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Correlating Multi-Pass Microscopy and Transmission Electron Microscopy for Biological Materials

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The aim of this work is to develop workflows for higher-contrast imaging with reduced specimen damage at the optical microscope. Because biological samples are weak phase objects and photons produce shot-noise, the prevailing method of increasing signal-to-noise and hence contrast has been to increase illumination at the risk of damaging the biological target. An optical wide-field, continuous wave multi-pass system has been described [1], where low-intensity illumination is passed through the sample a discrete number of times (up to m=4) to provide enhanced SNR. An enhancement in SNR was first demonstrated on red blood cells (6-8 μm). To further calibrate, we chose to image a strain of mini-cell-producing *E. coli* deficient in the minC gene product. These cells divide improperly, producing small (0.2-0.45 μm) cell-like entities. Their small size but intact membrane structures have been useful in cryo tomography studies [2]. Here the aim is to use them to calibrate what is visible in the multipass system by correlating with the same samples imaged by TEM. Not only do the cells provide a target for imaging, but their many pili and flagella can be clearly seen by TEM and can help to fine tune the multipass focusing and resolution. Initial results comparing normalized line scans of these bacteria show an increase in SNR under low illumination conditions. Additionally, their interaction with RBCs, and subsequent hemolysis, is a dynamic process that can be viewed in real-time, thus providing an important *in operando* test of this non-destructive method of imaging.

References:

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