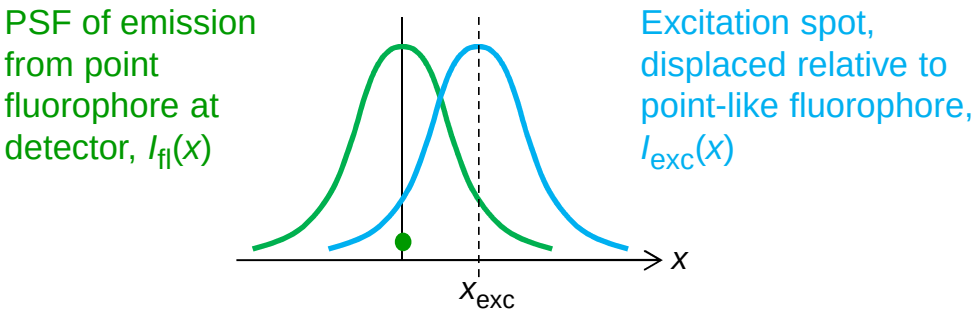
Supplementary Note: The super-resolution mechanism of microlenses aligned with scanned excitation and emission

York et al. [1,2] explain that acquiring images in a scanning confocal arrangement, shifting each image by ½ the displacement of the corresponding pinhole and summing them results in super-resolution imaging, with a twofold improvement in resolution over widefield microscopy. In the iSIM, this acquisition, shifting and summing takes place over the course of one camera exposure, as the scanning mirror sweeps the excitation pattern across the sample, and the resulting emission across the camera.

Here, we further elucidate how the microlenses and beam scanning combine to result in the shifted and summed intensities required for super-resolution. (Image acquisition occurs after the pinhole array (the “macropinholes” [2]) have rejected out of focus emission.)

Scanning excitation, without microlenses:



The PSF of light emitted by a point fluorophore has a point-spread function at the detector, $I_{fl}(x)$.

Assuming emitted power to be linear with excitation power, with a fluorophore imaged at $x = 0$ on the detector, for any given position of the excitation pattern,

$$I_{det}(x) = I_{fl}(x) \times I_{exc}(x = 0),$$

where $I_{det}(x)$ is intensity at the detector and $I_{exc}(x)$ is the distribution of the excitation pattern, as it would be imaged at the detector.

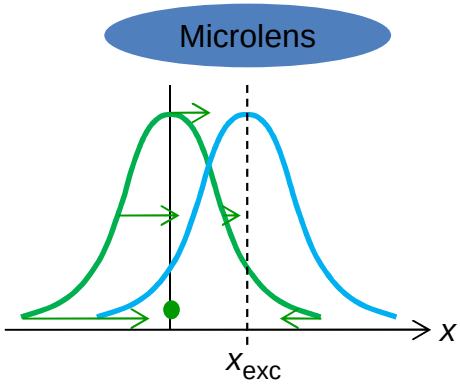
The image of the fluorophore position remains stationary on the detector while the excitation pattern is scanned across the object. In one exposure, the detector signal is summed over the different central positions of the excitation pattern, x_{exc}, so

$$I_{det}(x) = I_{fl}(x) \times \int I_{exc}(x = 0)\, dx_{exc},$$

which has the same distribution as $I_{fl}(x)$, so no resolution is gained (since $\int I_{exc}(x = 0)\, dx_{exc}$ is a constant).

In iSIM, a microlens is aligned to each excitation spot:

The axial position of the microlens causes the PSF of the fluorescent emission to be contracted by a factor of 2 towards the centre of the excitation spot (x_{exc}).



So at any x_{exc} of the excitation spot, each x of the fluorescence PSF is transformed to $(x + x_{exc}) / 2$. In other words, after contraction,

$$I_{fl}(x) = I_{fl}'(2x - x_{exc}),$$

where $I_{fl}'(x)$ is the PSF that would have been observed without the microlens in place. Therefore, for that x_{exc},

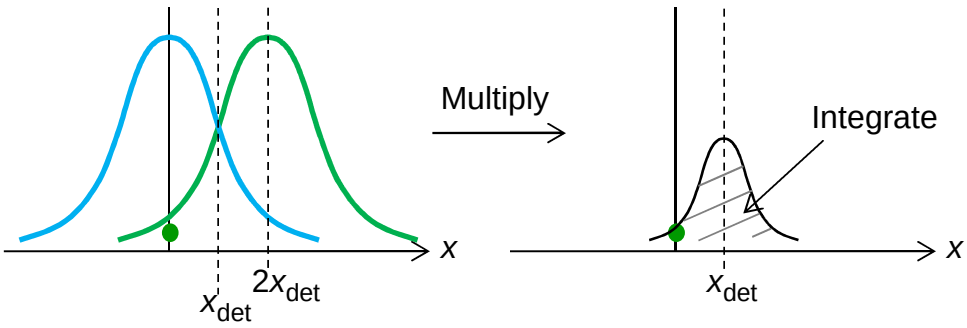
$$I_{det}(x) = I_{fl}'(2x - x_{exc}) \times I_{exc}(x = 0).$$

When we sum the fluorescence signal resulting from each excitation position, as it is scanned across the fluorophore, the camera signal at x,

$$I_{det}(x) = \int \{ I_{fl}'(2x - x_{exc})) \times I_{exc}(x = 0) \}\, dx_{exc}.$$

As x_{exc} is varied, this is equivalent to an emission PSF (without microlenses) centred at $2x$ and this is equivalent to an excitation PSF centred at 0,

and their product is the theoretical PSF of a confocal microscope with a nearly-closed pinhole displaced $2x$ from the fluorophore.



i.e. at a detector pixel displaced from an ideal fluorophore image by x_{det}, the microlens, combined with scanned excitation, has generated the intensity that would be recorded using a nearly-closed pinhole at $2x_{det}$. Therefore, the system has performed the “micro-pinholing” and scaling as described by York et al. [1,2].

References

[1] A.G. York et al., Nature methods, 9 (2012) 749-754.
[2] A.G. York et al., Nature methods, 10 (2013) 1122-1126.