VIDEO-RATE REMOTE-REFOCUSING VIA 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 Alpao 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 for the purposes of video-rate volumetric imaging with 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, creep was avoided by ensuring that the temporal average of the surface applied to the mirror was constant 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 5 minute oscillation warm up period 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 shows image volumes thus obtained as the same mask was translated towards the microscope objective at a constant velocity. This deformable mirror based refocusing system was then applied to a light-sheet fluorescence microscope and the system successfully achieved video frame rate volumetric imaging at 26.3 volumes/sec of pollen grains and fluorescent beads.

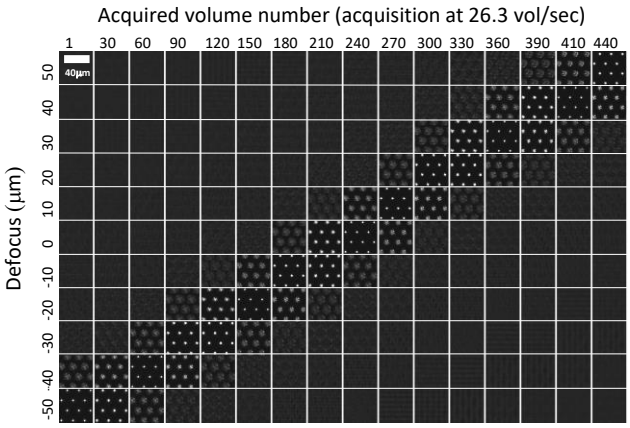

Figure 1. Image volumes obtained for a mask of 1 μm holes moving towards the objective at 6 μm/s. Each column shows the central sub-region of images acquired for DM refocus positions over the range -50 to 50 μm as DM refocussing scans at 26.3 sweeps/sec.

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