Thick samples image improvement from image aberration function correction

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Purpose

Deep-tissue observation is necessary to understand cell interactions with their 3D environment or to observe thick tissues. However, when the focus distance is increased, the image quality is strongly deteriorated mostly by the Spherical Aberration (SA) created because of the observation through a thick sample with a high Numerical Aperture (NA) objective. Adaptive Optics (AO) correctors are used to correct for these aberrations. The main difficulty is to determine the command to send to them to optimize the image.

Several solutions have been studied to determine this command. Some are based on the evaluation of the image quality from its spatial frequency components [1]. In this paper, we propose to directly measure the image aberration function in the microscope exit pupil and use it to control the wave front corrector.

Methods

We assume that all the rays issuing from the illumination aperture stop converge to the different field points in the image plane. If we are able to select only the rays that passed through a given field point in the object plane, then a phase measurement in the microscope exit pupil will lead to the aberration function for this specific field point.

We have implemented a solution where we filter the rays with a diaphragm in the image plane of a bright field microscope. The aberrations are measured with a PHASICS SID4 wave front sensor and they are corrected with a Spatial Light Modulator (SLM) from Hamamatsu (LCOS-SLM X10468 series) as explained in the reference [2]. The SID4 wave front sensor and the SLM are both conjugated with the microscope exit pupil.

Results

In this experiment, samples are observed on a Nikon inverted bright field microscope with a Köhler illumination. The image of the sample is obtained on a classical CCD camera (640×480 pixels; 1pixel = 7.4µm). The image aberration function correction is obtained by selecting, with the diaphragm located at the microscope exit, the rays issuing from the sample center. As expected in the case of thick samples observation, this aberration function is mostly composed of spherical aberration which strongly deteriorates the image quality (cf. figure 1). The figure 1 presents images obtained before correction (left) and after correction (right) on a mouse embryo observed through 2 millimeters of glass with a 40x objective (NA=0.6).



Fig. 1. Example of image quality improvement before correction (left) and after correction (right).

The comparison of the images of the figure 1 shows the quality improvement obtained after correction notably in terms of contrast. After correction (right), the image quality is equivalent to the one obtained in the case of thin sample and the image is anew obtained with the microscope resolution.

Conclusions

In this paper we present a method to enhance the image quality by AO in the case of the observation of thick samples in microscopy. In this method the aberration function is directly measured with a SID4 wave front sensor and used to control the SLM. In this method the aberration function is obtained for a specific field point. For this, the given field point is selected with a diaphragm at the microscope exit and the corresponding wave front is then measured. So, the aberration function is easily determinated and directly used as command for the correction. Moreover in this case the aberrations of the SLM are taken into account in the command and the image is optimized.

References

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- 2. B. Wattellier, and I. Doudet, "Hologram and image optimization using high resolution adaptive optics", in AOIM 2009