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Live In-Cell Visualization of Proteins Using Super Resolution Imaging

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1. Introduction

Fluorescence microscopy is a non-invasive technique that allows for the dynamic recording of molecular events in live cells, tissues and animals and is based on the principle that fluorescently-labelled material can be illuminated at one wavelength and emit light or fluoresce at another wavelength. The live material is selectively labelled with a fluorescent probe (the fluorophore) to generate a fluorescent image which is detected and recorded through the objective of a microscope. At the one end of the scale: positron emission tomography (PET), magnetic resonance spectroscopy (MRI) and optical coherence spectroscopy (OCT) provide real-time images from live animal or human subjects with resolutions up to about 1 mm, 100 μm and 10 μm respectively (Fernandez-Suarez and Ting 2008) (see Figure 1). At the other end of the scale: electron microscopy provides near molecular-level spatial resolution down to a few nanometers, but here cells must be fixed, which is invasive and prevents dynamic imaging. The most widely used fluorescent imaging methods in research are confocal and wide field microscopy which can provide resolutions down to a few hundred nanometers (ie can resolve intracellular organelles and track proteins in live cells). With the recent emergence of new far field fluorescence imaging techniques, it is now possible to achieve a higher level of resolution down to 10 nm to resolve single synaptic vesicles or pairs of interacting proteins (Fernandez-Suarez and Ting 2008; Huang, Babcock, and Zhuang 2010). In this chapter, we will focus on the relatively new field of far field or super resolution fluorescence imaging. The current limitations in terms of spatial and temporal resolution will be discussed together with recent fluorescent probe technology. A few applications of these techniques which have led to new discoveries will be presented.

2. The spatial resolution limit

In light microscopy, resolution is fundamentally limited by the properties of diffraction (Abbe 1873) or the "spreading out" of a light wave when it passes through a small aperture or is focused to a focal point. The diffraction barrier, which was first described by Ernst

Abbe in 1873, describes the inability of a lens-based optical microscope to discern details that are closer together than half the wavelength of light (Toomre and Bewersdorf 2010). As a result, a minutely small object that emits light will be detected as a finite-sized spot, the size of which is referred to as the point-spread function (PSF) (Figure 2). The PSF is

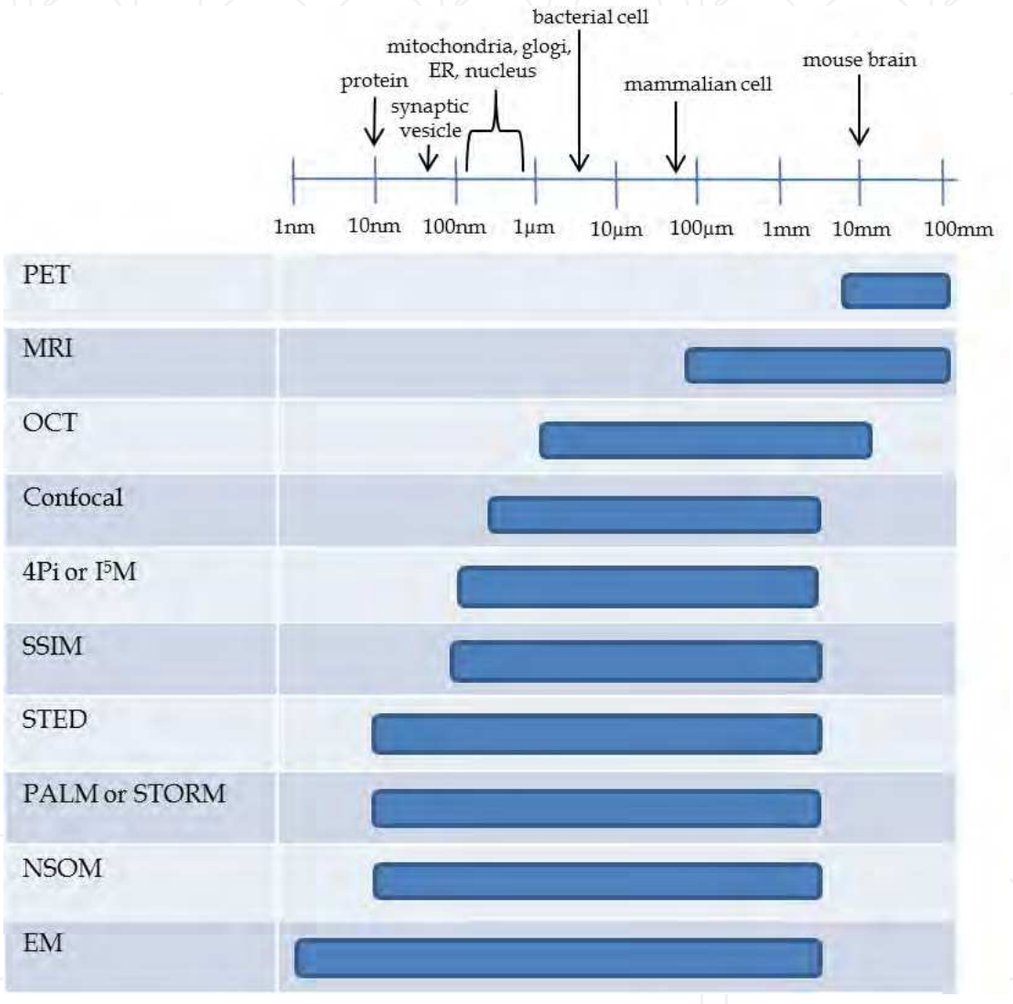

Fig. 1. **Comparison of the spatial resolutions of biological imaging techniques**. The size scale is logarithmic and approximate sizes of biological features are displayed. In addition, the spatial resolutions are estimates and are given for the focal plane. ER, endoplasmic reticulum; PET, positron-emission microscopy; MRI, magnetic resonance imaging; OCT, optical coherence tomography; SSIM, saturated structured-illumination microscopy; STED, stimulated emission depletion; PALM, photoactivated localization microscopy; STORM, stochastic optical reconstruction microscopy; NSOM, near-filed scanning optical microscopy; EM, electron microscopy. Adapted from (Fernandez-Suarez and Ting 2008).

elongated in shape along the optical axis due to the nature of the non-symmetrical wavefront that emerges from a conventional objective lens (Heilemann 2010). According to the diffraction barrier: the resolution obtainable with a wide field microscope is 200 – 250 nm in the x and y directions and 500 – 700 nm in the z-direction (Toomre and Bewersdorf 2010) which is suitable resolution to view organelles and proteins.

Imaging techniques such as multiphoton fluorescence microscopy and confocal laser microscopy have gently pushed the diffraction limit by using a focused laser beam to reduce the focal point size. In addition the confocal microscope uses a spatial pinhole to eliminate out-of-focus light thicker than the focal plane. 4Pi microscopy and I^5M is another branch of microscopy that makes use of two opposing objective lenses to sharpen the PSF along the optical axis through interference of the counter-propagating wavefronts (Heilemann 2010). Although all of the above mentioned methods improve resolution (down to about 100 nm), they are still fundamentally limited by diffraction (Huang, Babcock, and Zhuang 2010).

Diffraction-limited resolution applies only to light that has propagated for a distance substantially larger than its wavelength (i.e. in the far field). In 1992, the first super-resolution image of a biological sample was obtained using near-field scanning optical microscopy (NSOM) (Betzig and Trautman 1992). Here the excitation source or detection probe is placed near the sample to obtain resolutions in the 20 - 120 nm range. Although NSOM has been used to study the nanoscale organisation of several membrane proteins, it cannot be used for intracellular imaging as the probe or excitation source needs to be within tens of nanometers of the target object.

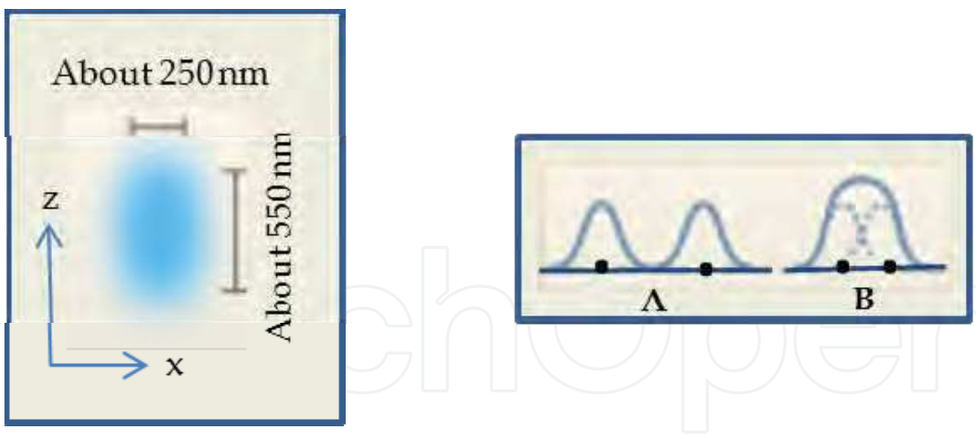

Fig. 2. **Diffraction-limited resolution of conventional light microscopy**. The focal spot of a typical objective with a high aperture is depicted by the ellispse with a width of about 250 nm in the x-direction and about 550 nm in the z-direction. The image of a point emitter imaged through the objective (the point spread function), has similar widths which define the diffraction-limited resolution. Two objects separated by a distance > the resolution limit are resolvable and appear as two separate entities in the image (i.e. in **A**) whereas images closer together than the resolution limit appear unresolvable (i.e. in **B**). Adapted from (Huang, Babcock, and Zhuang 2010).

3. Far field super resolution imaging

Features that are spectrally different are not challenged by diffraction. Likewise, Abbe's barrier does not prevent determining the coordinates of a molecule down to 1 nm with great precision (Kural et al. 2005) if there is no similar marker molecule within $\lambda/2n$ (the diffraction limited region). Overcoming the diffraction limit has been achieved by discerning groups of labelled features within a distance $<\lambda/2n$. This has been realised by modulating the emissions of fluorescent probes (i.e. transitions between bright and dark states) within a diffraction-limited region (Hell 2007).

One class of super-resolution techniques use patterned illumination to spatially modulate the fluorescence behaviour of a *population of molecules* within a diffraction-limited region, such that not all of them emit simultaneously. Microscopies utilizing this technique include stimulated emission depletion (STED), RESOLFT technology and saturated structured illumination microscopy (SSIM).

Another other class of super-resolution techniques uses photoswitching or other mechanisms to activate *individual molecules* within a diffraction-limited region. Images are then reconstructed with subdiffraction limit resolution from the measured positions of individual fluorophores. Microscopies utilizing this technique include stochastic optical reconstruction microscopy (STORM), photoactivated microscopy (PALM), and fluorescence photoactivation localization microscopy (FPALM).

4. Super-resolution imaging of a *population of molecules*

These techniques apply patterned light to a sample to manipulate its fluorescence emission. This spatial modulation can be applied in either a positive or negative manner. In the negative case, patterned light is applied to supress the population of molecules that can fluoresce. In the positive case, the light field used to excite the sample is patterned. In both of these techniques, the spatial information encoded into the illumination pattern allows neighbouring fluorophores to be distinguished from each other, leading to enhanced spatial resolution.

4.1 Principles of STED microscopy

In STED microscopy, fluorescence emission of a cluster of fluorophores is selectively "turned off" or quenched. The sample is illuminated with an excitation laser pulse which is immediately chased by a red-shifted pulse or STED beam (see Figure 3). The STED pulse quenches the fluorophores that reside within the excited state everywhere except those close to the zero intensity position to give a doughnut emission profile. When the two pulses are superimposed, only molecules close to the zero of the STED beam fluoresce, thereby lowering the PSF and increasing resolution. This approach offers improved resolution given a strong depletion light source, low scattering from the sample and good photostability of the fluorophores. In biological samples, STED images have achieved a resolution down to 20 nm in the case of organic dyes and 50 - 70 nm in the case of fluorescent proteins (Fernendez-Suarez and Ting 2008; Huang, Babcock, and Zhuang 2010).

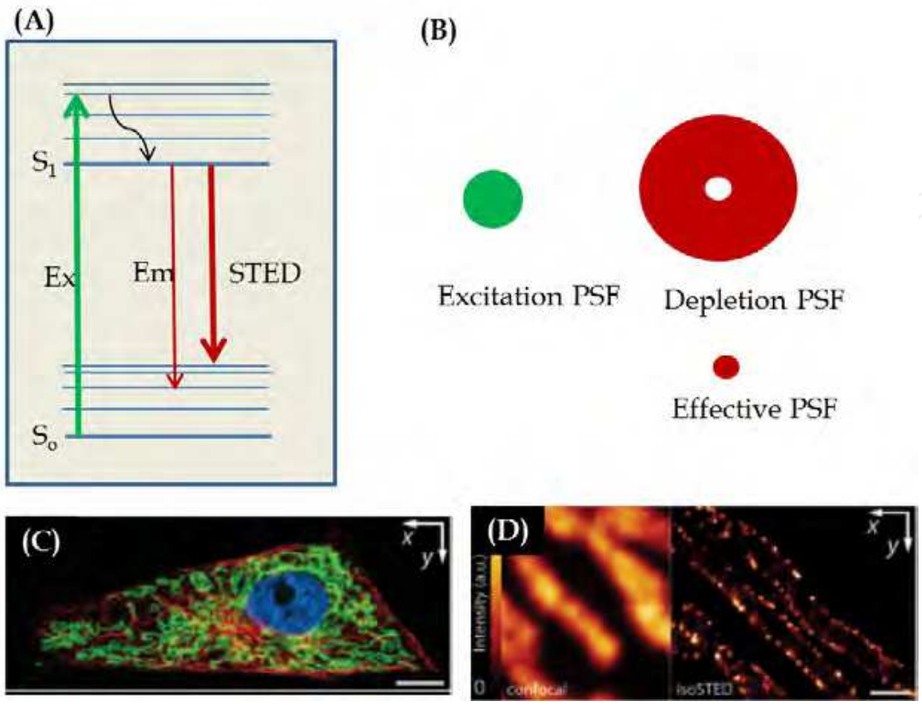

Fig. 3. **Super-resolution imaging with STED microscopy.** (**A**) A fluorophore can enter the first excited state S_1, following the absorption of a photon of appropriate energy. Fast relaxation to the vibrational ground state of S_1 can cause the emission of fluorescence to occur. The key principle of STED microscopy is that this excited state is locally depopulated by inducing stimulated emission. (**B**) A first laser that excites fluorophores into the S_1 state is overlaid with a depletion laser which has a doughnut-shaped intensity profile, where the area of zero-intensity scales with the irradiation intensity of the depletion beam. The resulting "effective" PSF represents the remaining area where fluorescence emission is still observed, and which is well below the diffraction limit. (**C**) Overview of the mitochondrial network of a PtK2 (kangaroo rat) cell. The mitochondria was labelled with antibodies against the translocase of the outer membrane of mitochondria (TOM) complex (green) and the microtubule cytoskeleton was labelled with antibodies against β-tubulin (red). The nucleus was stained with DAPI (blue). Scale bar 10 μm (**D**) Mitochondria labelled for the outer membrane with antibodies specific for the TOM complex imaged with a confocal microscope (left) and isoSTED nanoscope (right). Scale bar 500 nm. Both (**C**) and (**D**) are reprinted with permission (Schmidt et al. 2009). Copyright 2009 American Chemical Society.

4.2 Principles of SIM microscopy

SIM microscopy utilizes a positive sinusoidal pattern of excitation light by combining two light beams. A final image is computationally reconstructed from multiple snapshots collected by scanning and rotating the pattern. Spatial modulation from the excitation pattern brings about enhanced spatial resolution (see Figure 4).

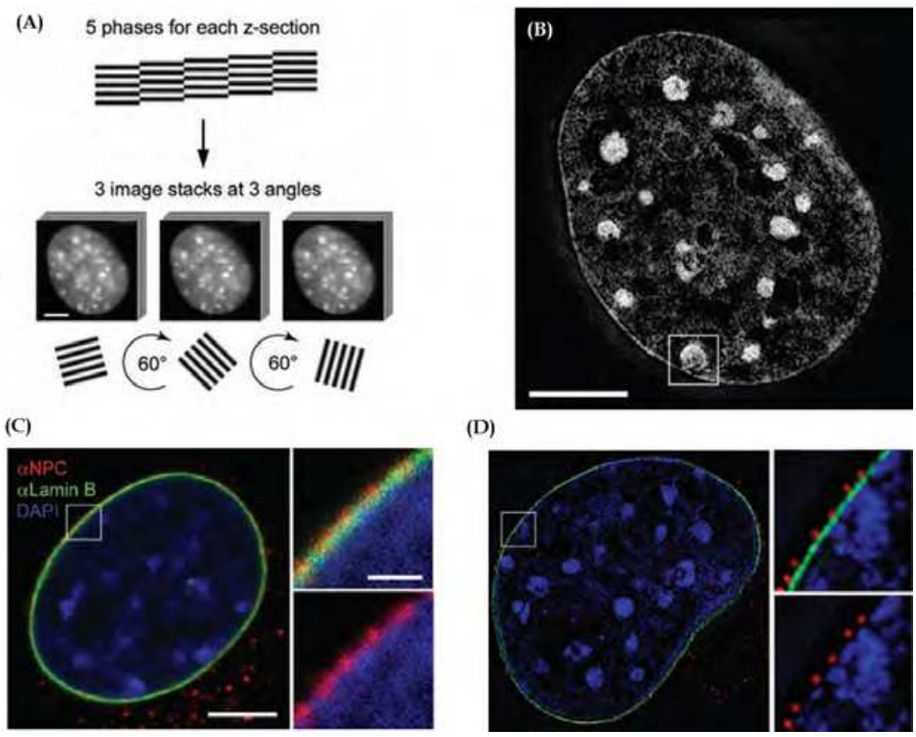

Fig. 4. **Super-resolution imaging with SIM spectroscopy.** Cross section through a DAPI-stained C2C12 cell nucleus aacquired with structured illumination. Five phases in the sine wave pattern were recorded at each z-position (in **A**), allowing the shifted components to be separated and returned to their proper location in space. Three image stacks are recorded with the diffraction grating rotated to three positions 60^{o} apart. The cross section is reconstructed to give the 3D SIM image (in **B**). Scale bar 5 µm. (**C**) Cross section of a confocal image of the nucleus stained for DNA (blue), lamin B (green), and the nuclear pore complex (red), The right panes show the magnified images of the boxed region. (**D**) 3D SIM image of a similarly stained nucleus. Reprinted with permission (Schermelleh et al. 2008).

4.3 Video rate super-resolution images of live cells using STED and SIM

Temporal resolution refers to the precision of a measurement with respect to time, which is critical for the dynamic imaging in living cells. There is an interplay between temporal and spatial resolution due to the finite speed of light and the time taken for the photons to reach the detector. During this timeframe, the system may have undergone a change, thus the longer the light has to travel, the lower the temporal resolution. Video rate STED imaging (28 frames per second) with 62 nm spatial resolution has been demonstrated in a field of view of about 5 µM^2, allowing the motion of individual synaptic vesicles in a dendritic spine to be followed (Westphal et al. 2008). This was achieved by increasing laser intensity (to 400 mW per cm^2) and reducing the number of photons collected per imaging cycle (resulting in increased spatial resolution). This situation is not ideal as the high laser intensities not damaging to living cells. Replacing the pulsed STED lasers with continuous wave lasers permits faster scanning and higher time resolution (Willig et al. 2007). In this regard, a 70 µM^2 image of an endoplasmic reticulum took only 0.19s to acquire (Moneron et al. 2010).

SIM is good for live-cell applications that require a large field of view but not very high spatial resolution as this technique is limited by how fast the illumination pattern can be modulated and the rate of camera speed (Huang, Babcock, and Zhuang 2010).

5. Super-resolution fluorescence microscopy by *single-molecule switching*

Fluorescent probes with photoswitchable properties have been developed to modulate the fluorescence emission profile of individual fluorophores such that only an optically resolvable subset of fluorophores are activated at any moment, allowing their localization with high accuracy. Over the course of multiple activation cycles, the positions of numerous fluorophores are determined and used to construct a high-resolution image. This is the basis of PALM, FPALM and STORM super-resolution spectroscopy. Here spatial resolution is dependent on the precision of a molecules' position which in turn is related to the number of photons which are detected. For example, in the absence of background, if 10 000 photons are collected from a single fluorophore before it bleaches or is turned off, its position can be determined to 2 nm precision (Yildiz et al. 2003).

Being able to localize a single molecule does not directly translate to super-resolution imaging as the labelled biological sample may contain thousands of fluorophores within a diffraction limited region. The fluorescence emissions of the fluorophores will overlap such that the overall image will appear as a blur. However, if the fluorescence emission from these molecules is controlled such that only one molecule is emitting at a time, individual molecules can be imaged and localized.

6. Fluorescent probes used in super-resolution imaging

STORM and PALM microscopy are made possible only though the use of fluorescent probes. Despite the high specifications required for these probes, a large number of switchable fluorophores are available. These probes must firstly have a fluorescent state that emits light at one wavelength and a dark state that does not emit light at this wavelength. Secondly, in order to achieve high precision of localization, the probes should emit a large number of photons before entering the dark phase. Thirdly, because only one fluorophore is activated within a diffraction-limited area at any time, the fluorophores within the dark state should remain as such to ensure high precision localization of the activated fluorophore. A low spontaneous rate of activation of the fluorophores in the dark state is also desired (i.e. spontaneous activation by thermal energy) (Huang, Babcock, and Zhuang 2010). Currently available probes range from organic dyes to fluorescent proteins. Some of these will be discussed below.

6.1 Fluorescent proteins

There are two classes of fluorescent proteins used in super-resolution imaging: those that convert from a dark to a bright fluorescent state upon irradiation (called photo-activatable proteins), and those whose fluorescence wavelength shifts upon irradiation (also called photoshiftable fluorescent proteins). All known photoshiftable proteins shift their wavelength emission irreversibly, whereas other non-photoshiftable fluorescent proteins emit both reversibly and irreversibly (Fernandez-Suarez and Ting 2008; Lukyanov et al. 2005).

EosFP is the most commonly used irreversible photoswitchable fluorescent protein which exhibits both a high contrast and brightness (Wiedenmann et al. 2004). This protein emits strong green fluorescence (516 nm) that changes to red (581 nm) upon near UV irradiation because of a photo-induced modification involving a break in the peptide backbone next to the chromophore (see Figure 5). This protein was used successfully to perform single-particle tracking of membrane proteins in live COS7 cells at an imaging speed of 20 frames per second using PALM (Manley et al. 2008). The main disadvantage of monomeric EosFP however, is that the chromophore formation occurs only at temperatures below 30 ^{o}C, which limits its use in mammalian cells (Wiedenmann et al. 2004). Even the brightest photoswitchable fluorescent proteins are still much dimmer than many of the small molecule organic fluorophores. For example EosFP provides about 490 collected photons per molecule (Schroff et al. 2007) whereas the switchable fluorophore pair Cy3-Cy5 provides about 6000 collected photons per molecule per switching cycle which lasts about 200 cycles (Bates et al. 2007; Bates, Blosser, and Zhuang 2005).

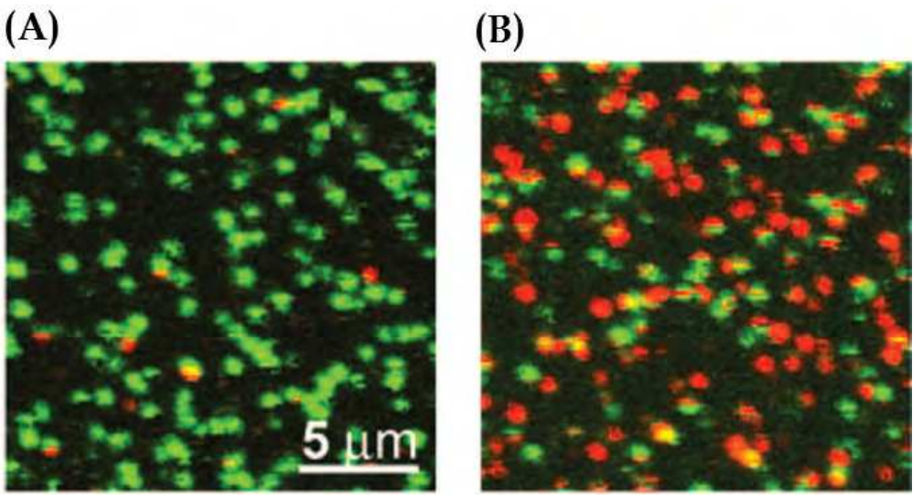

Fig. 5. **Single-molecule spectroscopy of EosFP immobilized on a BSA surface**. Confocal images were taken at 488 nm excitation (**A**) and 400 nm excitation (**B**). Reprinted with permission (Wiedenmann et al 2004)

Reversible fluorescent proteins are advantageous in super-resolution imaging as the same fluorophore can be imaged multiple times. Reversible photoswitching is a prerequisite in RESOLFT imaging, in which each molecule is switched on and off many times in order to reconstruct a subdiffraction image. The best known reversible fluorescent protein is the naturally occurring Dronpa (Ando, Mizuno, and Miyawaki 2004) and its variants of which Padron is one of them (Andresen et al. 2008).

6.2 Organic dyes

There are three main classes of non-genetically encoded probes that have been used in super-resolution imaging, namely inorganic quantum dots, reversible photoswitches and irreversible photocaged fluorophores.

Fluorescent molecules suitable for STED imaging need to have a high quantum yield and slow fluorescence decay, in which case ATTO or DY dyes are ideal. For RESOLFT imaging, the photoswitches FP595 and futyl fulgides are useful (Fernandez-Suarez and Ting 2008). The small molecule analogues to the reversible photoactivatable proteins (i.e. Dronpa) are photochromic probes which include rhodamines and diarylethenes and photoswitchable cyanines. These dyes have higher contrast ratios and higher extinction coefficients than their fluorescent protein counterparts, resulting in a larger number of photons collected per molecule. The photoswitchable cyanines have been used in both PALMIRA and STORM imaging (Bates et al. 2007; Huang et al. 2008) (see Figure 6 below). Cy5 is best used in combination with a secondary chromophore (or activator) that facilitates the switching. For example when Cy5 is paired with Cy3, the same red laser that excites Cy5 is also used to switch the dye to a stable dark state. Subsequently, exposure to green laser light converts Cy5 back to the fluorescent state, and this recovery depends on the close proximity of the secondary dye Cy3 (Bates, Blosser, and Zhuang 2005). Cy3 has also been found to facilitate switching of other cyanines which has greatly increased the amount of colours that are available for STORM imaging and has allowed for the simultaneous visualization of microtubules and clathrin-coated pits in fixed mammalian cells with 20 - 30 nm lateral resolution (see Figure 7) (Bates et al. 2007). The availability of several colours of photoswitchable cyanine dyes gives these fluorophores more diverse application than the photoswitchable fluorescent proteins of which only a few colours are available. Photoswitchable rhodamines are also an important class of photoswitches as they are membrane permeable which enables their use for live-cell imaging, compared to the cyanine dyes which are not.

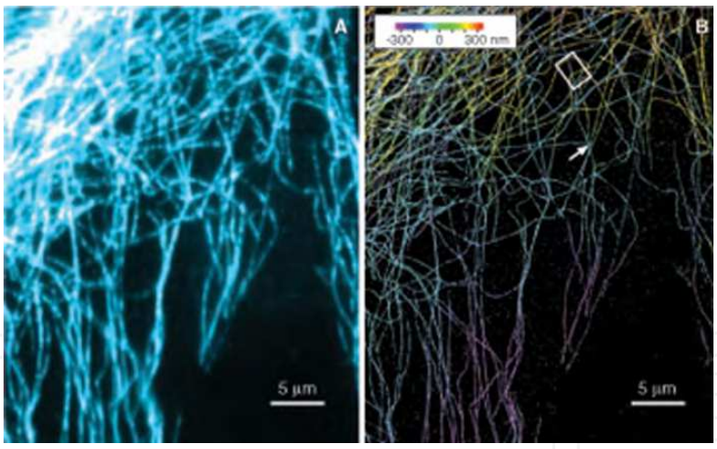

Fig. 6. **A three dimensional STORM image of microtubules in a BS-C-1 cell.**
(**A**) Conventional immunofluorescence imaging of microtubules. (**B**) The 3D STORM image of the same area using Cy3 and Alexa 647 photoswitchabe cyanine pair. A red laser (657 nm) was used to image Alexa 647 molecules and deactivate them to the dark state; a green laser (532 nm) was used to reactivate Alexa 646 in a Cy3-dependent manner. Reprinted with permission (Huang et al. 2008).

Another important class of dyes are the irreversible caged fluorophores such as the caged Q-rhodamine (Gee, Weinberg, and Kozlowski 2001) although these compounds have not been used for super-resolution imaging of biological samples.

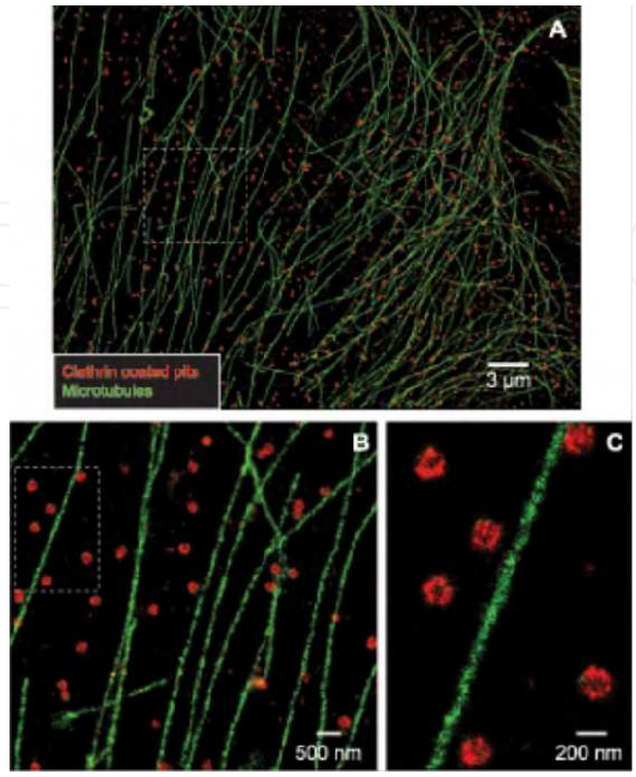

Fig. 7. **Two-colour STORM imaging of microtubules and clathrin coated pits in a mammalian cell**. (**A**) STORM image of a large area of a BS-C-1 cell. The microtubules were immunostained with Cy2 and Alexa 647, and those for clathrin with Cy3 and Alexa 647. The 457 nm and 532 nm laser pulses were used to selectively activate the two pairs of fluorophores. Each localization was false coloured according to the following code: green for 457 nm activation and red for 532 nm activation. (**B**) Enlarged STORM image of the boxed area. (**C**) Further magnification of the boxed area. Reprinted with permission (Bates et al. 2007).

7. Site-specific targeting of fluorophores to cellular proteins

Although non-genetically encoded probes generally show increased brightness and photostability compared to their fluorescent protein counterparts, they have their disadvantages. The lack of genetic encoding means that these probes require targeting to the biomolecule of interest inside the cell. These probes have been traditionally targeted using antibodies although their application is not widespread. Antibodies are not membrane permeable, and hence are not useful for labelling living cells intracellulary. Antibody staining also usually results in a low labelling efficiency and the large size of antibodies contributes to uncertainty in the spatial relationship between the label and target (Fernandez-Suarez and Ting 2008).

Some current approaches to site-specific labelling of biomolecules in living cells has been reviewed by Fernandez-Suarez and Ting (Fernandez-Suarez and Ting 2008). One method

involves fusion of a peptide that recruits a small molecule to the protein of interest (Martin et al. 2005; Lata et al. 2006). Other methodologies use proteins to recruit the small molecule tag (Marks, Braun, and Nolan 2004; Bonasio et al. 2007) which can improve the specificity of binding due to the larger interaction surface although the increased size of this protein can perturb protein/enzyme function. In a combination method which seeks to achieve high labelling specificity with minimal perturbation to the protein target, a peptide recognition sequence has been used comprising an enzyme to catalyse the attachment of the probe to the sequence (Fernandez-Suarez et al. 2007).

8. Perspectives on emerging applications of super-resolution microscopy in live cells

The major technological principles of super-resolution microscopy (SIM, STED and PALM/STORM) have now matured to the extent that they have been implemented in commercially available systems that are relatively easy to use and within reach for well-established research laboratories. Thus, it is likely that we are standing at the beginning of an era of groundbreaking discoveries, fuelled by a multitude of applications of these novel imaging approaches to the challenging questions in cell biology.

Substantial potential for super-resolution imaging exists, for example, in understanding the structural basis of signal transduction within cells. Aspects of the organization and function of lipid rafts or microdomains in the cell membrane have been controversially discussed in the past, and imaging with resolution on the nanometer scale now allows addressing questions such as the molecular composition and dynamics of putative signaling complexes (Lang and Rizzoli 2010) (see Figure 8), the dynamic cytoskeletal changes underlying cell motility and migration, the way plasmamembrane structures are linked to and interact with the cytoskeleton (Ahmed 2011), or how cells interact with substrate molecules.

Our understanding of how cells communicate *in vitro* or even in the context of live tissues is set to benefit substantially from super-resolution technologies. The STED approach has been used to analyse the subcellular distribution of Na-K-ATPase in neurons (Blom et al. 2011) and to map synaptic spines in live brain tissue (Nagerl and Bonhoeffer 2010) (see Figure 9). Protein localization in chemical synapses has been investigated using STORM imaging (Dani et al. 2010). In the context of immunology, super-resolution imaging has been applied to study the composition of the immunological synapse (Dani et al. 2010) (See Figure 10) and it is now well within reach to visualize the dynamic process of how viral particles interact with immune cells, as recently shown (Felts et al. 2010). It is even possible to map GFP-tagged proteins in live multicellular organisms, as has been demonstrated in the nematode C. elegans, using STED (Rankin et al. 2011).

Super-resolution microscopy will also enhance our ability to study molecular interactions, based on signal colocalization, FRET analysis or the genetic engineering of constructs that emit fluorescence when two interaction partners are in close proximity, as has been demonstrated (Ahmed 2011).

Beyond the study of proteins, super-resolution imaging, particularly STED due to its high temporal resolution and the fact that it is based on the simultaneous imaging of a number of fluorophores in a given volume, has the potential of becoming a powerful tool to study

cell physiology using diffusible fluorescent indicator dyes, e.g. for Ca^{2+} (Nagerl and Bonhoeffer 2010). Single-molecule super-resolution approaches using such dyes have been employed to visualize single ion channels (Patterson et al. 2010; Wiltgen, Smith, and Parker 2010).

In summary, each of the different approaches to super-resolution microscopy holds enormous potential in addressing key questions in current cell biology. However, they also have their characteristic advantages and drawbacks. SIM is a widefield technique easily implemented and not very demanding in terms of specimen preparation and labelling that can be used for multichannel fluorescence detection and is reasonably suitable for imaging of dynamic processes (image acquisition rates upward of 10 frames/s are possible), but it has comparatively low resolution upward of 50 nm. PALM, STORM and their derivatives are widefield fluorescence microscopy-based techniques that are currently achieving the highest resolution (in the range of 20 nm) and allow for multi-channel fluorescence imaging, but are largely confined to analysis of static or relatively slow processes in the order of minutes and in thin monolayers of cells or tissue sections. STED is a confocal laser scanning-based technique, allowing for imaging of fast dynamic processes in the range of milliseconds and analysis of relatively thick tissue slices with high lateral resolution. STED as well as PALM/STORM techniques have very specific requirements with regard to specimen preparation and labelling and the potential of these techniques is still limited to some extent by the availability of suitable fluorophores.

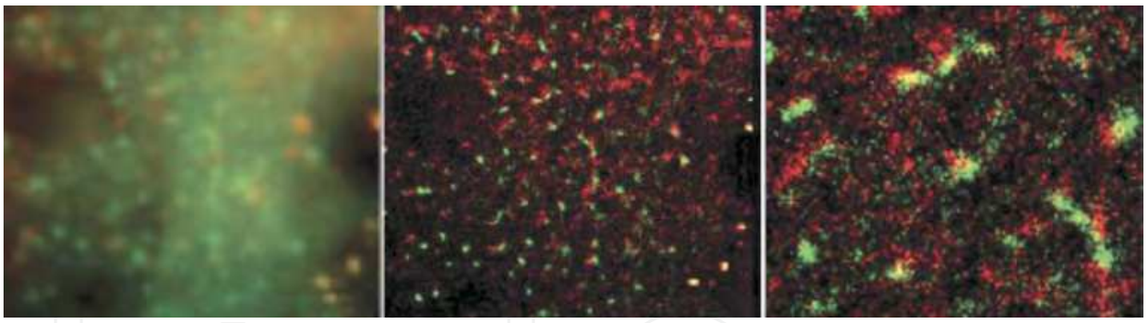

Fig. 8. **Application of TIRF and PALM imaging**. Demonstration of clusters of transferrin receptor (labeled with PalmCherry, red) and clathrin light chain (labeled with PAGFP, green) in the cell membrane by TIRF-microscopy (left) and PALM (middle; right, magnified view) (Lang and Rizzoli 2010).

As super-resolution microscopy techniques become established tools in cell biology research, a future challenge will be to design multimodal imaging approaches that combine the strengths of the different techniques. There is also a need to develop more fluorophores that are suitable for live-cell labelling, have sufficient quantum yield and provide a palette of spectral ranges suitable for the sensitive and simultaneous labelling of multiple cellular components. The drive towards a more sophisticated microscope, light source and computing hardware is still likely to lead to substantial improvements in the theoretically unlimited resolution beyond the diffraction barrier, and will enhance the capability of the systems for temporal resolution and 3-dimensional imaging.

Fig. 9. **Application of STED imaging**. STED-based 3-dimensional reconstruction of dendritic spines genetically tagged with GFP. Scale bar: 1μm (Nagerl and Bonhoeffer 2010).

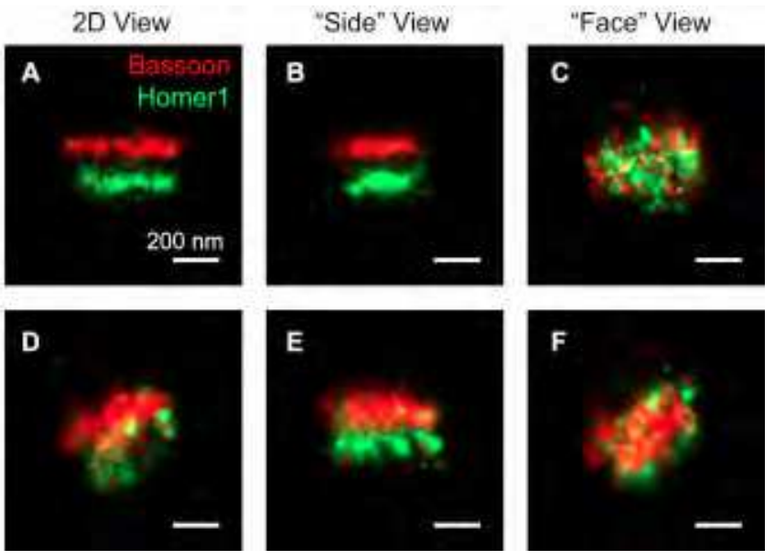

Fig. 10. **Application of STORM imaging**. STORM imaging of the pre-synaptic protein Bassoon and post-synaptic Homer1 using STORM super-resolution microscopy (Dani et al. 2010).

9. Conclusion

With the development of super-resolution imaging techniques it is now possible to image live cells down to tens of nanometers. STED imaging has allowed video rate tracking of synaptic vesicles in a dendritic spine down to 62 nm spatial resolution (Westphal et al. 2008), whereas STED, STORM and PALM have allowed cellular structures to be imaged in 3D and multiple colours. With such improved resolution, protein pairs have been visualised that contradict previous reports (Shroff et al. 2007) demonstrating the power of visualizing biomolecules of high resolution. For further improvements in spatial and temporal resolution, increased computational methods as well as fluorophores and site-specific live cell labelling are required.

10. Acknowledgments

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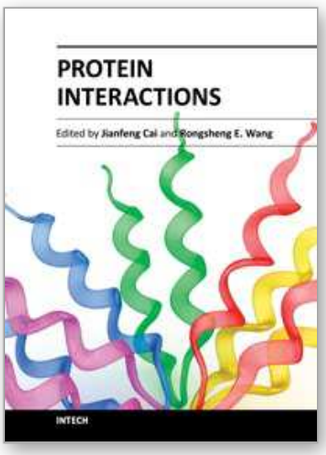

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Protein interactions, which include interactions between proteins and other biomolecules, are essential to all aspects of biological processes, such as cell growth, differentiation, and apoptosis. Therefore, investigation and modulation of protein interactions are of significance as it not only reveals the mechanism governing cellular activity, but also leads to potential agents for the treatment of various diseases. The objective of this book is to highlight some of the latest approaches in the study of protein interactions, including modulation of protein interactions, development of analytical techniques, etc. Collectively they demonstrate the importance and the possibility for the further investigation and modulation of protein interactions as technology is evolving.

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