LocalizeU

A small applet to visualize the build-up of localization microscopy images from single emitter images. Enjoy this "infographics".

Contact: Bernd Rieger (b.rieger@tudelft.nl)

Background on Localization Microscopy:

Imaging beyond the diffraction limit via a set of techniques nowadays termed localization microscopy has seen a sharp rise after the initial works around 2006; most notably methods introduced as (fluorescence) photoactivated localization microscopy (PALM) (Betzig, Hess) and stochastic optical reconstruction microscopy (STORM) (Rust). The common idea to achieve imaging below the diffraction limit in the optical far field is to localize single stochastically activated fluorescent molecules. These molecules are switched between a fluorescent on-state and a non-fluorescent off-state. The on-state molecules form a sparse subset of all molecules such that only one is active in a region the size of the size on the order of the diffraction limit. The positions of these emitting molecules are estimated, after which they return to the off-state and other molecules are activated and localized until all molecules have been imaged. Essential to this process is the localization of single fluorescent molecules, hence the common name for the techniques. The high resolution capability of these techniques follows from the precision with which the positions of the molecules can be estimated, which is much better than the diffraction limit (Ober, Smith) . Typically a fit of a simple Gaussian function is sufficient to approximate the complicated exact point-spread function (Stallinga2010). The localization precision is on the order of width of the point-spread function (PSF) of the optical system divided by the square root of the number of recorded emission photons (Mortensen, Stallinga2012). Typically, hundreds or thousands of photons can be recorded and with the width of the PSF ~250 nm this results in commonly achieved localization precisions on the order of tens of nanometers, although significantly smaller values in the range of nanometers have been reported (Pertsinidis, Vaughan, Xu). In comparison, Abbe's diffraction limit is given by half the wavelength divided by the half opening angle of the objective lens ~ 200 nm. This superior precision is what makes localization microscopy images crisper and sharper than regular fluorescent images and explains the widespread use of the technique nowadays. The final resolution in these kind of images is, however, not only dependent on the localization precision but also on the density of fluorescent emitters and even the imaged structure itself (Nieuwenhuizen). Even now, more and more flavors of localization based microscopy techniques are introduced.

Introductory reading:

- S.W. Hell. Far-field optical nanoscopy. Science, 316:1153-1158, 2007.
- S.W. Hell. Microscopy and its focal switch. Nature Methods, 6:24-32, 2009.
- T. Klein, S. Proppert, and M. Sauer. Eight years of single-molecule localization microscopy. Histochem Cell Biology, 2014.

Literature:

- E. Betzig, G.H. Patterson, R. Sougrat, O.W. Lindwasser, S. Olenych, J.S. Bonifacino, M.W. Davidson, J. Lippincott-Schwartz, and H.F. Hess. Imaging intracellular fluorescent proteins at nanometer resolution. Science, 313:1643-1645, 2006.
- S.T. Hess, T.P.K. Girirajan, and M.D. Mason. Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. Biophysical Journal, 91(11):4258-4272, 2006.
- K.I. Mortensen, L.S. Churchman, J.A. Spudich, and H. Flyvbjerg. Optimized localization analysis for single-molecule tracking and super-resolution microscopy. Nature Methods, 7(5):377-384, 2010.
- R.P.J. Nieuwenhuizen, K.A. Lidke, M. Bates, D. Leyton Puig, D. Grünwald, S. Stallinga, and B. Rieger. Measuring image resolution in optical nanoscopy. Nature Methods, 10:557-562, 2013.
- R.J. Ober, S. Ram, and S.E.Ward. Localization accuracy in single-molecule microscopy. Biophysical Journal, 86(2):1185-1200, 2004.
- A. Pertsinidis, Y. Zhang, and S. Chu. Subnanometre single-molecule localization, registration and distance measurements. Nature, 466:647-651, 2010.
- M.J. Rust, M. Bates, and X.W. Zhuang. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nature Methods, 3(10):793-795, 2006.
- S. Stallinga and B. Rieger. Accuracy of the Gaussian point spread function model in 2D localization microscopy. Optics Express, 18(24):24461-24476, 2010.
- S. Stallinga and B. Rieger. The effect of background on localization uncertainty in single emitter imaging. In IEEE International Symposium on Biomedical Imaging, pages 988-991, Barcelona, Spain, May 2-5 2012.
- C.S. Smith, N. Joseph, B. Rieger, and K.A. Lidke. Fast, single-molecule localization that achieves theoretically minimum uncertainty. Nature Methods, 7(5):373-375, 2010.
- J.C. Vaughan, S. Jia, and X. Zhuang. Ultrabright photoactivatable fluorophores created by reductive caging. Nature Methods, 9(12):1181-1184, 2012.
- K. Xu, G. Zhong, and X. Zhuang. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science, 339:452-456, 2013

ImPhys

Quantitative Imaging Group
Department of Imaging Physics
Delft University of Technology

TU Delft