Correlative super resolution using dSTORM and SIM

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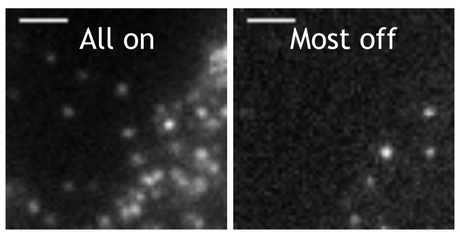
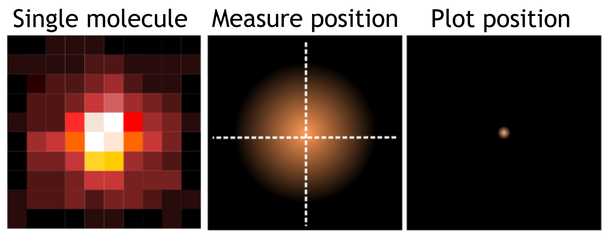
Super-Resolution Microscopy dSTORM

Recently, super-resolution microscopes have been developed, which can overcome the diffraction limit. One method is dSTORM (direct stochastic optical reconstruction microscopy), which can achieve resolutions up to 10 times better than traditional optical microscopes.

(1) Instead of having all the fluorescent molecules on, switch most of them off.



(2) Measure the middle position of each fluorescent molecule and plot its position.

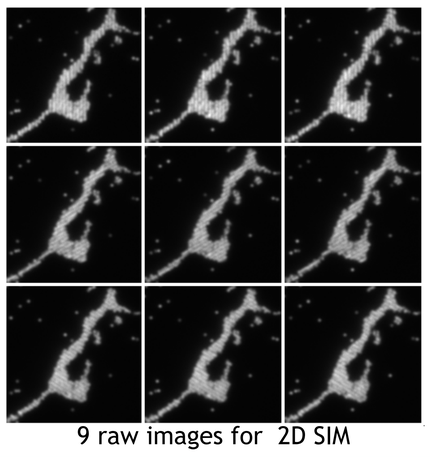


(3) Switch a different subset of the fluorescent molecules on and repeat steps 1 and 2 until all of them have been plotted. Typically this is done on 10000 or more frames.

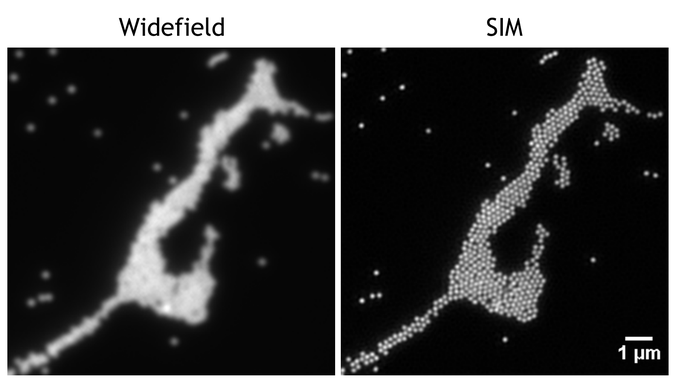
(4) Put together a super-resolution image using all the plotted molecular positions from the previous steps. Each plotted position is called a localisation and is represented by a super-resolution pixel. The brighter a pixel is the more localisations there are within that area.

SIM

Structured Illumination Microscopy (SIM) achieves resolution at twice the diffraction limit by exciting the sample with a known periodic field. High resolution information normally beyond the passband of the microscope is mixed into the observable passband. The high resolution information is recovered by taking multiple images with translated illumination patterns and solving a system of linear equations.



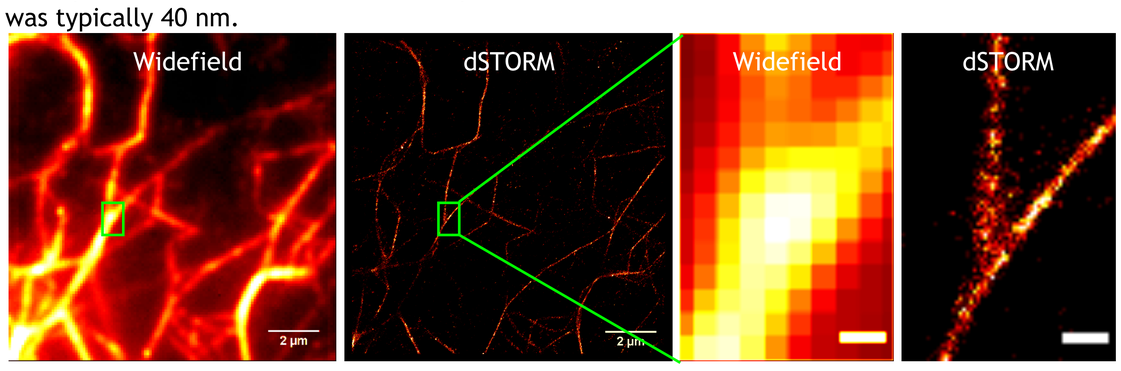
9 raw images for 2D SIM



A monolayer of 170 nm diameter fluorescent beads

Actin Filament Test Sample dSTORM

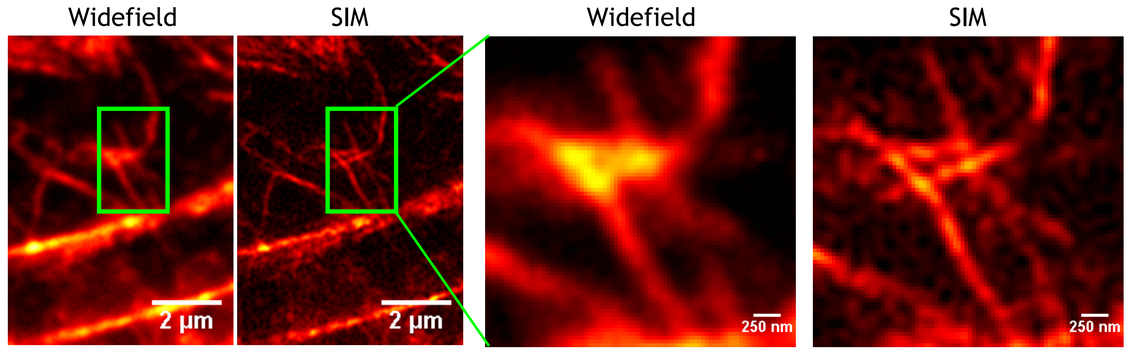
The dSTORM system was tested using a known test sample. Actin filaments were selected as these have a uniform diameter of 7 nm. A method for assembling and staining these filaments onto glass was optimised. The mean localisation preciesion was around ~30nm and the FWHM of the fibres was typically 40 nm.



▲ Fluorescently labelled actin filaments. Boxed regions are shown in the images on the right. Scale bars are 200 nm. dSTORM pixel size is 15 nm. Widefield pixel size is 100 nm.

SIM

To test our SIM system we imaged 170nm diameter fluorescent beads (shown above) and actin filaments in HeLa cells (below). Both samples show the expected increase of the passband of the microscope beyond the Abbe limit. FWHM of the actin fibres was typically 130nm.



▲ Fluorescently labelled actin filaments. Boxed regions are shown in the images on the right. SIM pixel size is 34 nm. Widefield pixel size was 68 nm but has been upscaled to 34 nm for better comparison.

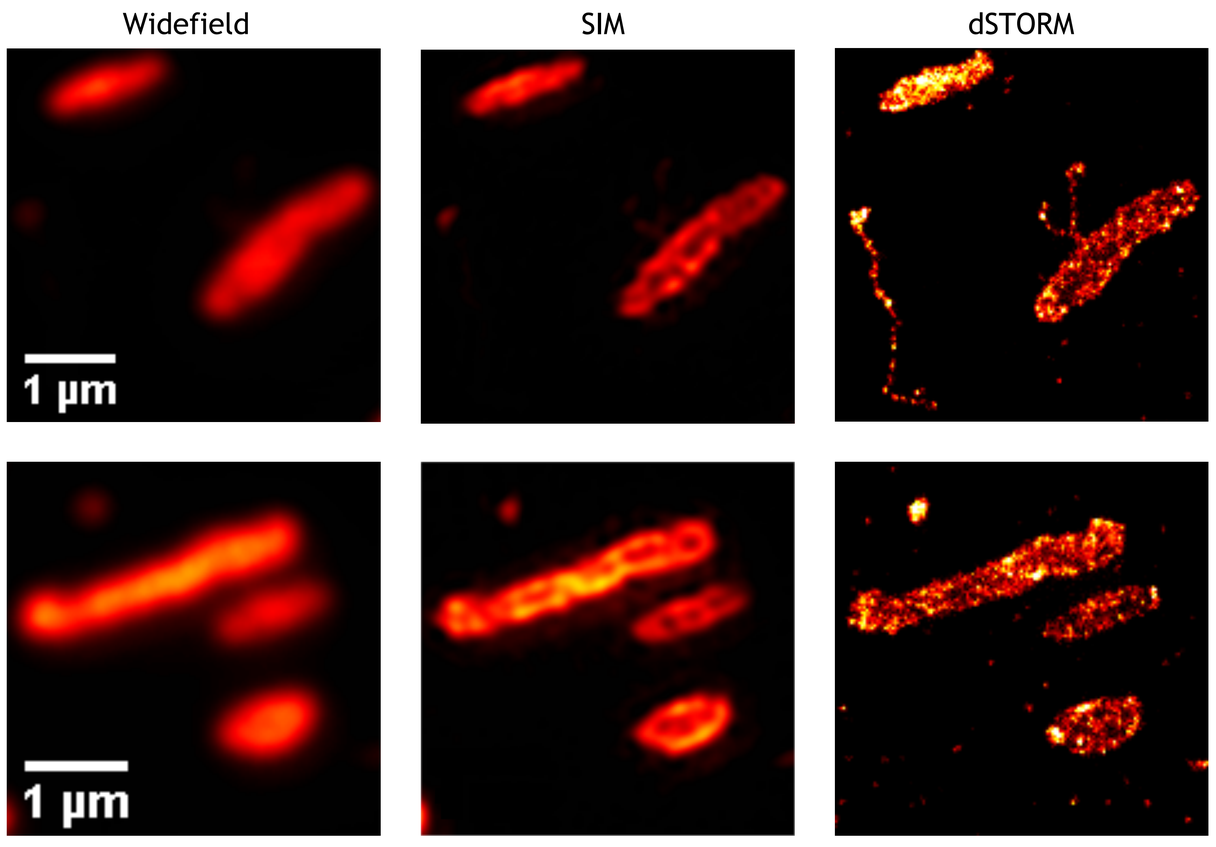
Correlative dSTORM and SIM

To perform correlative dSTORM we used dual immunolabeled samples prepared on gridded coverslips. Although a single label would provide better correlation, using a different label for each technique allows more flexibility in imaging sequence and reduces bleaching effects from multiple imaging. Once a sample was imaged on our dSTORM system the appropriate area was located and imaged on our SIM system.

A complication introduced in the processing stage is that the produced images do not have the same pixel size and may also be rotated with respect to each other. In order to register the images we padded the SIM image in Fourier space to obtain the correct pixel size and then performed sub-pixel image rotation and translation until the image corrrelation was maximsed.

After testing using fluorescent microspheres and actin fibres we performed correlative dSTORM and SIM on Weibel Palade Bodies (storage organelles of endothelial cells) by immunolabeling Von Willebrand factor with Alexa 647 (for dSTORM imaging) and Alexa 488 (for SIM imaging). This sample was chosen for its varying density and fine scale internal structure.

A striking result is the different response to fluorophore density. SIM images respond linearly to fluorophore density whereas dSTORM is non-linear and dependent on the reconstruction algorithm used (here we used rainSTORM). This non-linearlity can result in the supression of high density and amplification of low density regions. As such using a combination of linear and non-linear super resolution responses to fluorophore density can reveal more than using one technique alone.



Conclusions

Super resolution techniques such as dSTORM and SIM not only differ in their spatial and temporal resolution but also in their response to fluorophore density and spatial frequencies in the sample. Localisation microscopy particulary excels in sample regions that have low fluorophore density, whereas SIM reponds linearly to density albeit at a lower resolution. Potentially, correlated images taken with multiple techniques could be combined into one image, each technique enhancing different regions of the sample under observation.

Acknowledgements

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