THEORY AND PRACTICE OF REMOTE FOCUSING IN OPTICAL MICROSCOPY

Sharika Mohanan, Alex Corbett
College of Engineering, Mathematics and Physical Sciences
University of Exeter
e-mail: sm900@exeter.ac.uk

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ABSTRACT: In modern biology accurate spatiotemporal (x-y-z-t) information plays an important role in investigating complex dynamic process. Even though fast lateral (x-y) scanning is possible in the order of kHz, axial scanning (z) is limited to a few hertz due to the inertia introduced by the mechanical movement of the objective or the specimen. The Remote Focusing (RF) system described by Botcherby et al [1] eliminates the need to move the sample or the imaging objective and allows for high-speed, high resolution imaging. A typical RF system consists of lenses aligned in a 4f configuration which matches the pupil planes of the imaging and reference objectives (Fig.1). Selection of the magnification of the 4f configuration should follow the equation:

$$M_{4f} = \frac{M_1 n_2}{M_2 n_1}$$

where $M_{1,2}$ and $n_{1,2}$ are the magnification and immersion media refractive indices of the imaging and reference objective respectively. Deviation from this can lead to drastic reduction in the axial extent of aberration-free imaging [2].

Here, we determine the sensitivity of the RF system to a magnification mismatch. We have used MATLAB to numerically simulate the pupil functions of the two objectives and calculate the Strehl ratio for successive z planes. This gives us the maximum theoretical axial range for a given objective-4f configuration. The simulation has been performed for systems with increasing magnification mismatch and calculating the reduction in axial range. We validate these results using an experimental setup which measures the Point Spread Function (PSF) at different depths. The experiment has been repeated for setups with magnification mismatch and the results have been compared to the axial range predicted by the numerical simulation.

From the results it is seen that the suggested theoretical model can be utilised in optimising future RF systems and to obtain maximum dynamic range for diffraction limited imaging.

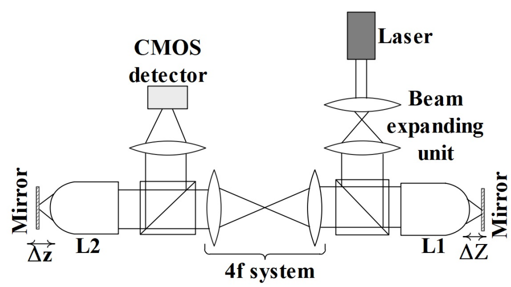

Figure 1: Experimental setup to measure the PSF at different depths. L1 and L2 are the imaging and reference objectives respectively.

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