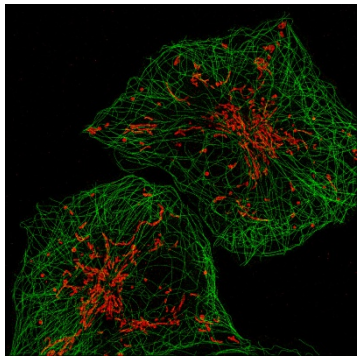
IMAGING

Extending super-resolution's FOV

An optimized illumination strategy allows for uniform single-molecule localization microscopy over large regions.

Single-molecule localization microscopy (SMLM) methods, such as photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), are becoming well established for their ability to generate super-resolution images of cells. However, one limitation of most current implementations of SMLM is the field of view (FOV), which is generally restricted to an area of ~50 × 50 μm^2, or about the size of one mammalian cell, because of technical challenges related to uneven illumination.



Large-FOV SMLM image of mammalian cells. Adapted from Douglass *et al.* (2016) with permission.

In imaging done with a standard expanded laser beam, illumination is stronger in the center and weaker at the edges of the image. This uneven illumination means that the fraction of fluorophores that are active will be different in different parts of the image, which ultimately affects the uniformity of the resolution and quality in the final image.

Suliana Manley, Kyle Douglass (a postdoctoral researcher in her lab) and their colleagues at École Polytechnique Fédérale de Lausanne sought to overcome these issues and develop a uniform illumination system that would enable imaging of large FOVs. The researchers recall being motivated by biological experiments requiring images of many cells. "With our new system we are able to image hundreds of HeLa cells with ~20-nanometer resolution in a single afternoon, something that would previously have taken weeks of hard work," says Douglass.

Their solution to the problem came in the form of a microlens-array-based epi-illumination system called flat illumination for field-independent imaging, or FIFI. According to the researchers, the work was inspired by beam-shaping used in UV lithography, which led them to insert a pair of microlens arrays into the illumination path of the microscope, forming what is known as a Köhler integrator. The team optimized the setup using custom computer simulations, which they note are freely available online. The end result of this design was 'flat' and even illumination of large areas.

Once the illumination system was developed and optimized, they began STORM imaging of biological samples, including COS-7 cells with labeled mitochondria and tubulin, and large fields of bacterial cells. Their results highlight the unprecedented size of the images obtainable with FIFI—according to the authors, theirs are the largest known localization microscopy images to date, with uniform resolution of around 20 nanometers across the entire FOV (120 × 120 μm^2).

Now that the FIFI system is available to acquire large amounts of data, a new problem has arisen: handling such data. "Because of the historically low throughput of these methods, the computational tools to store, process, and interpret large amounts of localization microscopy data are nowhere near as mature as other microscopy modalities, such as light sheet or cryo-EM," notes Douglass. The team thinks the next step will be developing the hardware and software tools needed to manage the "deluge" of data being generated.

Manley also hopes that it will be easy for other researchers to implement the FIFI system in their laboratories. Toward this end, the authors have released the design scripts and alignment guide along with the paper. They do note, however, that FOV is ultimately limited by laser power, and that they had to buy a more powerful laser to enable the large-FOV imaging they achieved. Overall, this work should enable high-quality, large-FOV super-resolution imaging and greatly improve the throughput of PALM/STORM.

Rita Strack

RESEARCH PAPERS
Douglass, K.M. *et al.* Super-resolution imaging of multiple cells by optimized flat-field epi-illumination. *Nat. Photonics* **10**, 705–708 (2016).