Supplementary Information

1 S1 - Determining the point spread function in confocal system

In this section, we investigate the point spread function using the imaging parameters on the FV3000 confocal system, which were employed for embryo imaging. Phantoms (200 nm fluorescence beads) were generated as described in the methods section of the main manuscript. Figure 1 shows the maximum intensity profile for a single bead and the line profile through it in the XY and YZ plane. As the imaging parameters generated images with a spatial resolution of 0.621 nm per pixel, the 200 nm beads were effectively limited to a single pixel, and consequently, the details of PSF could not be accurately resolved due to undersampling. To overcome this, we implemented Nyquist's theorem by increasing the optical zoom on the confocal system while maintaining all other parameters identical to that used for embryo imaging, increasing the spatial resolution from 0.621 nm to 0.13 um per pixel. This Nyquist-optimised imaging parameter was used for PSF quantification of phantoms, as shown in Figure 2 within the main manuscript.

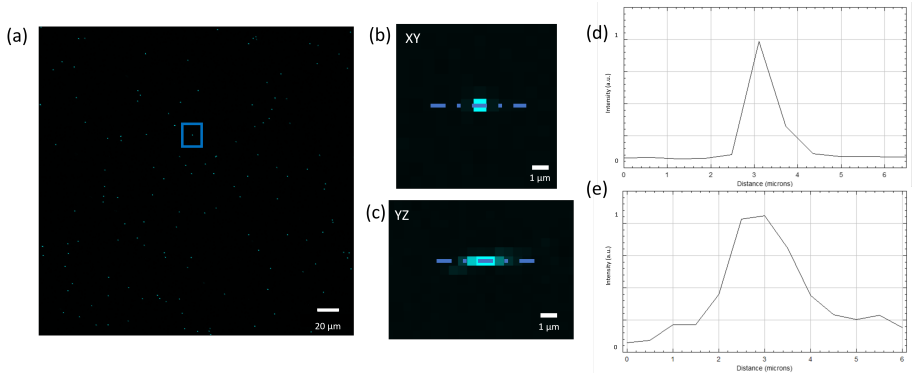

Figure 1: (a) Maximum intensity projection of 200 nm diameter fluorescence beads embedded in agarose imaged using confocal microscopy. Imaging parameters were the same used for embryo imaging. (b) and (c) show the intensity projection in xy and yz plane of a single bead as outlined in (a). (d) and (e) show the corresponding line profile across the bead in xy and yz plane respectively.