What Is Super Resolution Microscopy?

Light microscopes are used to observe and image objects that are too small for the human eye to see properly. However, there is also a limit to what these microscopes can see, as they are limited by the diffraction limit of light and can only observe/image samples that are larger than approximately 200 nm in size.

Human cells are typically 10 µm in diameter, meaning that microscopes can easily observe them but smaller components inside the cell (mitochondria, nucleus etc.) and other organisms like bacteria and viruses can be over 100x smaller, and other samples like DNA and proteins can be smaller still. If researchers want to observe these small samples they can't do it with normal 'diffraction-limited' microscope techniques, and need techniques that can break through this diffraction limit: **'super resolution'** techniques.

The Diffraction Limit Of Light

The smallest point that can be seen with a diffraction-limited system is called an Airy disk, named after the mathematician George Biddell Airy. Because light travels as a wave, when it passes through the small microscope aperture the light spreads out into a diffraction pattern, spreading the light out over a wide area, as seen in **Fig.1**.

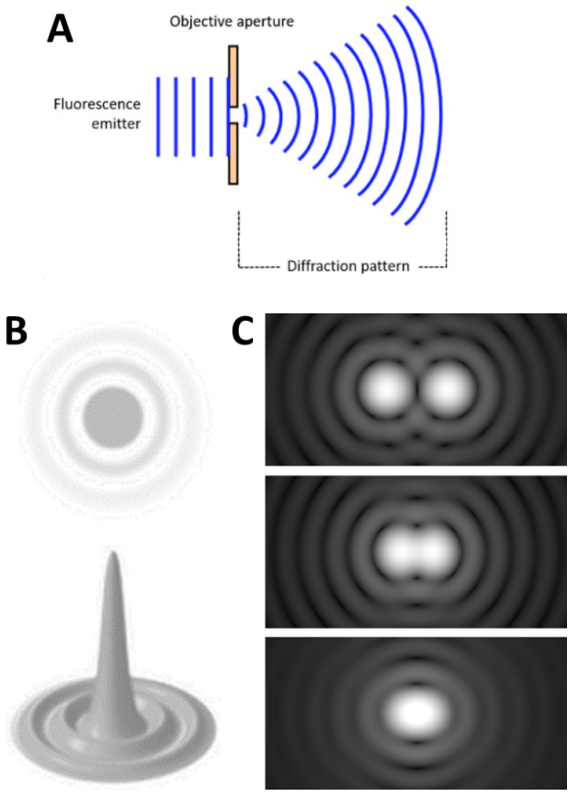

Figure 1 Diffraction patterns of light. A) After light passes through a small space, the diffraction pattern spreads out to a much wider area. B) Airy disks, shown in 2D (top) and 3D (bottom, height shows light intensity). C) If two Airy disks are close, they can merge and be seen as one. Top image shows two disks that can be seen as separate, middle image shows the Rayleigh limit before merging, bottom image shows two disks that are too close to distinguish between.

The spreading light forms an Airy disk **(Fig.1B)** and illuminates everything across a large area, making it impossible to pick out smaller sub-200 nm sized structures. Statistics can be used to convert the Airy disks into smaller points: because an Airy disk is brighter at the center, a point is fitted to the brightest spot. This point can be accurate down to a single nanometer which can improve image quality **(Fig.2)**.

However, the main issue is highlighted in **Fig.1C**: if two Airy disks get too close together they can merge into one, making it impossible to tell close objects apart. These merged disks are counted as one data point and information is lost, even with the statistical fitting. Microscope samples contain millions of molecules that can be various distances apart, if they are too close they will be observed as a single point instead of multiple points. Only with super resolution techniques can these samples be properly imaged.

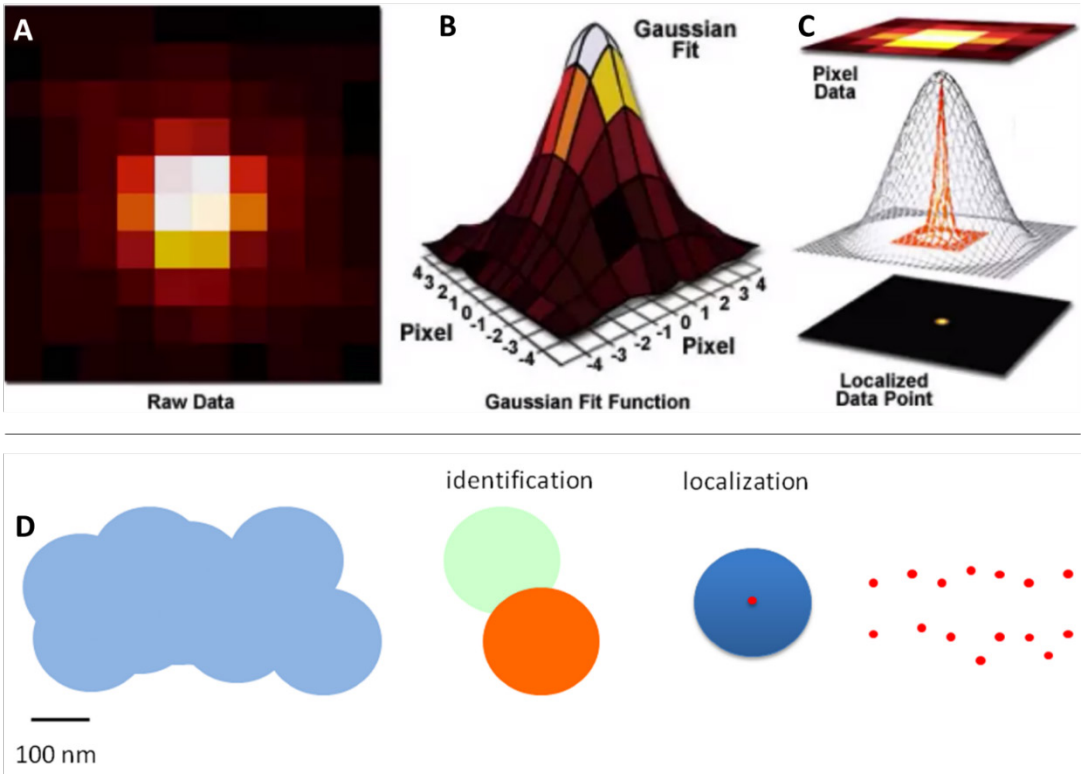

Figure 2 Localizing a single point from an Airy disk. A) A pixelated Airy disk, this is how the raw data appears. B) By applying a statistical function, the pixels can be fit to the intensity of the light, indicating the spot of most intense light. C) The data can be used to localize the central data point where the fluorophore is located, potentially down to ~1 nm. D) A schematic of this occurring across multiple Airy disks. Without super-resolution techniques, these disks are likely to merge and be counted as less points than shown.

Super resolution techniques

Super resolution techniques use a variety of methods to break the diffraction limit of light, and can be broken down into a few main categories.

Localization techniques overcome the problem of overlapping fluorophores by using fluorescent molecules that switch on and off at random. By taking thousands of images enough data is captured where all the molecules would have been "on" at least once. Molecules close together in space, are unlikely to both be "on" at the same time in the same image frame and can therefore be separated by time.

Techniques in this category include: **PhotoActivated Localization Microscopy (PALM)** (seen in **Fig.3A-B**), **STochastic Optical Resolution Microscopy (STORM)** (seen in **Fig.3E-G**), and **DNA-based Point Accumulation for Imaging in Nanoscale Topography (DNA-PAINT)** (seen in **Fig.3C-D**).

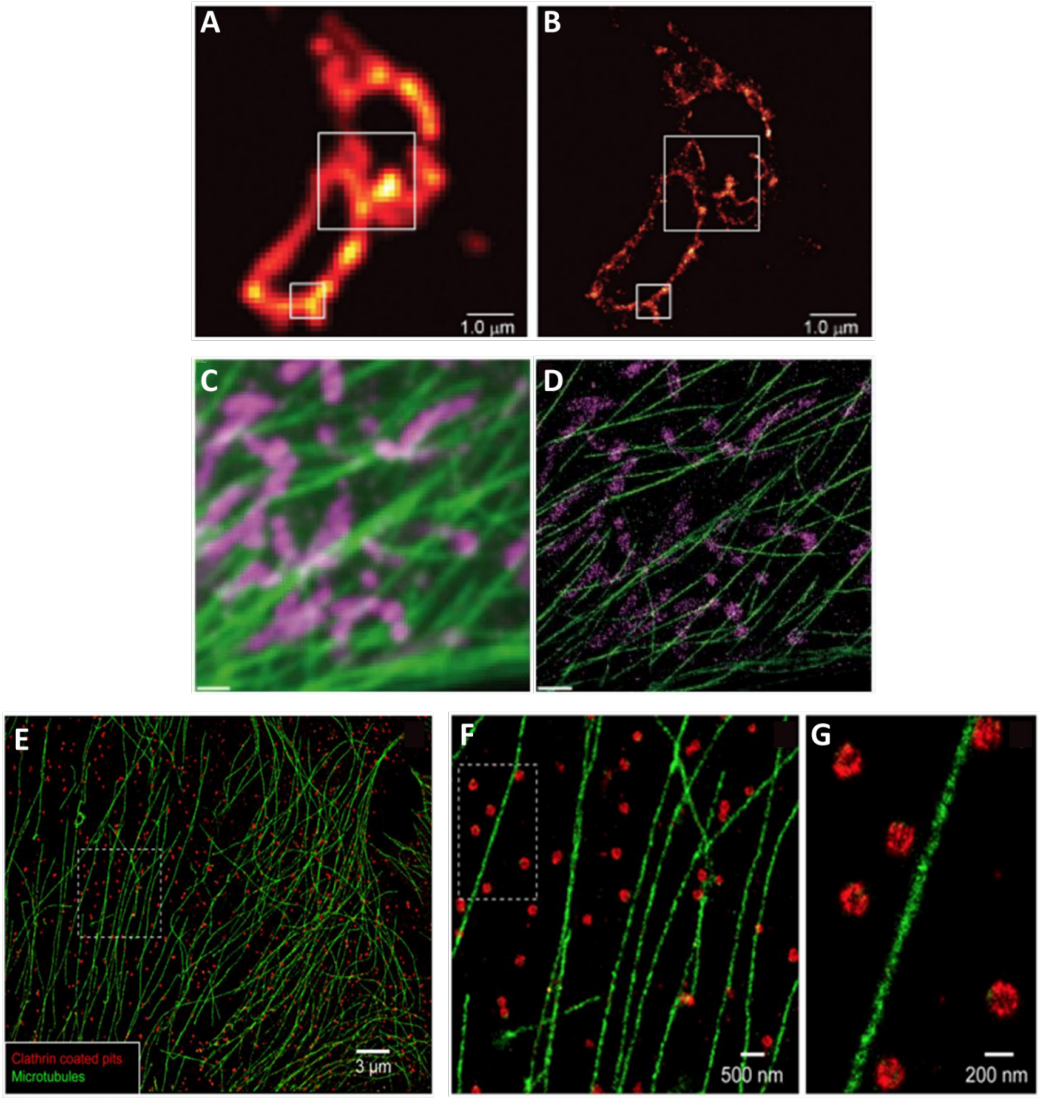

Figure 3 Images from different super resolution localization techniques, compared to standard microscopy. A) Standard microscopy and B) PALM images of transmembrane proteins (image from Betzig *et al.* 2006). C) Standard microscopy and D) DNA-PAINT images of mitochondria (magenta) and microtubules (green) from within a cell (image from Jungmann *et al.* 2014). E/F/G) Two-color STORM images of microtubules (green) and clathrin-coated pits from within a mammalian cell at increasing magnifications, area indicated by dashed box on previous image (image from Bates *et al.* 2007).

Structured light techniques use patterns of light instead of normal illumination. The best known technique in this category is **Structured Illumination Microscopy (SIM)**. When two patterns are overlaid it creates an interference pattern called a Moiré pattern, by imaging through these Moiré patterns super resolution images can be achieved. A useful variation of SIM is **instant SIM (iSIM)** which is up to 10,000x faster than PALM/STORM, and uses patterns of light that are scanned across the sample. Examples of iSIM images are shown in **Fig.4**. This technique is similar to **Spinning Disk Confocal Microscopy**, which can also achieve super resolution imaging.

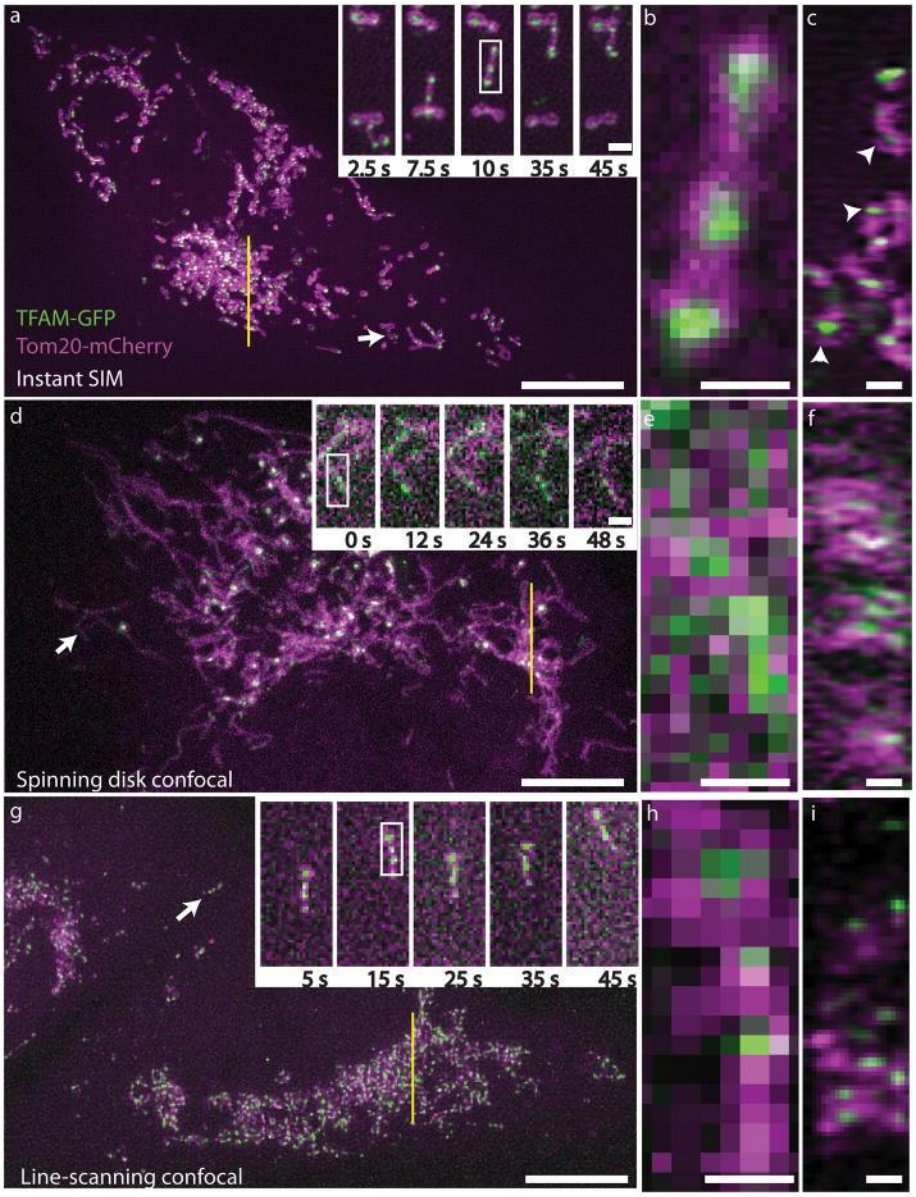

Figure 4 Imaging with iSIM vs confocal microscopy. Images of live human cells showing peroxisomes in green and mitochondria in purple. A-C) iSIM images at different magnifications, D-F) Spinning disk confocal images at different magnifications, G-I) Line scanning confocal images at different magnifications. A good comparison of standard vs super resolution images is seen from B (super resolution) vs E+H (standard).

Summary

By breaking through the diffraction limit of light, super resolution microscopy can image small samples with high resolution, pushing the boundaries of scientific research.