulation of tip-scattered infrared radiation which then measured by the detector. It was noted that the standing wave’s wavelength is varying with the number of crystal layers in the inorganic nanotube (INT). The measured dispersion of waves is appearing to be governed by the INT diameter.

The WS$_2$ nanotubes are prepared in high temperature gas phase reaction which often resulted in strain related defects, mixture of phases and more. These structural variations are strongly affecting the optical properties of the INT. Using a balanced detection scheme together with acusto-optic-tunable filter (AOTF) and a supercontinuum laser source, the extinction spectrum of individual WS$_2$ nanotube was observed for the first time (Fig. 2). Furthermore, this method provides a unique tool for studying the influence of phase mixture and structural defects on the optical properties of a single WS$_2$ nanotube.
THE GEOMETRY AND MECHANICS OF SELF-ASSEMBLED CHIRAL NANOSTRUCTURE

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We quantitatively study the time-evolution of the self assembly from micelles of C12-β12 (N-α-lauryl-lysyl-aminolauryl-lysyl-amide) to closed nanotubes, passing through several types of intermediates. The process consists of the evolution of elongated fibrils into twisted ribbons, and later, into coiled helical ribbons.

Using the framework of “incompatible elasticity,” we model the self-assembled ribbon as a thin strip with a saddle-like intrinsic curvature. We provide quantitative predictions for the twisted-to-helical transition in this system, based on the experimental study of natural and synthetic macroscopic ribbons.

We find a good agreement between the theoretical predictions and the experimental measurements and suggest that similar modeling could be used for other chemical systems. In particular, it could be used for the engineering of synthetic self-shaping nanodevices.
PHASE TRANSFORMATIONS IN EQUIATOMIC Al-Co-Cr-Fe-Ni HIGH ENTROPY ALLOY

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High Entropy (HEA) or multicomponent alloys consist of four - six elements in equiatomic (or close to equiatomic) ratios [1]. In these systems, solid solution with simple crystallographic structures (e.g. Face Centered Cubic (FCC) or Base Centered Cubic (BCC)) is promoted instead of the formation of intermetallic compounds due to the high mixing entropy effect. HEA exhibit exceptionally high hardness, good wear and oxidation resistance [2]. These qualities make HEA attractive candidates for variety of engineering applications.

In the current work, equiatomic AlCoCrFeNi HEA was studied thoroughly. Previously, it was reported that AlCoCrFeNi solidifies in a dendritic regime, forming two areas: dendrite (DR) and interdendrite (ID) [3] differing in their microstructure. Understanding how such differences affect the properties of each are and the evolution of the microstructure upon heating is essential for the production of an homogenous alloy.

By analyzing the microstructure of the as cast AlCoCrFeNi HEA, we noted differences in the phase content between the DR and ID areas, fact that explains the different morphology and hardness measured in these areas. Investigation of this alloy after heat treatments (followed by quenching) performed at 850°C and 1200°C has shown that these variations dictate the evolution of different microstructures in the DR and ID areas. At 850°C sigma phase was formed in both areas but with a different morphology, along with the growth of the FCC phase. At 1200°C the microstructure consisted of FCC, BCC and ordered BCC, the same phases obtained in the as cast condition, yet with very complex morphology, requiring high-end microscopy methods for investigating their structure and chemical composition. Conclusions were drawn regarding phase transformations occurring in the AlCoCrFeNi system.

References:
Adiabatic shear banding (ASB) is a synonym for a unique dynamic uncontrolled failure mechanism. It implies a concentrated shear deformation mode that ultimately results in catastrophic failure after violent impacts or high-speed machining for example.

The leading paradigm is that a competition between strain (rate) hardening and thermal softening determines the onset of the failure. But more important is the fact that adiabatic shear is universally considered as an instability (material or structural), and therefore modeled on the premise of stability analyses. As an instability, ASB is hardly controllable or even predictable to some extent.

However, as opposed to this purely mechanistic approach, it has recently been shown that instead of thermal softening mechanisms, microstructural softening transformations such as dynamic recrystallization (DRX) are responsible for adiabatic shear failure. Those transformations are dictated by the stored energy of cold work, so that energy considerations can be used to macroscopically model the failure mechanism.

Yet, one question persists, namely how is the shear band formed? What are the initial mechanisms that will later lead to final failure? And most of all, is adiabatic shear failure an abrupt instability or rather a gradual transition as would be dictated by microstructural evolutions?

This paper reports fine scale microstructural characterizations that clearly show the gradual character of the phenomenon, best described as a nucleation and growth failure mechanism, and not as an abrupt instability as previously thought. These observations are coupled to a simple numerical model that illustrates them.
DAVID (MICHELANGELO) NONLINEAR METAMATERIALS FOR HOLOGRAPHY

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Here we demonstrate a nonlinear multi-layer metamaterial hologram. By using e-beam lithography we fabricate polarization-sensitive nonlinear hologram, where the phase information of an object is encoded in computer generated plasmonic nanoantennas in the multi-layer metamaterial. By illumination the sample with a polarized infrared laser, the image of the object is reconstructed at a frequency that is the third-harmonic of the incoming beam. This is obtained by the nonlinear process of Third Harmonic Generation.

-Illustration of the concept of a multilayer nonlinear metamaterial hologram.

-Non Linear formed images of a three-layer hologram displaying the word MET (indicating the name of the Metropolitan Museum in New York). Each character is formed at a different focal distance corresponding to different light polarization (0, 45 and 90 degrees).

-Chinese characters for “Peace and harmony”. Each image of character is formed at different focal distance for different light polarization.

-SEM image of the top layer of Multilayer device.

-Higher magnification of (d). Layers underneath can be observed as well. Scale bar is 200nm.

**Fabrication process**

The samples are prepared by multilayer e-beam lithography on a borosilicate glass substrate. To avoid electron charging on the substrate during SEM, 3 nm thick chromium (Cr) layer was initially deposited on the substrate by e-beam evaporation. A 180 nm silica layer was grown by Plasma Enhanced Chemical Vapor Deposition (PECVD) on top of the Cr film. Then the desired design of 30 nm thick antennas is patterned by e-beam lithography. In order to fabricate a nearly flat patterned surface of gold antennas, the silica pattern is etched via Inductively Coupled Plasma (ICP) after developing the PMMA resist. Then a 2 nm thick chromium adhesion layer and a 30 nm thick gold layer were deposited on the patterns by e-beam evaporation. The resist is then lifted off in acetone in an ultrasonic bath, leaving a gold antenna buried in the SiO\(_2\) layer. A 180 nm thick silica layer is deposited on the metallic pattern by PECVD and serves as a dielectric spacer between two adjacent active nanoantenna layers. The entire fabrication process is repeated for the additional layers.
OUR GROUP INVESTIGATED THE ELECTRICAL AND MECHANICAL PROPERTIES OF THE P(VDF-TRFE-CFE) ELECTRO ACTIVE POLYMER AND DEVELOPED CUSTOM PROCESSING TOWARDS INTEGRATING WITH CONVENTIONAL FABRICATION TECHNOLOGIES

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Process Characterization

Vertical side walls, residue control

Precise, non destructive sub-micron thickness control with Spectroscopic Ellipsometry

Cross sections
PEDOT/PVDF Grids – Under Development

P(VDF-TRFE-CFE)

Down to 2um feature size –
Optical Lithography

PEDOT:PSS

Sub-Micron feature sizes – E-Beam/Nano Imprint Lithography
A PARALLEL AND SIMPLE FABRICATION OF MULTIPLE PHOTODETECTORS BASED ON GUIDED GROWTH OF ZnSe NANOWIRES

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The parallel fabrication of multiple photodetectors is based on the guided growth of ZnSe nanowires. In the first step we choose the starting point of the nanowires by designing a photolithography mask in order to create a pattern of a gold catalyst. This step is done with standard photolithography followed with electron-beam-deposition of a thin gold film and a lift off process.

In the second step guided nanowires are grown from the gold catalyst in a standard chemical-vapor-deposition growth.

In the third and last step we exploit our a-priori knowledge of the growth direction of the nanowires in order to design a second photolithography mask that defines an electrode pattern that is compatible to the catalyst pattern of the guided nanowires. Now followed by a standard photolithography process, Ti/Au (10 nm/50 nm) electrodes were deposited over the guided Nanowires using electron-beam-deposition to form photodetectors.
We design and fabricate an electrical biasing TEM holder and samples, for electron-optical experiments. There are many variants of commercial TEM sample holders today, for *in-situ* experiments. Some include heating or cryo capabilities, liquid cell, nanoindentation, and electrical biasing. These, however, are meant for materials or biological research. We are in the process of fabricating a specialized electrical biasing holder, with the aim of modulating the electron beam *in-situ*. Such a modulated beam could be used as a tool for specialized research in electron optics or fabrication of nano elements. We begin with an old heating holder, which has the benefit of being hollow for putting conductive wiring through. The tip is replaced with a CAD-planned new tip, fabricated in a university’s mechanical workshop. I then use a femtosecond laser micromachining machine to carve a rectangular depression into the tip, which would accommodate a replaceable chip (a). At the same time, a vacuum-friendly printed circuit board is also laser-milled to produce electrical copper contacts leading to the chip (a-inset). To fabricate the chips, I use a standard photolithography process, starting with a commercial silicon wafer coated with 200nm of silicon nitride. The four contacts are gold-evaporated, whereafter the wafer is diced and the silicon is chemically etched to form a thin silicon-nitride membrane (b). When put together, the device is complete, see (c) and (d). With many chips now available, I use electron beam lithography to fabricate small conductive structures on the membrane itself, e.g. (e) and (f). In (f), for example, the membrane is coated with aluminum, and the visibly marked lines are where the aluminum has been etched; this results in four areas, each controlled with a different electrical contact. The snake-like curve is intended to be used as a dynamic Bragg grating for electrons, giving the ability to control diffraction efficiency with variable voltage.
THE MICROTUBULES HIGHWAY MESH OF THE CELLULAR MILIEU

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This image shows the layout of the cell’s microtubules, the main infrastructure for translocation of organelles within the dense environment of the amoeba. This image was taken using super resolution microscopy that allows sub-diffraction limit resolution of the microtubule filaments. Green represents microtubules surrounding the purple heart of the amoeba (nucleus).
EXPERIMENTAL ELECTRON RONCHIGRAM AT THE EDGE OF A BN 001 CRYSTAL SHOWING KIKUCHI LINES WHEN THE PROBE IS WELL UNDERFOCUSED

Vladimir Ezersky
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Inspired by works of Marc Chagall - the quintessential Jewish artist of the twentieth century

What I liked best was geometry.
At that I was unbeatable.
Lines, angles, triangles, squares carried me far away to enchanting horizons.

Marc Chagall

Experimental electron Ronchigram at the edge of a BN <001> crystal showing Kikuchi lines when the probe is well underfocused.

Dr. Vladimir Ezersky
Ilse Katz Institute for Nanoscale Science & Technology
Ben-Gurion University of the Negev, Beer-Sheva
DO YOU WANT TO BUILD A NANO SNOWMAN?

Youngjin Jang
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The image shows two quantum dots attached by chance. Quantum dots are SnTe/CdTe nanocrystals prepared by a colloidal synthesis. This image was obtained with a FEI Titan transmission electron microscope in Technion. The TEM sample was prepared by using a spray method proposed by Dr. Yaron Kauffmann and coworkers.
CARDIOMYOCYTE SHOWING COMPLEX SARCOMERIC PROTEIN NETWORK

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Cardiomyocyte isolated from adult rat heart, displaying an intricate mesh-like network of sarcomeric proteins. Immunostaining - alpha-sarcomeric actinin (green pseudocolor); troponin-I (red pseudocolor); DAPI (blue). Image was captured at the Technion Biomedical Core Facility, using a Zeiss LSM 510 confocal microscope. Objective used was a EC Plan Apochromat x63/1.4NA. Image shown is a max intensity projection of 30 z-stack slices.
MICROSCOPIC WONDERLAND

Ilana Shtein
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A trichome (leaf hair) on Arabidopsis thaliana leaf. Note the little bumps on the surface of the trichome and the complex multicellular base.
EVOLUTION IN THE MERISTEM

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1. CREATION
2. LOVE
3. NEXT GENERATION

What is it? These images are of the apical meristems of Arabidopsis mutant seedling. These mutants are affected in cell growth parameters in the shoot apical meristem. Bar=50µm.
ACTING FILAMENTS OF THE GIANT AMOEBA

Liran Ben Yaakov
Structural Biology, Weizmann Institute of Science, Rehovot, Israel

This image shows the 3D structure of the Amoeba’s pseudopodia being used for its motility and ingesting of nutrients. This image was taken using super resolution microscopy that allows sub-diffraction limit resolution of the actin filaments that form the pseudopodia unique and dynamic structure. Color gradient represents the depth of actin within the different pseudopodia protrusions at the tip of the Amoeba.
LITTLE SHOP OF HORRORS (CARNIVOROUS PLANT)

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This Carnivorous plant was created in chemical bath deposition (CBD) method. CBD is used as a simple and cost-effective route to study chemical epitaxy of cadmium chalcogenide thin films on GaAs substrates. Little Shop of Horrors is a famous rock musical composed in the early 1960s in which an infamous carnivorous plant participate.

The Image was taken using a JEOL JSM-7400F high-resolution scanning electron microscope equipped with field emission gun (FEG)-SEM. Image enhancement was conducted by designer, Alon Gruss.
Solid state dewetting (also known as agglomeration) of thin films is a process in which a substrate beneath a thin film is exposed and the film itself transforms into an array of isolated particles at the temperatures below the melting point of the film material.

Here thin Au thin layer were dewetted at 700°C for 5 hours. Among the round islands that were formed, an island which resembles to the great britain island was also observed.
This SEM micrograph image shows Electrical Fuse (eFUSE) bits, on silicon substrate. Each "glass" structure is one bit which composed with a poly silicon layer on top of the silicon substrate.

eFUSE is a One-Time-Programmable (OTP) solution for System-on-Chip (SoC) Integration. Programming of an eFUSE block is normally done by driving relatively high current pulses through its submicron dimension fuse structures (narrow section). The correct trimming of the voltage, as well as the resulting current and the pulse duration of the blowing electrical pulse is critical for achieving an accurate and reliable code during operation of the product in the field.

Programmed eFuses are electrically high resistance due to the short between two sides of the narrow section, while unprogrammed eFuses remain in a low resistance state due to the disconnection between the two parts of the fuse.

* This image captured with Hitachi S-4700 SEM.
BIG OR SMALL THEY ALL HAVE POWERS, ROSES, DAISIES OR NANOFLOWERS

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Presented herein is HRSEM image of Mo(S\textsubscript{x}Se\textsubscript{1-x})\textsubscript{2} alloy nanoflower produced by colloidal synthesis. The nanoflowers are produced for photocatalytic hydrogen production. The morphology was designed to expose multiple edges, resulting in the nanoflower appearance, since hydrogen is catalytically generated on the alloys’ edges.
REVERSE CONNECTION

Maria Koifman Khristosov
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This image shows nanoporous gold single crystals. These gold particles were obtained due to eutectic decomposition of gold and germanium. The porous gold received this way is a single crystal. The image was taken with a high resolution scanning electron microscope, Zeiss Ultra-Plus FEG-SEM. Scale bar - 1 micron.
SCIENTIFIC VOLCANO (IN THE NIGHT)

Vladimir Yu. Kolosov  
Physics Dept., Institute of Natural Sciences, Ural Federal University, Ekaterinburg, Russia

Dark field (Hollow-cone) image of thin-film microcrystal (α-Fe2O3) in transmission electron microscope. Unusual “transrotational”* crystal has strong internal bending of the crystal lattice planes of complex geometry revealed by the analysis of bend-contour patterns presented.

HAMANTASHEN

Hadar Nahor, Wayne D. Kaplan
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Left: A secondary electron HRSEM micrograph of a single-crystal Ni particle on a (111) YSZ substrate. The Ni particle is oriented with the (111) plane parallel to the substrate surface and is surrounded by grains of a second phase containing mostly Cr and O.

In this work agglomeration of a Cr-doped Ni film ("solid state dewetting") on YSZ substrates was carried out at 800 °C, in Ar/H2, for 72 hr to form equilibrated Ni(Cr)-YSZ interfaces. During Ni agglomeration, Cr segregated to the Ni surface and reacted with oxygen from the environment.

Right: A Nikon S8200 micrograph of a hamantash on a napkin. Hamantash is a filled-pocket cookie or pastry recognizable for its triangular shape, usually associated with the Jewish holiday of Purim. The shape is achieved by folding in the sides of a circular piece of dough, with a filling placed in the center.

In this case the dough is made of butter, sugar and flour and the filling is dates spread. The baking was carried out at 180 °C, in air, for 10 min.
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