IOPscience iopscience.iop.org

Home Search Collections Journals About Contact us My IOPscience

Super-resolution fluorescent methods: where next for super-resolution?

This content has been downloaded from IOPscience. Please scroll down to see the full text.

2015 Methods Appl. Fluoresc. 3 030201

(http://iopscience.iop.org/2050-6120/3/3/030201)

View the table of contents for this issue, or go to the journal homepage for more

Download details:

IP Address: 86.7.160.234
This content was downloaded on 16/08/2015 at 08:47

Please note that terms and conditions apply.

Methods and Applications in Fluorescence

RECEIVED
24 June 2015

REVISED

ACCEPTED FOR PUBLICATION
24 June 2015

PUBLISHED
13 August 2015

EDITORIAL

Super-resolution fluorescent methods: where next for super-resolution?

Alex Knight, Clemens F Kaminski and Markus Sauer
Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB2 3RA, UK
E-mail: cfk23@cam.ac.uk

Revolutionary experimental techniques often wait decades before receiving formal recognition. However, the awarding of the 2014 Nobel Prize in Chemistry to some of the originators of super-resolution microscopy is an exception, and shows how rapidly these new techniques—which overturn what was for long thought to be a fundamental experimental limitation—have made an impact.

In fact, one of the techniques whose inventor was recognised by the award—stimulated emission depletion (STED)—was first demonstrated by Stefan Hell and colleagues in 2000 [1]. The other approach, localisation microscopy, first embodied as photo-activated localization microscopy (PALM), was first described by Eric Betzig and colleagues in only 2006 [2], although in both cases the groundwork had been laid earlier. The other recipient of the prize, William Moerner, is recognised for his ground-breaking work in single molecule fluorescence, which has taken somewhat longer to receive recognition; the first detection of a single fluorophore was described in 1989 [3]. Although not a super-resolution method per se, single molecule fluorescence imaging is fundamental to single molecule localisation microscopy.

Another key figure in the development of super-resolution microscopy was the late Mats Gustaffson [4], who was one of the originators of structured illumination microscopy (SIM), a family of methods that, according to the Royal Swedish Academy of Sciences, 'although stretching Abbe's limit of resolution… remain confined by its prescriptions.' Nevertheless, these approaches have many advantages and have proven very popular because of their relatively high imaging speed, which makes them ideal for live cell applications, and their lower barrier to adoption than other approaches. In fact, Gustaffson had already in 2005 shown that non-linear variants of structured illumination microscopy achieve theoretically unlimited resolution [5] and the technique is thus in principle no more confined by the prescriptions of the Abbe limit than the other superresolution methods.

Although Nobel recognition for super-resolution microscopy has come relatively quickly, the progress made in the past decade has been frankly astonishing, and indeed forms part of a wider renaissance in light microscopy, which also includes innovations such as light sheet microscopy. The articles presented here reflect this rapid progress and the technical breadth of the field.

Despite offering the lowest resolution of the methods presented here, **structured illumination microscopy** has seen rapid developments in the past few years. As noted, one of the most significant advantages of SIM is its high acquisition speed, and Lu-Walther *et al* [6] have described a SIM instrument that can achieve super-resolution frame rates of 15 frames per second with a biological sample (or 20 frames per second with a restricted field of view). Although this article describes the familiar wide-field SIM approach, an innovation that has attracted a lot of interest recently is the family of multifocal SIM (MSIM) techniques that offer many of the advantages of conventional SIM whilst using physical or virtual pinholes to reject background fluorescence that can decrease pattern contrast and prevent SIM from working in thicker samples. The work by Ströhl *et al* [7] describes an improved parallel algorithm for processing MSIM data based on joint Richardson–Lucy deconvolution.

Localisation microscopies are based on finding the positions of single fluorophores based on the fitting of point spread functions to the images of the molecules. The coordinates that are obtained are typically used to render images, but more sophisticated forms of analysis can be performed based on the coordinates themselves. Broeken *et al* [8] describe how the coordinates from many similar particles (here, nuclear pore complexes) can be registered in 3D and averaged together to obtain higher-resolution structural information in a method analogous to the particle averaging approaches used in cryoelectron microscopy.

Multi-colour localisation microscopy can pose challenges including cross-talk between different probes or chromatic offsets between channels. Lampe *et al* [9] describe a 'spectral demixing' method for two-colour localisation microscopy, where both channels are imaged simultaneously and fluorescent molecules are identified in both channels before being assigned to one or the other. This avoids the need to correct for

© 2015 IOP Publishing Ltd

chromatic offsets and low cross-talk between the channels is obtained, albeit at the cost of rejecting many ambiguous localisations.

Correlative microscopy—often combining light and electron-based imaging—has been a growth area in recent years. Spahn *et al* [10] have demonstrated some correlative super-resolution methods that they use to investigate the distribution of RNA polymerase and DNA within growing bacterial cells. In their first approach, they combined three localisation microscopy techniques sequentially, each of which provides different information: PALM maps the distribution of RNA polymerase; PAINT shows the cell membrane; and dSTORM images DNA. In their second approach, they combined single particle tracking PALM (sptPALM) of RNA polymerase with dSTORM of DNA to investigate the accessibility of condensed DNA regions to the polymerase.

The limits of most super-resolution experiments are imposed not by the instrumentation, but by the probes that are used to label the molecules of interest. Of particular interest are classes of fluorescent molecule that can be 'switched' on and off, and the limitation here is often the rate at which molecules photobleach, or effectively switch-off irreversibly. Duan *et al* [11] took a reversibly switchable fluorescent protein and engineered it to increase the number of times the molecule can cycle, with detailed characterisation of the structure and properties of the mutant and, importantly, insights into the mechanism of bleaching.

The field of particle tracking is intimately related to localisation microscopy, but it aims to produce trajectories and quantify motion rather than producing images. However, many of the underlying challenges, such as probes and data analysis algorithms, are very similar. Albrecht *et al* [12] described an improved labelling approach for dual-colour particle tracking using nanobodies. Pandzic *et al* [13] took a different approach using a variant of image correlation spectroscopy to obviate the need to track individual particles, a computationally intensive and error-prone process requiring high illumination intensities. They showed how spatio-temporal image correlation spectroscopy (STICS) with photoactivatable fluorescent proteins ('paSTICS') can be used to measure diffusion and flow with lower excitation intensities and fewer artefacts than other methods.

Finally, we come to **STED**, which is a raster imaging method analogous to confocal microscopy. Central to STED is the superimposition of two laser foci: a conventional excitation spot and a toroidal depletion focus, which 'de-excites' fluorophores around the edge of the spot to 'trim down' the excited volume and therefore increase the resolution of the microscope. Patton *et al* [14] described how aberrations and misalignments can affect the quality of the images produced and provide guidance on how to avoid these problems to obtain the highest quality images, for example, by the use of a spatial light modulator rather than a physical phase mask to shape the STED focus.

The adoption of super-resolution microscopy has been extremely rapid since the availability of commercial instruments for all the main techniques. These commercial instruments, of course, are inevitably in some way behind the state of the art, which these articles show is advancing rapidly, a progression that seems likely to continue at least into the near future.

References

[1] Klar T A, Jakobs S, Dyba M, Egner A and Hell S W 2000 Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission *Proc. Natl. Acad. Sci. USA* **97** 8206–10

[2] Betzig E *et al* 2006 Imaging intracellular fluorescent proteins at nanometer resolution *Science* **313** 1642–5

[3] Moerner W E and Kador L 1989 Optical detection and spectroscopy of single molecules in a solid *Phys. Rev. Lett.* **62** 2535–8

[4] Gustafsson M G L 2000 Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *J. Microsc.* **198** 82–7

[5] Gustafsson M 2005 Nonlinear structured-illumination microscopy: wide-field fluorescence imaging with theoretically unlimited resolution *Proc. Natl. Acad. Sci. USA* **102** 13081–6

[6] Lu-Walther H-W, Kielhorn M, Förster R, Jost A, Wicker K and Heintzmann R 2015 fastSIM: a practical implementation of fast structured illumination microscopy *Methods Appl. Fluoresc.* **3** 014001

[7] Ströhl F and Kaminski C F 2015 A joint Richardson–Lucy deconvolution algorithm for the reconstruction of multifocal structured illumination microscopy data *Methods Appl. Fluoresc.* **3** 014002

[8] Broeken J, Johnson H, Lidke D S, Liu S, Nieuwenhuizen R P J, Stallinga S, Lidke K A and Rieger B 2015 Resolution improvement by 3D particle averaging in localization microscopy *Methods Appl. Fluoresc.* **3** 014003

[9] Lampe A, Tadeus G and Schmoranzer J 2015 Spectral demixing avoids registration errors and reduces noise in multicolor localization-based super-resolution microscopy *Methods Appl. Fluoresc.* **3** 034006

[10] Spahn C, Cella-Zannacchi F, Endesfelder U and Heilemann M 2015 Correlative super-resolution imaging of RNA polymerase distribution and dynamics, bacterial membrane and chromosomal structure in Escherichia coli 2015 *Methods Appl. Fluoresc.* **3** 014005

[11] Duan C, Byrdin M, El Kathib M, Henry X, Adam V and Bourgeois D 2015 Rational design of enhanced photoresistance in a photoswitchable fluorescent protein *Methods Appl. Fluoresc.* **3** 014004

[12] Albrecht D, Winterflood C M and Ewers H 2015 Dual color single particle tracking via nanobodies *Methods Appl. Fluoresc.* **3** 024001

[13] Pandžić E, Rossy J and Gaus K 2015 Tracking molecular dynamics without tracking: image correlation of photo-activation microscopy *Methods Appl. Fluoresc.* **3** 014006

[14] Patton B R, Burke D, Vrees R and Booth M J 2015 Is phase-mask alignment aberrating your STED microscope? *Methods Appl. Fluoresc.* **3** 024002