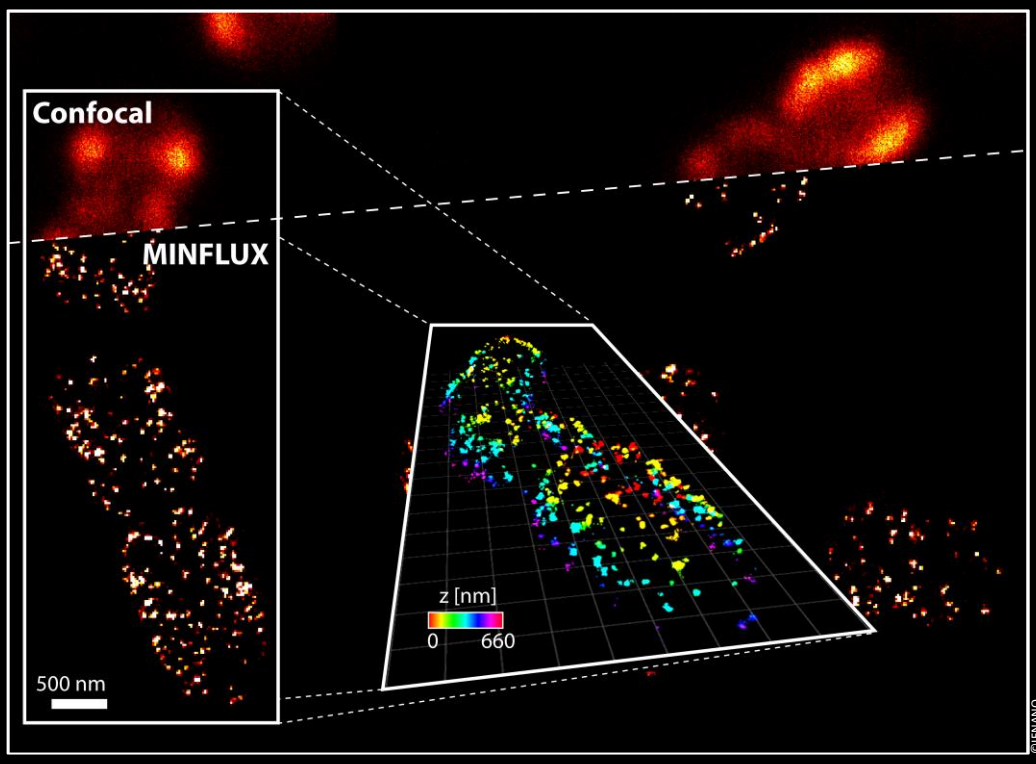
Open session within the framework of the NanoVIB Horizon 2020 project

Near-IR Nanoscopy and Vibrational Microscopy

16th May 2023, FB54, Albanova University Center, Stockholm
and on Zoom at https://kth-se.zoom.us/j/62429164481



13:00 - 13:05 : Welcome, Introduction and brief project presentation
Jerker Widengren, *KTH Royal Institute of Technology, Stockholm*

13:05 - 13:55 : MINFLUX nanoscopy and related matters
Stefan W. Hell, *Max Planck Institute for Multidisciplinary Sciences, Göttingen & Max Planck Institute for Medical Research, Heidelberg*

Short break

14:00 - 15:00 : Next generation super-resolution MINFLUX platform
Andreas Schönle, *Abberior Instruments GmbH, Göttingen*
Single photon counting detector arrays
Michel Antolovic, *PI Imaging Technology SA, Lausanne*
Lasers for Stimulated Raman Scattering (SRS) imaging
Ingo Rimke, *Angewandte Physik & Elektronik (APE) GmbH, Berlin*
Multimodal instrument integration
Alexander Egner, Institut für Nanophotonik (*IFNANO), Göttingen*
Fluorophore photophysics and imaging procedures
Jerker Widengren, *KTH Royal Institute of Technology, Stockholm*
Biomedical perspectives and lead application
Birgitta Henriques-Normark, *Karolinska Institutet, Stockholm*

Read more at:
https://www.nanovib.eu/

MINFLUX nanoscopy and related matters

Stefan W. Hell

Max Planck Institute for Multidisciplinary Sciences, Göttingen &
Max Planck Institute for Medical Research, Heidelberg

I will show how an in-depth description of the basic principles of diffraction-unlimited fluorescence microscopy (nanoscopy) [1-3] has spawned a new powerful superresolution concept, namely MINFLUX nanoscopy [4]. MINFLUX utilizes a local excitation intensity minimum (of a doughnut or a standing wave) that is targeted like a probe in order to localize the fluorescent molecule to be registered. In combination with single-molecule switching for sequential registration, MINFLUX [4-7] has obtained the ultimate (super)resolution: the size of a molecule. MINFLUX nanoscopy, providing 1–3 nanometer resolution in fixed and living cells, is presently being established for routine fluorescence imaging at the highest, molecular-size resolution levels. Relying on fewer detected photons than popular camera-based localization, MINFLUX and related MINSTED [8,9] nanoscopies are poised to open a new chapter in the imaging of protein complexes and distributions in fixed and living cells. MINFLUX is also set to transform the single-molecule analysis of dynamic processes, as already demonstrated by tracking in detail the unhindered stepping of the motor protein kinesin-1 on microtubules at up to physiological ATP concentrations [10], and providing answers to longstanding questions with respect to the kinesin-1 mechanochemical cycle.

[1] Hell, S.W., Wichmann, J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. **Opt. Lett.** 19, 780-782 (1994).

[2] Hell, S.W. Far-Field Optical Nanoscopy. **Science** 316, 1153-1158 (2007).

[3] Hell, S.W. Microscopy and its focal switch. **Nat. Methods** 6, 24-32 (2009).

[4] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S.W. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. **Science** 355, 606-612 (2017).

[5] Eilers, Y., Ta, H., Gwosch, K. C., Balzarotti, F., Hell, S. W. MINFLUX monitors rapid molecular jumps with superior spatiotemporal resolution. **PNAS** 115, 6117-6122 (2018).

[6] Gwosch, K. C., Pape, J. K., Balzarotti, F., Hoess, P., Ellenberg, J., Ries, J., Hell, S. W. MINFLUX nanoscopy delivers 3D multicolor nanometer resolution in cells. **Nat. Methods** 17, 217–224 (2020).

[7] Schmidt, R., Weihs, T., Wurm, C. A., Jansen, I., Rehman, J., Sahl, S. J., Hell, S. W. (2021) MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. **Nat. Commun.** 12:1478.

[8] Weber, M., Leutenegger, M., Stoldt, S., Jakobs, S., Mihaila, T. S., Butkevich, A. N., Hell, S. W. MINSTED fluorescence localization and nanoscopy. **Nat. Photon.** 15, 361-366 (2021).

[9] Weber, M., von der Emde, H., Leutenegger, M., Gunkel, P., Sambandan, S., Khan, T. A., Keller-Findeisen, J., Cordes, V. C., Hell, S.W. MINSTED nanoscopy enters the Ångström localization range. **Nat. Biotechnol.**, 41, 569-576 (2023).

[10] Wolff, J.O., Scheiderer, L., Engehard, T., Engelhardt, J., Matthias, J., Hell, S.W. MINFLUX dissects the unimpeded walking of kinesin-1 **Science**, 379, 1004-1010 (2023).