Wide field adaptive optics microscopy based on image sharpness optimization in a sensorless and sensored configuration

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Purpose

Effects such as refraction index mismatch and sample induced aberrations limit the imaging resolution of optical microscopy and, in particular, optical sectioning methods such as confocal or multi-photon microscopes in deep biological samples. The use of adaptive optics (AO) in such cases can allow a partial correction of these aberrations and an improvement of the image quality (resolution and contrast for example) leading to a deeper penetration in the sample. Most of these systems used an optimization algorithm in some form to determine the aberration correction required, and this continues to be the general situation though this has recently come to be termed “sensorless” correction.

We report on recent developments in the use of AO in wide-field microscopy to remove both system and sample induced aberrations. Two configurations have been studied. First we describe a sensorless configuration where the optimization relies on image sharpening metric techniques used with a simplex search algorithm. We compare the optimization efficiency based on 5 commonly used image sharpness metrics and discuss the advantage of guiding the search with deformable mirror shapes based on low order Zernike polynomials. Then we describe a closed loop configuration with a wavefront sensor where the aberration is measured via the help of a probe laser beam working at 633nm, reflected either on the microscope slide or on the sample.

Methods

In the sensorless configuration, five metrics have been studied. These metrics include the intensity square and image variance metrics, a Fourier based high pass filter, and two edge detection metrics including a Sobel and a Wavelet based metrics. Software synchronizing the science camera and the deformable mirror has been created which allows the user to select, in the field of view, a region of interest on which the optimization will be performed. The simplex algorithm used to find the optimum deformable mirror voltage map can take two different routes. The first one uses random voltage map as starting point and the second one uses low order Zernike terms with random amplitudes.

In the closed loop configuration, which includes a wavefront sensor, the optical set-up is described in Fig. 1. A 633nm beam is used to measure the residual aberration in the set-up and the sample induced aberration. We demonstrate the stability of the loop by first using the light reflected on the top face of the slide. The wavefront reference, defined as the non aberrated beam, has been obtained after optimizing the spot image on the science camera using the intensity square metric. Secondly we tested the closed loop using the light reflected by the sample layers and filtered by pinhole a positioned at the focus of the wavefront sensor, to get rid of the out of focus light.

Results

A typical optimization result performed on back skin mouse tissue, is given in Fig. 2. Results shows that, although each metric has a different relative improvement (which varies from 10% for the intensity square metric to 50% for the wavelet metric), the final image is visually the same. This is demonstrated by the fact that, for whatever metrics used in the optimization process, the relative improvement remains unchanged if calculated with a given metric. The comparison between the low order Zernike guided search (LOZGS) and the random search (RS) shows that with LOGZS, the optimization is more reliable and repeatable. As tilt can be removed of the various starting point required by the simplex, the image remains fixed during the process.

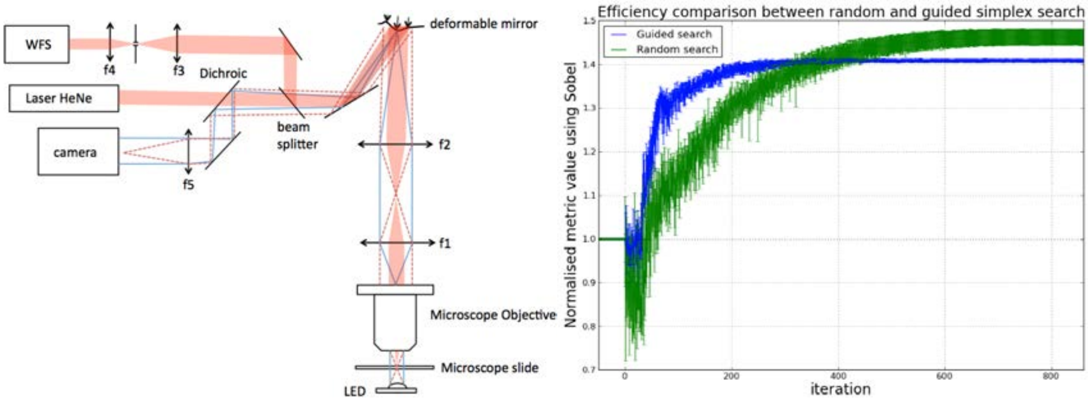

Fig. 1. Overall system configuration for optimization AO in a closed loop wide field microscope (outer left), Convergence efficiency comparison between a pure RS and LOZCS.

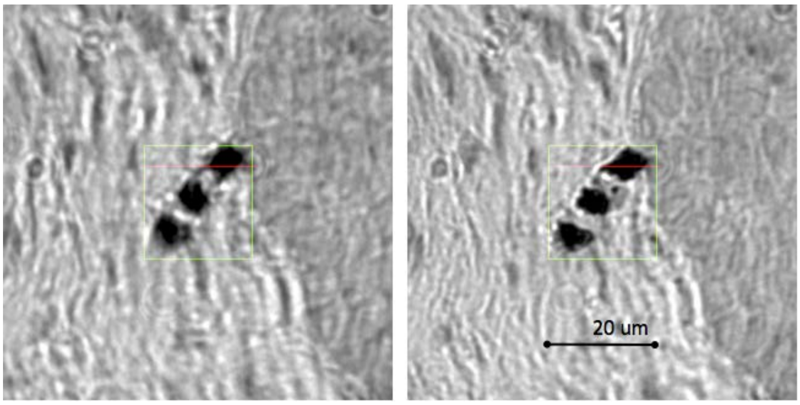

Fig. 2. Before (left) and after (right) optimization. The green square defines the region of interest on which the optimization is performed.

The closed loop, when using the reflection on the first face of the slide, is showing a stable behaviour and allows the correction of the focus when the microscope objective is manually defocus.

Conclusions

We have reported on the implementation of an AO transmission microscope working either in a sensorless configuration or in a closed loop configuration with a wavefront sensor.