

## WAVEFRONT SENSING IN STRONGLY SCATTERING SAMPLES

Markus Rueckel, Marcus Feierabend, Winfried Denk  
Max-Planck Institute for Medical Research  
Jahnstr. 29, 69120 Heidelberg, Germany  
E-mail: mrueckel@mpimf-heidelberg.mpg.de

**KEY WORDS:** adaptive optics, wavefront sensing, multi-photon microscopy

The reason for combining adaptive optics with multi-photon microscopy [1] is to achieve a diffraction-limited focus and thus the optimal fluorescence yield, by correcting the strong wavefront distortions that are introduced by refractive-index inhomogeneities in the specimen. We present a direct method, which we call coherence-gated wavefront sensing (CGWS), to determine the wavefront in strongly scattering samples [2]. The need for gating arises from the fact, that only light scattered back from the focal region carries the appropriate information needed to correct for wavefront aberrations. The experimental setup used for CGWSs is based on a low-coherence interferometer (Fig 1). Selection is based on the fact, that only that portion of the scattered light interferes with the reference light that has traveled for the same amount of time (within the coherence time) as has the reference light. One has to ensure, that the travel time in the reference arm matches that for the light scattered back from the focus volume. The complex amplitude of the in-focus light is encoded in the interference term and can be extracted by a four-step phase-shifting algorithm [3]. The step size is  $\lambda/4$  and is performed by shifting the mirror M2. To extract the wavefront we used a virtual Shack-Hartmann wave-front sensor (SHS) [4], which mimics the physical properties of a real SHS by numerically propagating the measured electric field through a virtual lenslet array.

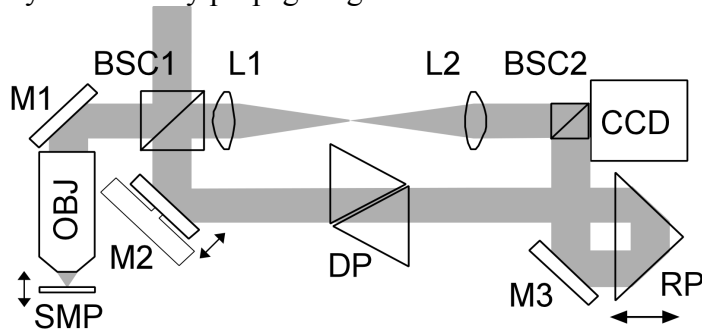


Fig.1: Experimental setup. Low-coherence light is split by the beam splitter cube BSC1 into reference and sample light. The sample light is reflected by mirror M1 into the objective OBJ. The light backscattered by the sample (SMP) is traveling through L1 and L2 onto the CCD chip, which is conjugate to the back focal plane. Via another beam splitter (BSC2) the CCD also receives reference light that has been reflected by mirror M2, which is mounted on a piezo actuator, and has passed through 4 glass dispersion matching prisms (DP). The length of the reference arm is adjusted by moving the right angle prism RP as indicated.

[1] Denk, W., J.H. Strickler, and W.W. Webb, Two-Photon Laser Scanning Fluorescence Microscopy. *Science*, 1990. **248**(4951): p. 73-76.

[2] Feierabend, M., M. Ruckel, and W. Denk, Coherence-gated wave-front sensing in strongly scattering samples. *Optics Letters*, 2004. **29**(19): p. 2255-2257.

[3] Malacara, D., Optical Shop testing. Second Edition ed. Wiley Series in Pure and Applied Optics, ed. J.W. Goodman. 1992, New York: John Wiley & Sons, Inc.

[4] Shack, R.V. and B.C. Platt, Lenticular Hartmann-screen. *Optical Sciences Center Newsletter*, 1971. **5**(1): p. 15-16.