

REMOTE FOCUSING MICROSCOPY: TOLERANCE ANALYSIS

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ABSTRACT: When imaging live biological samples it is essential that the sample is not perturbed when refocusing to different depths. Remote focusing (RF) is an optical technique that allows for the sample and imaging objective (O1, Fig. 1(a)) to remain stationary as refocusing is performed at a remote location in the optical train of the microscope [1].

COMPUTATIONAL MODEL: To understand the magnification tolerances of an RF system we have built a computational model to quantify the amount of spherical aberration present at different depths of the sample for non-ideal magnifications [2]. In Fig. 1b, $\beta=1$ signifies an RF system with ideal magnification and deviation from this decreases the overall diffraction limited axial range of the RF system.

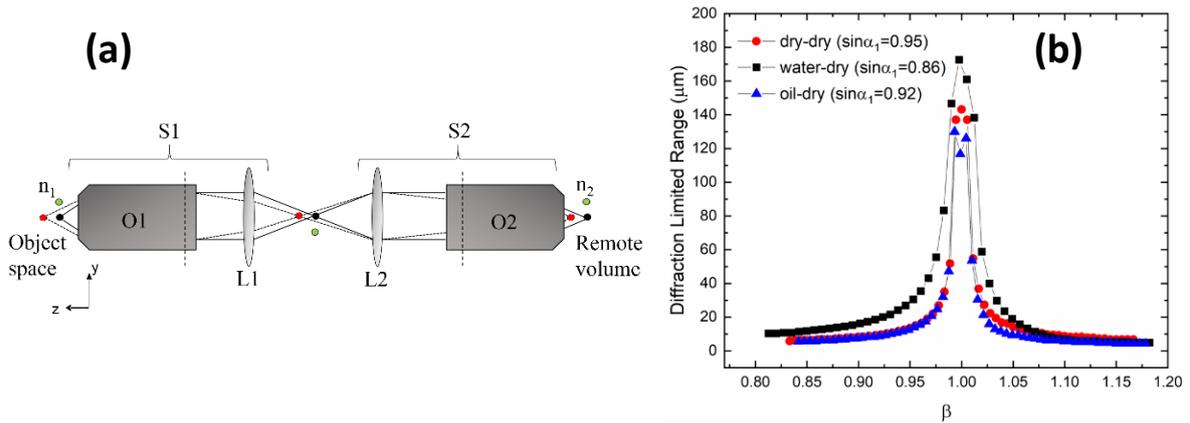


Fig 1: (a) An RF system with two microscopes S1 and S2 placed back to back. The remote volume has an extended diffraction limited axial range if the magnification of the RF system is n_1/n_2 (refractive index of the immersion medium of O1 and O2). (b) Shows the reduction in the diffraction limited range if the RF system deviates from ideal magnification ($\beta=1$). The simulation was performed for three different O1-O2 pairs.

EXPERIMENTAL VALIDATION: The computational model was verified experimentally for an RF system with non-ideal magnifications and for a range of objectives. The simulations predict that high numerical aperture (NA) RF systems are sensitive to small deviations from ideal magnification where a 1% change in magnification results in a 50% decrease in the diffraction limited range. We will show how these tolerances can be relaxed by compromising the effective NA of O1.

REFERENCES:

- [1] Botcherby, Edward J., et al. "Aberration-free optical refocusing in high numerical aperture microscopy." *Optics letters* 32.14 (2007).
- [2] Mohanan, Sharika, and Alexander David Corbett. "Sensitivity of remote focusing microscopes to magnification mismatch." *Journal of Microscopy* (2020).