Technology Offer

Reflection Microscopy: Ready to Use Neural Networks for Wavefront Correction in Adaptive Optics - Ref.-No.: 2022-6510-FG

Advantages

- Improves resolution and intensity of the image in an efficient way
- Enables modelling of complex optical paths which otherwise are difficult to be modelled
- Suitable for epi-detection configurations

Applications

- Laser scanning microscopy, particularly multi-photon microscopy
- Biological imaging

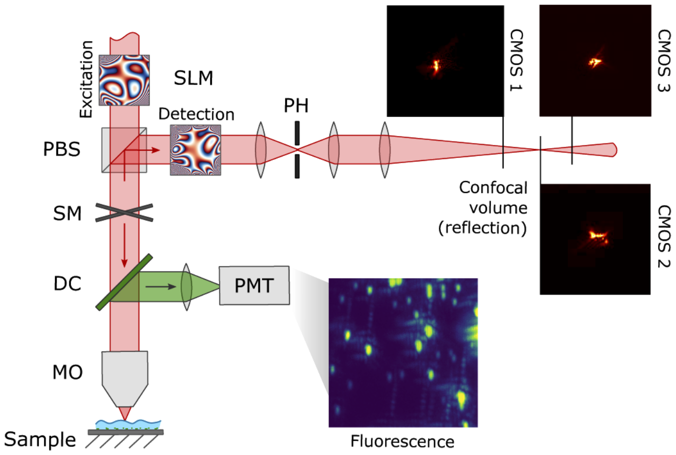

Fig. 1: setup: excitation and detection pathways are each controlled with a spatial light modulator (SLM); reflected light is imaged in an epi-detection configuration onto three cameras (CMOS 1-3); fluorescence is observed using two-photon scanning microscopy and a photomultiplier tube (PMT); MO = microscope objective, DC = dichroic mirror, SM = scanning mirrors, PBS = polarizing beam splitter, PH = pinhole.

Background

Imaging in many samples, particularly in biological tissue, with resolution performance at the diffraction limit of the microscope is limited due to aberrations and scattering. In this situation, laser scanning combined with adaptive optics can be used to improve the image resolution; thereby the adaptive optics shapes the wavefront in such a way as to correct for aberrations. In reflection-mode imaging, however, the excitation point spread function (PSF) and the detection PSF can undergo different aberrations, and therefore, for an accurate aberration correction, the contributions to aberrations accumulated in the excitation and reflected detection pathway need to be disentangled. This can be achieved by using matrix methods (for strongly scattering samples) or a wavefront sensor (for weakly scattering samples), which is technically demanding and/or restricted to a combination of sample and detection characteristics. Approaches for wavefront sensing based on deep neural networks, which have been developed so far, work only for configurations that require the correction of a single pass through a scattering medium, and hence cannot be applied for epi-detection detection configurations. Hence, there is a need to extend these approaches to reflection-mode imaging in an epi-detection configuration.

Technology

The two-photon laser scanning microscope depicted in Fig. 1 includes spatial light modulators (SLM) and reflected light detection for wavefront sensing and correction. The reflected focal spot is monitored at three different focal planes using cameras. As the microscope has a SLM in the excitation and detection path, it can disentangle the aberrations accumulated in these paths. The microscope corrects a reflection image and/or fluorescence image by: a) radiating a light distribution into an excitation path of the microscope, wherein the excitation path guides the light distribution I_0 to a sample and, while entering the sample, I_0 is distorted to a light distribution $I_{A,Probe}$, by scattering; b) reflecting the light distribution $I_{A,Probe}$ at the sample into the detection path, wherein, while exiting the sample, $I_{A,Probe}$ is distorted to form the light distribution $I_{D,Probe}$; c) recording the reflected light distribution $I_{D,Probe}$; d) transferring $I_{D,Probe}$ to a mathematical model which models the light propagation

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in the microscope; e) outputting a 2-tuple (M_A, M_D), wherein M_A describes the distortion of the light distribution I_0 while entering the sample and M_D describes the distortion of the light distribution $I_{A,Probe}$ while exiting the sample; f) setting the complementary distortion M_A^* on the SLM in the excitation path and the complementary distortion M_D^* on the SLM in the detection path. The mathematical model is implemented as a neural network, which is trained by the following steps: a') radiating a light distribution I in the excitation path; b') modulating the light I with the modulation M_A with an SML to generate the light distribution I_A; c') reflecting the light I_A at the sample's position into the detection path; d') modulating the reflected light I_A with the modulation M_D with another SML to generate the light distribution I_D; e') recording the light distribution I_D; repeating the steps a') to e') n times, thereby generating n tuples (M_A, M_D, I_D); and adapting the neural network, i.e. the mathematical model that describes the light propagation in the microscope, based on the n tuples (M_A, M_D, I_D). Fig. 2 shows the effect of the aberration correction achieved with the microscope described above.

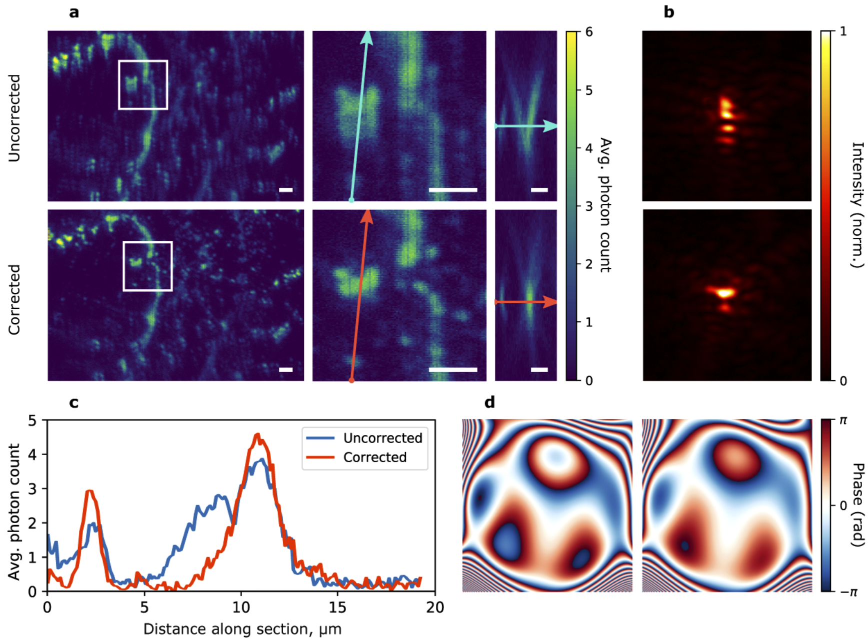

Fig. 2: aberration correction: **a, left:** corrected and uncorrected image of fluorescent beads distributed on a reflector surface imaged through a layer of vacuum grease; **a, center:** white frame in left figure; **a, right:** axial cross section through lines in the center figure recorded in a z-stack with 1 μ m step size; **b, top:** uncorrected focus at center of field-of-view in "a"; **b, bottom:** corrected based on reflected light (colorscale in the corrected image is saturated); c: cross sections for uncorrected (black) and corrected (red) images along the lines indicated in "a, center"; **d, left:** excitation phase mask; **d, right:** detection phase mask; all scale bars are 5 μm.

Patent Information

Patent DE 10 2020 109 734 B4
Application WO 2021/204663 A1

Other Publication

Ivan Vishniakou and Johannes D. Seelig, Wavefront Correction for adaptive optics with reflected light and deep neural networks, Optics Express, Vol. 28, No. 10 / 11 May 2020 / 15459

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