Molecule-scale resolution and dynamics in fluorescence microscopy

Stefan W. Hell & co-workers

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

I will discuss MINFLUX [1-4], a recent molecular localization and superresolution method that has reached Angström localization precision and resolution of the size of a fluorophore molecule. MINFLUX and the related MINSTED concept [5,6] are being established for routine applications in cell and molecular biology, structural biology and neuroscience. Relying on much fewer fluorescence photons than the widely used camera-based localization methods, these techniques are poised to characterize dynamic processes of single proteins, as demonstrated by tracking the nanometer conformational changes of the motor proteins kinesin-1 [7] and dynein in living cells [8]. MINFLUX has also been demonstrated to measure intramolecular distances with Angström precision, providing a precise and reliable alternative to FRET [9]. Harnessing confocal detection, MINFLUX also provides nanometer-range resolution deeper down in layers of cells and (mildly) scattering tissue [10]. Finally, I will show an arguably surprising ability of MINFLUX to separate individual identical fluorophores without sequential ON/OFF switching or activation of fluorescence. Thus, the simultaneous, uninterrupted, nanometer-scale tracking and imaging of multiple, identical (same-color) fluorophores becomes possible for the first time [11]. This novel superresolution principle should allow MINFLUX to reveal the conformational changes of individual proteins in their native environment.

[1] 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).

[2] 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).

[3] 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).

[4] 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.

[5] 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).

[6] 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).

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

[8] Schleske, J. M., Hubrich, J., Wirth, J. O., D'Este, E., Engelhardt, J., Hell, S. W. MINFLUX reveals dynein stepping in live neurons. **PNAS** 121, e2412241121 (2024).

[9] Sahl, S. J., Matthias, J., Inamdar, K., Weber, M., Khan, T. A., Brüser, C., Jakobs, S., Becker, S., Griesinger, C., Broichhagen, J., Hell, S. W. Direct optical measurement of intramolecular distances with angstrom precision. **Science** 386, 180-187 (2024).

[10] Moosmayer, T., Kiszka, K. A., Pape, J. K., Leutenegger, M., Steffens, H., Grant, S. G. N., Sahl, S. J., Hell, S. W. MINFLUX fluorescence nanoscopy in biological tissue. **PNAS** 121, e2422020121 (2024).

[11] Hensel, T. A., Wirth, J. O., Schwarz, O. L. Hell, S. W. Diffraction minima resolve point scatterers at few hundredths of the wavelength. **Nat. Phys.** https://doi.org/10.1038/s41567-024-02760-1 (2025).