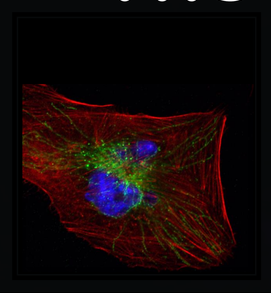
The Perfect STORM: Super Resolution Imaging of Cellular Structures



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Introduction

- The resolution of a conventional light microscope is limited by the wave like nature of light and is described by the Abbe equation
- Diffraction limit XY = wavelength imaged / 2 x Numerical Aperture of the lens used
- Stochastic Optical Reconstruction Microscopy (STORM) has the potential to image structures below the diffraction limit, tenfold improvements in resolution have been reported by others
- STORM microscopy has the potential to allow biologists to study structures and processes in amazing detail without the need to use more complicated imaging approaches such as electron microscopy

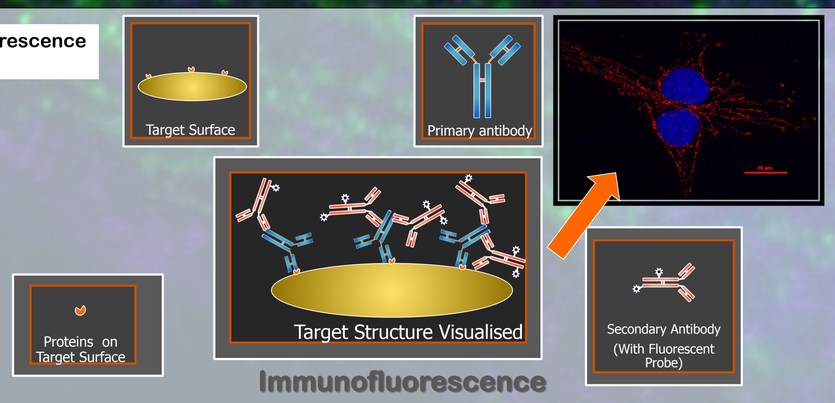
Aims

- To characterise various antibodies and fluorescent probes for use in super resolution imaging
- To image cells using a variety of microscopy techniques and evaluate the resolution limitations of each
- To create the perfect conditions for an ideal STORM image

Methods & Imaging Techniques

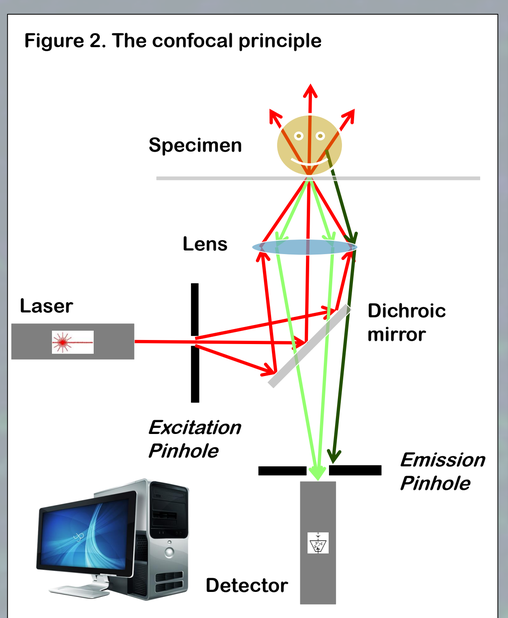
- Fibroblast cells were used for the various imaging techniques
- Cells were fixed using paraformaldehyde and permeabilised using Triton X100
- Cytoskeletal (Actin) and Mitochondrial proteins (TOM-20) were probed with labelled phalloidin and conventional immnofluorescence techniques (figure 1)
- Cells were examined using confocal microscopy and super resolution imaging techniques.
- Full width half maximum measurements were taken for actin filaments imaged using the different imaging techniques

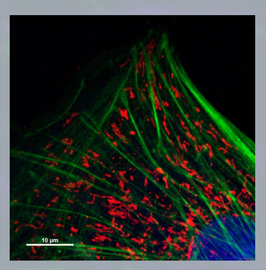
Figure 1. Immunofluorescence imaging techniques

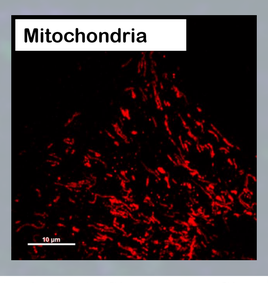


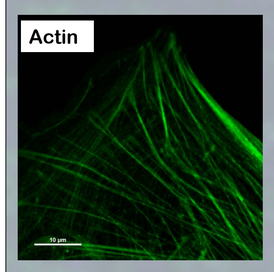
1. Confocal Microscopy

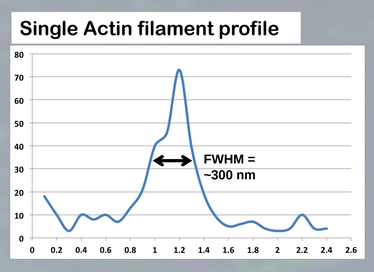
Figure 2. The confocal principle





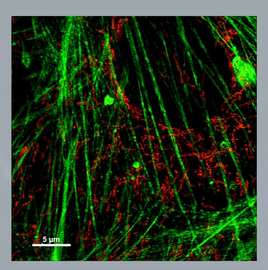


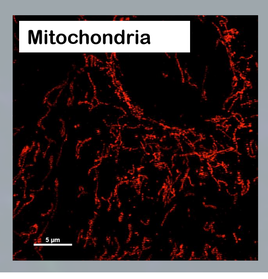


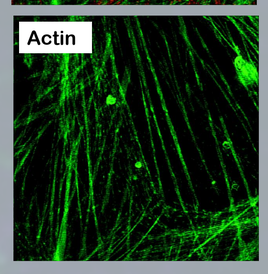


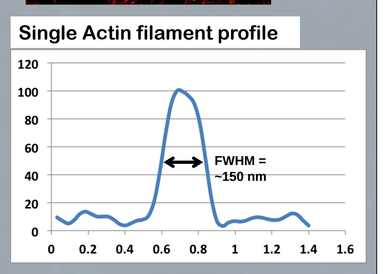
✧ Resolution is limited by the wavelength of light ~250-300 nm imaging at ~520 nm

2. N-SIM (Structured Illumination Microscopy)



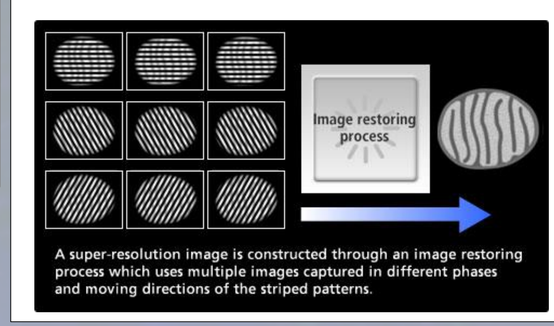






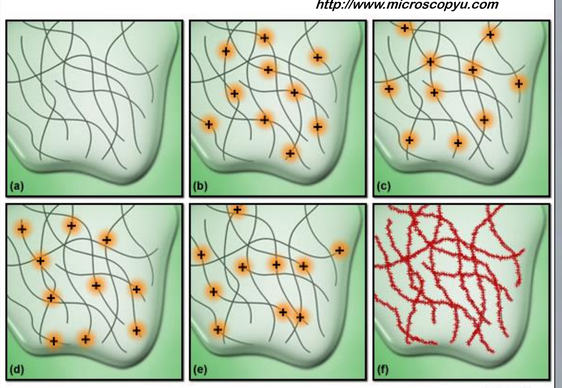
✧ two-fold improvement in resolution over confocal microscopy

Figure 3. Structured illumination (SIM) principle
"*The Moire effect*"

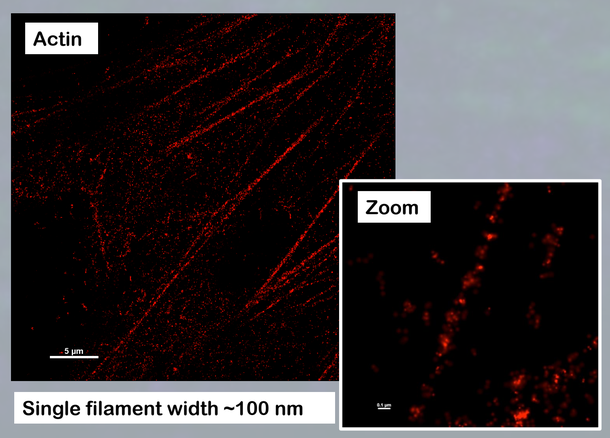


3. N-STORM (Stochastic Optical Reconstruction Microscopy)

Figure 4. STORM imaging



a) densely labeled filamentous network of intracellular structures *e.g.* cytoskeletal actin
b) a sparse set of the fluorescent probes are activated to produce single-molecule images (represented by orange circles) that do not overlap.
After capturing the images with a digital camera, the point-spread functions of the individual molecules are localized with high precision based on the photon output before the probes spontaneously photobleach or switch to a dark state. The positions of localized molecular centers are indicated with black crosses.
c-e) The process in b) is repeated through until all of the fluorescent probes are exhausted due to photobleaching.
f) Final super resolution image is constructed by plotting the measured positions of the fluorescent probes.

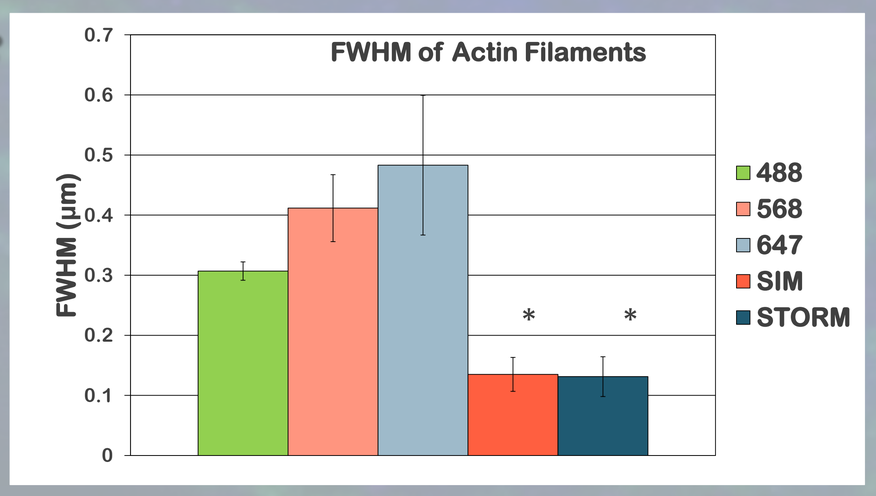


Single filament width ~100 nm

✧ Further improvement in resolution (three- fold)
✧ Ten-fold improvements have been achieved by others (~20 nm)

Results

- Full Width at Half Maximum pixel intensity profiles were measured for actin filaments



- Confocal Resolution: 300 nm, 400 nm & 490 nm imaging at 520, 600 and 700 nm
- N-SIM Resolution: 135 nm imaging at 520 nm
- N-Storm Resolution: 131 nm imaging at 700nm

Conclusions

- Resolution decreases as wavelength increases for optical microscopes
- N-STORM offers the best resolution of the various imaging techniques we tested, and theoretically is unaffected by the wavelength of light
- Fluorophores and buffers needed to produce STORM images can be improved

Acknowledgements

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