

VIDEO-RATE REMOTE-REFOCUSING THROUGH CONTINUOUS OSCILLATION OF A MEMBRANE DEFORMABLE MIRROR

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There are numerous applications in microscopy where it is desirable to refocus a high numerical aperture objective lens rapidly [1]. In this work, an Alpa DM97-15 membrane deformable mirror was used to refocus a 40×/0.85 air objective and a 40×/0.80 NA water-immersion objective through a defocus range of -50 to 50 μm at 26.3 sawtooth sweeps per second (Figure 1). The ultimate aim is to use this deformable mirror to achieve video-rate volumetric imaging in a light-sheet fluorescence microscope. Such deformable mirrors have been used for rapid refocusing before [2], but are known to exhibit viscoelastic creep and temperature dependent variations in the mirror response [3]. In this work, viscoelastic creep was avoided by ensuring that the temporal average of the surface applied to the mirror was flat over timescales comparable to the creep time constant. Optimisation of the mirror surface to correct for the high-NA defocus of the objective was performed with the mirror continuously refocusing at the desired refocus sweep rate. An initial warm-up period of 5 minutes of oscillation was used to allow thermal effects to stabilise prior to the start of the mirror optimisation procedure. The PSF across the FOV and as a function of defocus was characterised by imaging a mask of 1 μm holes, achieving a mean Strehl metric greater than 0.6 over a 200×200 μm^2 field of view in the sample and an 80 μm defocus range.

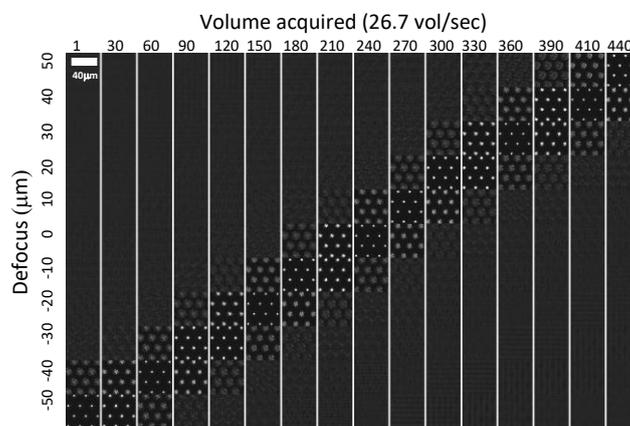


Figure 1. Each column shows the central sub-region of images acquired for DM refocus positions over the range -50 to 50 μm as DM refocussing scans at 26.3 sweeps/sec. The mask of holes moves at 6 $\mu\text{m s}^{-1}$

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