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The cell biologist's guide to super-resolution microscopy

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microscopy is limited by the diffraction of light, which precludes the visualization of details below ~ 200 nm (Vangindertael et al., 2018). Since the early 2000s, super-resolution microscopy (SRM) approaches have been devised that bypass this diffraction limit to resolve biological objects down to a few tens of nanometers (Sahl et al., 2017; Schermelleh et al., 2019; Sigal et al., 2018). A variety of techniques are now available as either commercial or open, do-it-yourself, instruments and software (Thorley et al., 2014), and cell biologists interested in applying SRM to their question of interest can feel lost among the number of options available. In particular, the different SRM techniques vary greatly in the resolution they attain, the constraints they place on sample type, and their general versatility and ease of use (Bachmann et al., 2015; Wegel et al., 2016). In this Cell Science at a Glance article and accompanying poster, we focus on SRM methods that we consider accessible to researchers, by being commercially available or that are easy to implement by non-experts. In Box 1, we highlight the general elements to be considered when preparing samples for SRM. We briefly explain the principles behind each SRM technique, then summarize its performance for relevant cellular imaging parameters using an indicative scoring system represented by radar plots on the poster (using a '1 to 5' scale of less adapted to more adapted; see 'Techniques and Principles' on poster). The advice we propose is based on our collective experience of applying various SRM techniques to different cell biological questions (Almada et al., 2019; Carisey et al., 2018; Gustafsson et al., 2016; Jacquemet et al., 2019; Stubb et al., 2019; Vassilopoulos et al., 2019). The aim of this Cell Science at a Glance article and the accompanying poster is to provide a short overview that will foster further reading or discussion with imaging facility specialists and help put these powerful techniques in the hands of diverse users.

Fluctuation-based super resolution microscopy

Principle

When excited with continuous light, the emitted light of every fluorophore randomly varies over time. This is due to transitions between the non-fluorescent states of the fluorophore (van de Linde and Sauer, 2014) or their interactions with the surrounding environment (Bagshaw and Cherny, 2006). After capturing these oscillations over a sequence of tens to hundreds of images, algorithms such as super-resolution optical fluctuation imaging (SOFI) (Dertinger et al., 2009) and super-resolution radial fluctuations (SRRF) (Gustafsson et al., 2016) use the temporal correlations in these oscillations to predict the presence and location of fluorophores at improved resolution (see 'Techniques and Principles' and 'Representative Images' on poster). The accuracy and resolution will considerably improve when samples are decorated with highly fluctuating probes, such as reversibly photoswitchable fluorescent proteins (Zhang et al., 2016) or organic dyes in a photoswitch-inducing buffer (van de Linde et al., 2011).

Versatility – 4/5

Fluctuation-based methods are purely analytical approaches that are compatible with most microscopes and do not depend on hardware modifications. They are thus easy to implement, and free software versions exist, but these require knowledge to properly tune the processing (Dedecker et al., 2012; Gustafsson et al., 2016). Moreover, the need to acquire a fast sequence of images (typically hundreds) can make multidimensional protocols complex.

Box 1. Sample preparation for optimized imaging

Experimental design

- The same pitfalls as in conventional microscopy apply to SRM and must be known to the experimentalist to avoid bias during image acquisition, analysis and data validation (Jost and Waters, 2019).
- For multicolor imaging, online tools should be used to predict the compatibility between the different fluorophores or fluorescent proteins (Lambert, 2019) and the microscope to be used.
- The speed of acquisition needs to be taken into consideration when choosing the most appropriate SRM technique. Slow acquisition strategies may lead to motion blur and/or artifacts during live imaging.
- Sample size should be computed ahead of the experiment using a pilot dataset.
- Consistency is key for preparation of fluorescent samples for SRM, but this does not eliminate the need for biological and technical replicates.

Sample labeling

- Fixation methods need to be carefully optimized as each fixative has specific advantages, drawbacks and dye compatibility. Live-cell imaging can be used to validate that no fixation artifacts have been introduced (Pereira et al., 2019).
- Reagents should be carefully validated, including antibodies and dyes.
- Aldehyde-based fixatives should be quenched using inert amine-containing molecules (glycine or sodium borohydride).
- The nonspecific adsorption of fluorescent compounds should be reduced by adding detergent and sera in the washing buffer. Ultimately, the ratio between signal of interest and background is more important than the overall brightness of the staining.
- Thick samples can be cleared to remove light-scattering and light-absorbing components of a tissue (Kolesova et al., 2016; Qi et al., 2019), allowing access to deeper structures.

Choice of vessel

- Glass coverslips should be used rather than plastic, as plastics introduce optical aberrations and are not compatible with immersion oils for long-term imaging.
- The thickness of the glass must match the expected value for the microscope used; 170 μm -thick cover glass (#1.5H) is often recommended. To ensure reliable performance, high quality, high tolerance cover glass should be chosen.
- Positioning of the sample must be designed to reduce the distance between the objective and the region of interest. This will help improve the resolution by reducing light dampening and distortion.
- Most techniques can accommodate a slide–cover-glass sandwich format, but some require access to the sample. In these cases, glass-bottom imaging dishes and multiwell slides are recommended.

Choice of the imaging medium

- For fixed samples, multiple mounting media are available, all designed to bring the refractive index (RI) of your sample close to the RI of the cover glass (RI=1.52).
- Curing mounting media allow for longer conservation and have an RI that is better for image quality, but lead to a slight shrinkage of cellular structures.
- Non-curing mounting media may be preferred due to convenience of use, and preservation of sample structure, despite the compromise in RI.
- For live-cell imaging, the priority of the medium is to ensure the survival of the biological sample.
- Imaging media need to be nutrient rich and provide a pH-controlled environment. For instance, adding 25 mM HEPES in imaging media may help to ensure cell survival when CO_2 access is non-optimal during imaging (Frigault et al., 2009).
- Consider removing any non-essential compounds from the medium that are autofluorescent (e.g. Phenol Red, flavins, nicotinamide adenine dinucleotide and lipofuscin) (Surre et al., 2018).

XY resolution – 2/5

The resolution improvement considerably depends on the capacity of the algorithm used to detect fluctuations in fluorophores. It is thus

difficult to predict the resolution that will be achieved prior to actual imaging, but an enhancement of two- to three-fold can typically be expected (Culley et al., 2018a).

Z resolution – 1/5

Resolution improvement in the Z-axis cannot yet be directly achieved by the algorithms, but specialized multiplane-imaging setups are being developed to tackle this limitation.

Thick-sample friendliness – 4/5

The resolution and quality of the data reconstructed will depend on the density of fluorophores captured in each image. As such, optical sectioning techniques, such as total internal reflection fluorescence (TIRF), confocal or light-sheet considerably improve the data generated and enable these methods to super-resolve samples with a thickness of tens of micrometers.

Live-cell friendliness – 4/5

Fluctuation-based SRM is one of the least phototoxic methods in existence; enhanced resolution can be achieved using an illumination intensity that is similar to conventional fluorescence imaging (mW/cm² magnitude). Imaging from minutes to hours without significant photobleaching or apparent light-induced cell stress has been demonstrated (Movie 1, Culley et al., 2018a), but slow speed can be an issue with moving samples (see below).

Image fidelity – 3/5

Intensity in the generated images weakly relates to the local stoichiometry of fluorophores. While images will represent the structure (with some degree of unwanted defects), care needs to be taken when employing further analysis routines that take pixel brightness into account (Culley et al., 2018b).

Multicolor – 4/5

Multicolor imaging is possible and straightforward since fluctuation imaging is compatible with most fluorophores.

Temporal resolution – 2/5

A temporal stream of a few hundred images needs to be collected to capture a single channel, generally taking one to five seconds.

Availability

Free implementations can be found for both SOFI and SRRF (Dedecker et al., 2012; Gustafsson et al., 2016), and SRRF has been implemented as onboard processing in some electron multiplying charge coupled device (EMCCD) cameras (Cooper et al., 2019).

Pixel reassignment super resolution microscopy

Principle

In this technique, single or multiple focal spots are used to scan the sample, as in confocal microscopy. However, the fluorescence signal is not captured by a single-point detector, such as a photomultiplier tube, but by an array detector (camera, concentric detector array or single-photon detector array) (Ströhl and Kaminski, 2016; Wu and Shroff, 2018). The signal detected in each element of the detector array is reassigned in space to achieve a smaller point-spread-function and thus higher resolution. After data processing, a 1.4-fold improvement in resolution can be achieved laterally with a minor axial improvement (Vangindertael et al., 2018). A number of variants exist for pixel reassignment, such as image scanning microscopy (ISM) (Müller and Enderlein, 2010), Zeiss Airyscan (Huff, 2015), rescan confocal microscopy (RCM)

(Luca et al., 2013) and multifocal structured illumination microscopy (MSIM) (York et al., 2012). Pixel reassignment has been adapted to spinning disc and swept-field confocal microscopy (Azuma and Kei, 2015; Hayashi and Okada, 2015; York et al., 2013). There is an expectation that the majority of confocal systems will, in the future, integrate these concepts to improve resolution.

Versatility – 4/5

A lateral resolution improvement of 1.4-fold can be achieved purely through optics without the need for an additional analytical step. This means researchers can observe the resolution-enhanced sample in real-time. MSIM implementations (York et al., 2012), however, require the digital analysis of the images being collected.

XY resolution – 2/5

The 1.4-fold lateral-resolution improvement can be increased to two-fold through additional and careful deconvolution of the collected data.

Z resolution – 2/5

Minor (~25%) axial resolution improvement has been demonstrated (York et al., 2013), and further improvement necessitates additional deconvolution of the collected data.

Thick-sample friendliness – 4/5

Reassignment systems are expected to generate images of similar fidelity to those collected in laser scanning confocal or spinning disc microscopy for thick samples.

Live-cell friendliness – 5/5

Pixel reassignment allows single-shot super-resolution imaging at low-illumination (Movie 2). Reassignment implies additional magnification, which requires the use of sensitive detection systems.

Image fidelity – 4/5

The pure-optical resolution enhancement does not generate additional image defects, such as those commonly occurring in super-resolution techniques that require data processing to generate a final image. However, the additional use of deconvolution can lead to image artefacts.

Multicolor – 5/5

The technique does not rely on specific fluorophores and can be used for multicolor imaging. It has the same multicolor capacity as laser scanning confocal or spinning disc microscopy.

Temporal resolution – 5/5

Owing to the mostly optical nature of the resolution improvement process, high-speed imaging is possible and expected to be similar to that of laser scanning confocal or spinning disc microscopes.

Availability

Commercial offerings include dedicated setups, and add-ons to a widefield or spinning disc microscope. Most commercial systems are relatively recent, so they are not yet widely available in imaging facilities.

Structured illumination microscopy

Principle

In the structured illumination microscopy (SIM) technique, the sample is illuminated using a patterned light. For each focus plane, multiple images are taken using a different pattern and are then

combined by a computer algorithm to reconstruct a super-resolved image (Schermelleh et al., 2019). The most common patterns are parallel lines, but hexagonal or even random patterns can be used (Heintzmann and Huser, 2017). In the case of parallel lines, multiple shifted and rotated patterns are obtained using a grid or a spatial light modulator: 9 images for a 2D image (Gustafsson, 2000) and 15 images per plane for a 3D volume (Gustafsson et al., 2008).

Versatility – 4/5

SIM is generally easy to use, suitable for a wide variety of biological samples and is compatible with most fluorophores with the conditions that they are relatively resistant to photobleaching and non-blinking (Demmerle et al., 2017). SIM performances are affected by both the sample and the imaging conditions, and therefore dedicated training and optimizations are required to achieve good results.

XY and Z resolution – 3/5

Linear SIM typically doubles the spatial resolution in all three dimensions (Gustafsson et al., 2008). Non-linear SIM approaches that bypass this resolution use fluorophore saturation, but are not yet commercially available (Li et al., 2015; Rego et al., 2012).

Thick-sample friendliness – 3/5

The SIM design is often based on widefield microscopy. In this case, SIM performance can be strongly affected by the sample thickness, as well as by the presence of out of focus light. ‘Grazing incidence’ illumination (Guo et al., 2018) or TIRF (Kner et al., 2009; Li et al., 2015) can be used to image objects that are close to the coverslip with a better signal. In addition, lattice light-sheet (Chen et al., 2014) or slit-confocal (Schropp et al., 2017) arrangements can be combined with SIM to image deeper into cells.

Live-cell friendliness – 4/5

SIM is commonly used for live-cell imaging (Burnette et al., 2014; Carisey et al., 2018; Fiolka et al., 2012). Traditional 3D SIM often requires the acquisition of hundreds of images per time point (depending on the volume imaged) and can be phototoxic. Therefore, SIM-TIRF or grazing-incidence SIM are better suited for live-cell imaging (Movie 3). In addition, improvement in reconstruction algorithms are enabling the imaging of biological samples using low laser power over hour-long time lapses (Huang et al., 2018).

Image fidelity – 4/5

When using linear SIM, the fluorescence intensities in reconstructed images are not directly transposed from the intensities of the raw images (Heintzmann and Huser, 2017). The intensity of the reconstructed image typically correlates well with the brightness of the original structures, but SIM can attenuate constant signals and is thus not suited to image and quantify diffuse (cytoplasmic) staining.

Multicolor – 4/5

As SIM is compatible with most fluorophores, its setup can typically accommodate three to four different color channels (Jacquemot et al., 2019; Vietri et al., 2015). However, optimal resolution requires a precise tuning of the oil refractive index that can be slightly different for distinct channels (Demmerle et al., 2017).

Temporal resolution – 4/5

The temporal resolution of SIM widely depends on the setup used. Traditional 3D SIM requires the acquisition of hundreds of images per time point (depending on the volume imaged) and is relatively

slow (several seconds per time point) (Fiolka et al., 2012). Other SIM implementations such as SIM-TIRF offer a faster acquisition speed of 11 Hz (Kner et al., 2009). Recent developments such as instant TIRF-SIM allow acquisitions as fast as 100 Hz, with the use of a sliding window over the varying patterns (Guo et al., 2018; Huang et al., 2018).

Availability

SIM microscopy requires a dedicated microscope and therefore can be expensive to implement. Multiple commercial systems are available.

Stimulated emission depletion microscopy

Principle

Stimulated emission depletion (STED) microscopy is based on laser scanning confocal microscopy where a second hollow beam (a donut-shaped STED beam) is overlaid on top of the excitation laser beam (Heine et al., 2017; Hell and Wichmann, 1994; Klar et al., 2000). In the zone of overlap between the two beams, the STED beam depletes the fluorophores before fluorescence takes place, thinning the emission area to a sub-diffraction sized spot. The donut-shaped STED beam is obtained using a vortex phase mask and the most recent systems are combining femtosecond lasers and detector time gating to improve the signal-to-noise ratio and significantly reduce the background (Hernández et al., 2015; Moffitt et al., 2011).

Versatility – 3/5

STED microscopy is very similar to, and has the same sample requirement as, classical confocal microscopy. STED does not need specific buffers, but special care should be taken during sample preparation (e.g. fixation) to ensure maximal preservation of cellular structures at the STED resolution (Blom and Widengren, 2017).

XY resolution – 4/5

In practice, in biological systems, the best resolution achieved are ~40 nm in living tissue and cells (Bottanelli et al., 2016; Willig et al., 2006), and 20 nm in fixed samples (Göttfert et al., 2013), and a typical ~60 nm resolution can be obtained in core facility settings. Recently, 1 nm resolution was achieved by using a hybrid microscopy technique combining STED and single-molecule localization microscopy (SMLM) (Balzarotti et al., 2017; Gwosch et al., 2020).

Z resolution – 4/5

In 3D STED, it is possible to also thin the emission spot along the Z-axis using a second phase mask, which provides up to a four-fold improvement in Z resolution when compared to point scanning confocal (Klar et al., 2000). For instance, one study achieved 90 nm in the Z dimension, while maintaining 35 nm in the XY dimensions (Osseforth et al., 2014).

Thick-sample friendliness – 2/5

Both the intensity and the geometry of the excitation and depletion beams are differently affected while traveling across the biological sample, leading to difficulties when imaging deep into tissues. This can be partially improved by using multiphoton excitation lasers, which allow for a deeper penetration imaging capability.

Live-cell friendliness – 3/5

Owing to the requirement for high-intensity illumination by the STED beam, this technique remains a challenge for live-cell imaging. From the various configurations available, depletion using a 775 nm laser line is the most live-cell friendly and has been shown to work for a panel of fluorescent proteins and probes

(D'Este et al., 2015). Gentler approaches called RESOLFT, based on photoswitchable probes, allow resolution enhancement with a lower light dose (Grotjohann et al., 2011; Masullo et al., 2018).

Image fidelity – 5/5

STED microscopy does not require post-processing of the images, which strongly limits the risk for artifact generation. Image quality and signal-to-noise ratio can however be further improved by photon reassignment using computational deconvolution.

Multicolor – 2/5

The number of fluorophores compatible with STED remains limited and many commonly used dyes are irreversibly bleached by the high intensity STED beam. In addition, in multicolor experiments, special care must be taken when selecting fluorophores to ensure that no overlap exists between their excitation spectrum and the depletion laser to improve the resolution of the other channels. Sequential scanning can be used as a workaround but acquisitions are limited to a single time point and focal plane.

Temporal resolution – 3/5

STED microscopy allows the user to directly visualize the object of interest in super resolution without the need for offline computation or post-processing of multiple images. It is therefore suitable for the imaging of fast cellular events, such as organelle dynamics and protein trafficking (Bottanelli et al., 2016).

Availability

STED is commercially available from two companies as complete setups, with a range of costs that makes them more suited to shared facilities.

Single-molecule localization microscopy

Principle

During single-molecule localization microscopy (SMLM) experiments, the emission of individual fluorophores are recorded using a camera. The resulting images are composed of diffraction-limited spots (typically ~200 nm in width) that are then fitted to precisely pinpoint the fluorophore position (typically within ~10–15 nm) (Diezmann et al., 2017). In practice, tens of thousands of images of blinking fluorophores are acquired in rapid succession (Jimenez et al., 2019). A processing software is then used to fit the blinking events and create a super-resolved image (Baddeley and Bewersdorf, 2018). To detect single molecules from densely labeled samples, only a small fraction of the fluorophores can be emitting photons at any one time (Li and Vaughan, 2018). This can be achieved by using sparsely activated photoactivatable/photo-convertible fluorescent proteins in (fluorescence-) photo-activated localization microscopy [(F)PALM] (Betzig et al., 2006; Hess et al., 2006). Organic dyes can also be induced to blink using specific buffers in (direct) stochastic optical reconstruction microscopy [(d)STORM] (Heilemann et al., 2008; Rust et al., 2006) or ground-state depletion (GSD) microscopy (Fölling et al., 2008). Alternatively, blinking can be generated by the transient interaction between two short DNA sequences, one labeled and one unlabeled, in so-called DNA point-accumulation in nanoscale topography (DNA-PAINT) (Jungmann et al., 2014).

Versatility – 2/5

Owing to its high spatial resolution, SMLM requires specific care during sample preparation to ensure optimal ultrastructural

preservation (Jimenez et al., 2019; Whelan and Bell, 2015). In addition, as single molecules are recorded, the density of labeling must be high enough to delineate the final structure of interest (Patterson et al., 2010).

XY resolution – 5/5

SMLM reconstructs images at 10 to 15 nm resolution. The final resolution achieved depends on the brightness of the fluorophores detected, their labeling density and the capacity to accurately detect individual fluorophores (Culley et al., 2018b). Importantly, at this scale, the size of the probe (antibody or fusion protein) can start to degrade the precision of the imaging (Magrassi et al., 2019).

Z resolution – 5/5

A common way of retrieving the Z coordinate of fluorophores is to deform the point-spread function into an ellipse using a cylindrical lens (Huang et al., 2008). This provides a typical Z localization precision of 20–30 nm (Diezmann et al., 2017).

Thick-sample friendliness – 2/5

SMLM is sensitive to light diffusion and spherical aberrations when imaging structures that are more than a few microns above the coverslip. More complex setups using light-sheet illumination schemes or adaptive optics allow to reach deeper in cells and tissue, but they are not yet broadly available (Liu et al., 2018).

Live-cell friendliness – 2/5

The time necessary to accumulate enough localizations and the high illumination intensities needed to visualize single molecules makes SMLM challenging to use on live samples (Tosheva et al., 2020; Wäldchen et al., 2015), although it has been performed (Huang et al., 2013; Jones et al., 2011).

Image fidelity – 2/5

The complexity of the required post-acquisition analysis (localization and image reconstruction) and the high precision attained by SMLM makes it prone to artifacts. Care must be taken to ensure proper quenching of fluorophores and to limit the density of their blinking.

Multicolor – 3/5

Multicolor remains a challenge for most SMLM strategies – photoactivatable and/or convertible proteins rapidly occupy all available channels in PALM (Shroff et al., 2007), and the photophysics of organic fluorophores makes it difficult to identify those that are spectrally distinct and have good blinking properties (Dempsey et al., 2011; Lehmann et al., 2015). However, DNA-PAINT allows for virtually unlimited sequential imaging of distinct targets, and is easily used for imaging of three to four colors (Jimenez et al., 2019; Jungmann et al., 2014).

Temporal resolution – 1/5

The necessity to acquire thousands of images for a single reconstruction is a strong impediment to fast acquisition in SMLM. Higher laser intensities and fast cameras can be used (Lin et al., 2015). Furthermore, the use of artificial intelligence shows promise in the ability to infer structure from a limited number of acquired images (Ouyang et al., 2018).

Availability

A regular epifluorescence microscope equipped with lasers is all that is needed to perform SMLM, with high-power lasers required

for STORM; several free options exist to process and analyze SMLM data (Jimenez et al., 2019; van de Linde, 2019).

Expansion microscopy

Principle

Expansion microscopy (ExM) is a sample preparation technique that physically increases the size of the specimen (Chen et al., 2015). Multiple variations of the ExM protocol have been described, but they all share a common workflow. After fixation, the sample is embedded and cross-linked to a swellable gel that is then expanded using water (Wassie et al., 2019). The resulting enlarged specimen can then be imaged using classical microscopy techniques (Chen et al., 2015; Chozinski et al., 2016; Ku et al., 2016; Tillberg et al., 2016).

Versatility – 3/5

ExM has been successfully applied to a wide variety of samples, including cells (Chen et al., 2015), tissue sections (Zhao et al., 2017) and model organisms (*Drosophila* and zebrafish embryo) (Cahoon et al., 2017; Freifeld et al., 2017), as well as whole intact organs (Gao et al., 2019; Ku et al., 2016). ExM has also been used to observe RNA (Chen et al., 2016) and lipids (Karagiannis et al., 2019 preprint). Each new application of ExM needs careful and specific optimization.

XY and Z resolution – 4/5

The final 'resolution' of the image depends on the final size of the expanded sample, as well as on the microscopy strategy used to acquire the images. ExM protocols typically lead to a 4.5-fold expansion in all dimensions (an expected resolution of ~70 nm), but others attain a 10-fold expansion (an expected resolution of 25–30 nm) (Truckenbrodt et al., 2018). Samples can also be expanded sequentially (an expected resolution of ~25 nm) (Chang et al., 2017). ExM is also compatible with other super-resolution modalities, including STED (Gao et al., 2018), SMLM (Shi et al., 2019 preprint) and SIM (Cahoon et al., 2017; Halpern et al., 2017).

Thick-sample friendliness – 5/5

ExM can be used on thick samples, such as small model organisms or whole mouse organs (Cahoon et al., 2017; Freifeld et al., 2017; Gao et al., 2019; Ku et al., 2016). However, it is important to note that post-expansion, samples will be at least 100-fold more voluminous and, therefore, they can be challenging to image at high magnification using classical microscopes. Light-sheet microscopy is especially well suited for imaging such samples (Gao et al., 2019).

Live-cell friendliness and temporal resolution – 0/5

ExM is only compatible with fixed samples.

Image fidelity – 3/5

ExM leads to an isotropic expansion and has been shown to preserve the structural integrity of the various cellular structures imaged. In addition, using the nuclear pore as a reporter, ExM was found to have a uniform accuracy in the range of 20 nm (Pesce et al., 2019). Nevertheless, one concern that remains is whether all the structures of interest within a sample expand at the same rate or to the same extent. For instance, in bacteria, the expansion efficacy varies across species (Lim et al., 2019). Moreover, the digestion procedure typically attenuates the fluorescence of organic or protein-based fluorophores, and the expansion itself dilutes the fluorescent signal spatially (a 4-fold expansion results in 64 times less fluorescence per volume unit), requiring sensitive microscopes for ExM sample imaging.

Multicolor – 5/5

ExM is compatible with standard dyes and fluorescent proteins and is therefore well adapted to multicolor experiments.

Availability

ExM sample preparation only requires commercially available reagents and is therefore readily available; it is also relatively inexpensive to implement (Asano et al., 2018).

Conclusions – going above and beyond

Regardless of the technology used to acquire the fluorescent signal, powerful signal post-processing methods, such as deconvolution or image restoration, can be used to increase the resolution of the final image. Various algorithms have been implemented directly in the acquisition software commercially available, while others, for instance SACD (Zhao et al., 2018 preprint), can be run independently of the pipeline. Another promising development is the use of artificial intelligence to improve the quality of the final image; here, large datasets of high-resolution images are used to train neural networks that can then be applied to low-resolution and/or noisy datasets (Belthangady and Royer, 2019; Moen et al., 2019; von Chamier et al., 2019; von Chamier et al., 2020 preprint). The resulting image gains a dramatic resolution improvement thanks to the prior knowledge of the structure obtained from high-resolution images.

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Supplementary information

Supplementary information available online at <http://jcs.biologists.org/lookup/doi/10.1242/jcs.240713.supplemental>

Cell science at a glance

A high-resolution version of the poster and individual poster panels are available for downloading at <http://jcs.biologists.org/lookup/doi/10.1242/jcs.240713.supplemental>

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