Clearing the Haze
A Computational Method for Fast Optical Sectioning on the Mesolens Using HiLo Microscopy

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We have developed a computational method for optical sectioning in widefield fluorescence microscopy on the Mesolens, a recently presented objective lens with a large **field of view (~5mm)** and high **numerical aperture (0.47)** (McConnell et al. 2016), allowing **subcellular resolution (700nm)** on the full field of view. The method is based on HiLo microscopy (Lim et al. 2011), using two images, one uniformly illuminated and one with structured illumination, to generate optical sections comparable to confocal laser scanning microscopy limited only by the acquisition speed of the CCD camera detector. The optical sectioning is achieved by extracting information from the fluorescence response of the structured illumination image and subsequently filtered to only allow the in-focus information to propagate to the final image. With this method in its current implementation, optical sections of 10µm thickness, close to matching the axial resolution of the Mesolens of 7µm, could be easily achieved at up to **60 times faster acquisition** compared to Laser Scanning Confocal Microscopy. Additional computation time is required which varies with size of the images, however, this is done post acquisition and has no negative effect on the time required for imaging. We aim to use this method to observe marine invertebrates in large, unrestricted volumes (5mm x 5mm x 3mm) and investigate the impact of environmental factors on their motility (Chan et al. 2016). Optical sectioning will help to determine more accurately the orientation of the organisms and thus the evaluation of their motility under the influence of negative environmental factors, e.g. water (ocean) acidification.

References

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