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Video-rate remote refocusing through continuous oscillation of a deformable mirror.

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Abstract Text

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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 (Figure 1). Such mirrors are known to exhibit viscoelastic creep and temperature dependent variations in the mirror response [2]. 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 achieve refocusing with a high-NA 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. This deformable-mirror-based refocusing system was incorporated in a light-sheet fluorescence microscope in order to perform video-rate volumetric imaging.

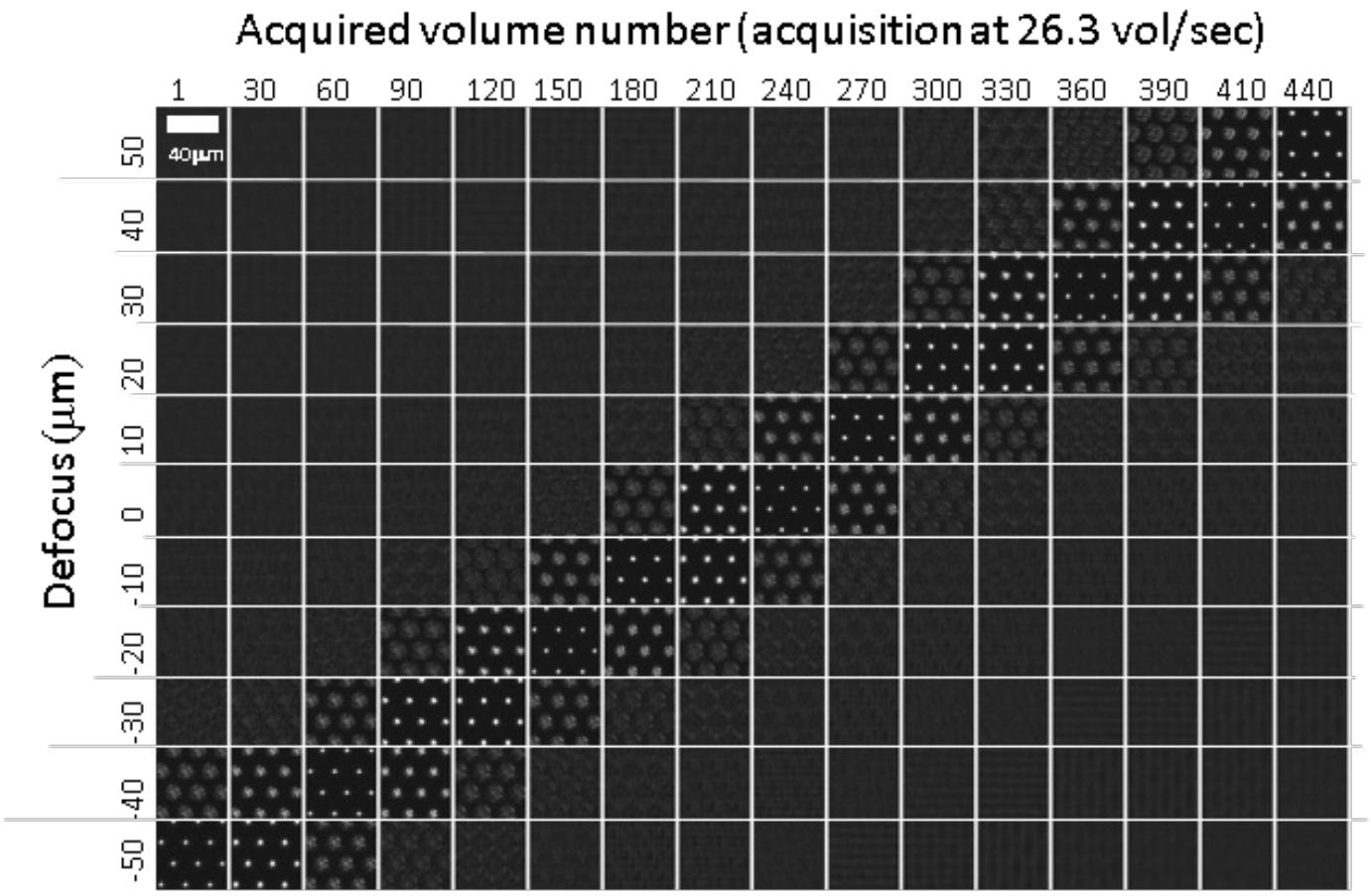

Figure 1. An array of 1 µm pinholes was illuminated with an LED and imaged in transmission by the system. The pinholes were on a motorised stage and were moved towards the microscope objective at 6 µm/s. The objective was refocused by the DM at a rate of 26.3 refocusing scans/s over a range of -50 to 50 µm. Each column shows the central 50×50 μm^2 sub-region of the field of view of images acquired for DM refocus positions over the range -50 to 50 µm.