FOCAL SERIES RECONSTRUCTION FOR LOW-DOSE TEM IMAGES OF ORGANIC CRYSTALS

Idan Biran

Faculty of Chemistry, Weizmann Institute of Science, Israel

The study of crystals, crystallization, is a well-established topic. It covers many of the life science fields, and its importance varies from understanding nanoparticles arrays[1] to protein formations,[2] and even drug engineering through molecular design.[3] One of the most popular methods for direct imaging of crystal formation, defects, ext., is transmission electron microscopy (TEM). It is most abundant in inorganic materials, since the electron dose is not as limiting as in organic materials. While challenging, high resolution electron microscopy of organic material is possible. [4–9] Earlier studies on several phthalocyanine derivatives[10–18] addressed the need to reveal organic crystal defects, polymorphs and grain boundaries, but with outdated equipment and technology, if compared to our days. The recent resolution revolution[19,20] has brought high, less than two angstrom resolutions in single particle reconstruction of identical protein structures. It is mainly due to the stability of the new electron microscopes and novel direct electron detectors.[21,22] Real-space images of organic crystals and their aperiodic features obtained by a modern cryo-electron microscope potentially show the detailed structure, but high resolution details still remain hidden because strong defocus conditions need to be applied to produce contrast at low dose. Aberration correctors that are successful in materials science target at sub-Angstrom resolution while effectively diminishing the contrast at lower frequencies, therefore are not dose efficient when it comes to materials that damage fast and require cryo-microscopy.

We used phase retrieval by focal series reconstruction (FSR) developed originally for inorganic samples[23,24] and adapted the method for fast series recording of hundreds of frames within few seconds on a direct-electron detector, all at low dose conditions in cryogenic temperatures. Practically, the objective lens focus ramps fast while the direct electron detector acquires a stack of images at a high duty cycle. For the reconstruction of the exit-plane wavefunction we implemented relevant parts of a Python code, eventually allowing to retrieve aberration-fixed **in-focus phase images of small organic crystals** with a resolution up to the information limit of the microscope. This code, to our best knowledge, is the first one operating on sparse low dose images with less than 1 e^-/pixel and later frames, aligns with sub-pixel accuracy, and successfully reconstructs the wavefunction.

The application examples in the talk will show that our work enabled **high-resolution real-space crystallography** on individual organic nanocrystals, and imaging of crystal defects, grain boundaries and

sub-molecular features such as aromatic rings with 1.6 Å resolution. The impact is evident for devices based on thin layers of organic crystals, for example membranes[25] and field-effect transistors[26]. In those devices the organic crystals can be only few nanometers in size, and therefore with different properties than if they were in a larger bulk.[27]

FSR for organics represents a general direct method for structural analysis of organic matter with near-atomic resolution, enabling to elucidate unknown structures at the nanoscale.

References

[1] S. Mann, *Nat. Mater.* **2009**, *8*, 781–792.
[2] P. G. Vekilov, *Prog. Cryst. Growth Charact. Mater.* **2016**, *62*, 136–154.
[3] J. D. Rimer, Z. An, Z. Zhu, M. H. Lee, D. S. Goldfarb, J. A. Wesson, M. D. Ward, *Science (80-.).* **2010**, *330*, 337–341.
[4] D. Zhang, Y. Zhu, L. Liu, X. Ying, C. E. Hsiung, R. Sougrat, K. Li, Y. Han, *Science (80-.).* **2018**, *359*, 675–679.
[5] D. S. Sholl, R. P. Lively, *J. Phys. Chem. Lett.* **2015**, *6*, 3437–3444.
[6] X. Gong, K. Gnanasekaran, Z. Chen, L. Robison, M. C. Wasson, K. C. Bentz, S. M. Cohen, O. K. Farha, N. C. Gianneschi, *J. Am. Chem. Soc.* **2020**, *142*, 17224–17235.
[7] Y. Peng, Y. Huang, Y. Zhu, B. Chen, L. Wang, Z. Lai, Z. Zhang, M. Zhao, C. Tan, N. Yang, F. Shao, Y. Han, H. Zhang, *J. Am. Chem. Soc.* **2017**, *139*, 8698–8704.
[8] X. Gao, Y. Zhu, D. Yi, J. Zhou, S. Zhang, C. Yin, F. Ding, S. Zhang, X. Yi, J. Wang, L. Tong, Y. Han, Z. Liu, J. Zhang, *Sci. Adv.* **2018**, *4*, 1–8.
[9] D. C. Martin, J. Chen, J. Yang, L. F. Drummy, C. Kübel, *J. Polym. Sci. Part B Polym. Phys.* **2005**, *43*, 1749–1778.
[10] J. R. Fryer, *J. Porphyr. Phthalocyanines* **1999**, *3*, 672–678.
[11] J. R. Fryer, *J. Electron Microsc. Tech.* **1989**, *11*, 310–325.
[12] J. R. Fryer, F. Holland, *Proc. R. Soc. London, Ser. A Math. Phys. Sci.* **1984**, *393*, 353–369.
[13] J. R. Fryer, *Mol. Cryst. Liq. Cryst.* **1986**, *137*, 49–65.
[14] D. J. Smith, J. R. Fryer, R. A. Camps, *Ultramicroscopy* **1986**, *19*, 279–297.
[15] T. Kobayashi, Y. Fujiyoshi, F. Iwatsu, N. Uyeda, *Acta Crystallogr. Sect. A* **1981**, *37*, 692–697.
[16] J. R. Fryer, D. J. Smith, *J. Microsc.* **1986**, *141*, 3–9.
[17] T. Kobayashi, S. Isoda, *J. Mater. Chem.* **1993**, *3*, 1–14.
[18] T. Kobayashi, Y. Fujiyoshi, N. Uyeda, *J. Cryst. Growth* **1983**, *65*, 511–517.
[19] W. Kühlbrandt, *Science (80-.).* **2014**, *343*, 1443–1444.
[20] D. Elmlund, H. Elmlund, *Annu. Rev. Biochem.* **2015**, *84*, 499–517.
[21] G. McMullan, A. R. Faruqi, R. Henderson, *Direct Electron Detectors*, Elsevier Inc., **2016**.
[22] A. R. Faruqi, G. McMullan, *Q. Rev. Biophys.* **2011**, *44*, 357–390.
[23] T. Duden, A. Thust, C. Kumpf, F. S. Tautz, *Microsc. Microanal.* **2014**, *20*, 968–973.
[24] A. Thust, W. M. J. Coene, M. Op De Beeck, D. Van Dyck, *Ultramicroscopy* **1996**, *64*, 211–230.
[25] T. Wolf, A. Niazov-Elkan, X. Sui, H. Weissman, I. Bronshtein, M. Raphael, H. D. Wagner, B. Rybtchinski, *J. Am. Chem. Soc.* **2018**, *140*, 4761–4764.
[26] N. Stingelin-Stutzmann, E. Smits, H. Wondergem, C. Tanase, P. Blom, P. Smith, D. De Leeuw, *Nat. Mater.* **2005**, *4*, 601–606.
[27] A. A. Benzerga, N. F. Shaver, *Scr. Mater.* **2006**, *54*, 1937–1941.