

# Biological imaging by Digital Holographic Adaptive Optics

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**Abstract** The basic principles of DHAO have been presented and demonstrated in a recent paper [Opt. Lett. **36**, 2710-2712(2011)]. In this paper, the application of DHAO in biological imaging is investigated.

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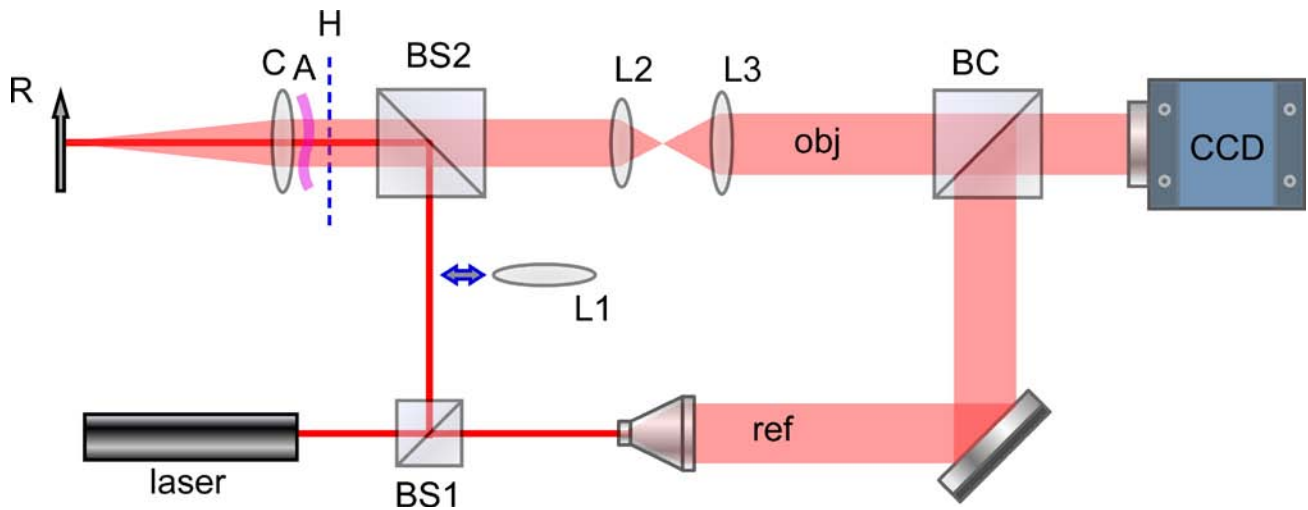
## 1. Introduction

Adaptive optics(AO) was first adopted in astronomy to correct for the aberrations due to the atmosphere [1]. A second successful application of AO is in vision science. In 1997, AO was successfully applied in vision science by Liang and colleagues [2]. With AO system, unprecedented resolution was achieved. Since that time the field of AO in vision science has been rapidly growing with more and more AO systems being developed [3]. A typical AO system includes several critical hardware pieces: spatial light modulator or deformable mirror, lenslet array and a second CCD camera in addition to the camera for imaging [2, 3].

The recently proposed digital holographic adaptive optics (DHAO) system may replace these hardware components with numerical processing for wavefront measurement and compensation of aberration through the principles of digital holography [4]. The wavefront sensing and correction by DHAO have almost the full resolution of the CCD camera. It is inherently faster than conventional AO because it does not involve feedback and iteration, and the dynamic range of deformation measurement is essentially unlimited [4-5]. Although the DHAO was originally aimed at emulating the conventional AO at lower cost, it opens applications in astronomy and biological imaging. In this paper, the application of DHAO in biological imaging is investigated. By measuring and removing the phase aberration of a flawed lens, the final image of the biological sample can be greatly improved.

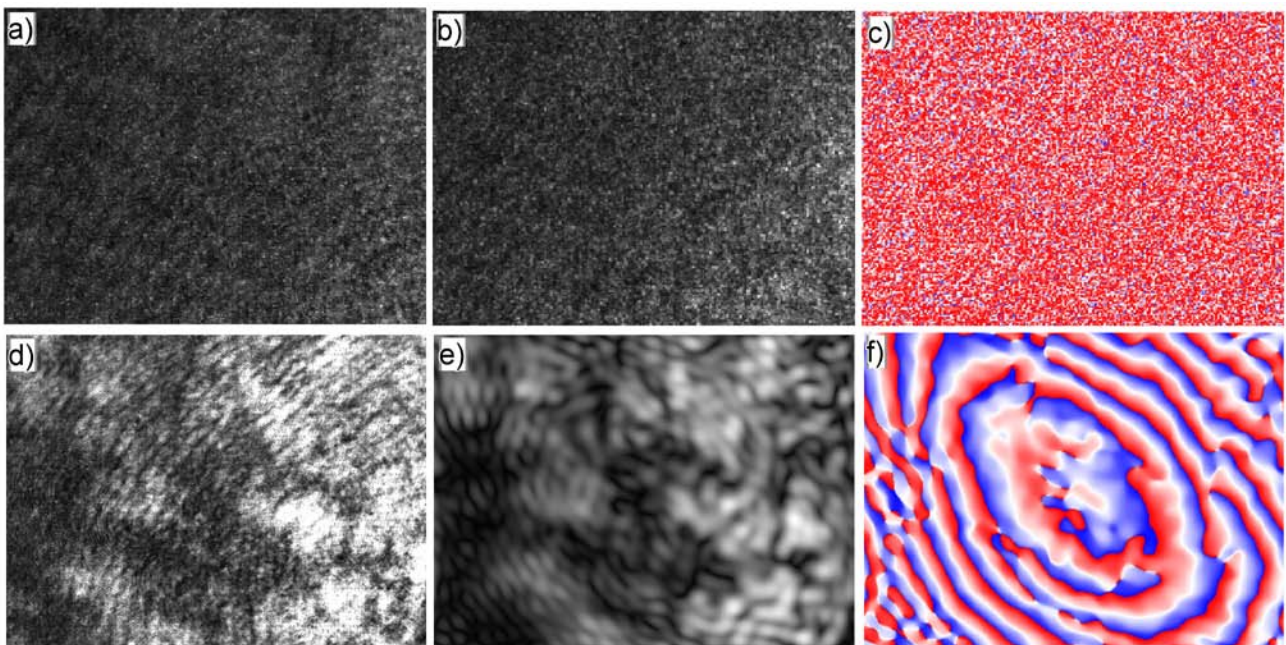
## 2. DHAO setup

The DHAO setup has been described in ref.4. For convenience of the readers, we give a brief review of the DHAO system that is illustrated by Fig.1. The lens, C, and the artificial phase aberrator, A, constitute a lens with aberration. The biological sample is placed at back focal plane(R) of the lens C. The CCD is focused at the front pupil plane (H) of the lens C. The DHAO imaging system consists of phase aberration sensing, full field imaging and phase aberration correction. Fig.1 shows phase aberration sensing. The collimated beam will be brought to a focus at the sample by the lens C. This focused spot emits a spherical wave that is collimated by C. The output collimated wave will experience the aberration of the flawed lens simulated by C and A. The CCD will capture a hologram from which the distorted phase at the CCD plane can be retrieved and stored. Then, the lens L1 is inserted in the setup and focuses the collimated beam at the front focal point of lens C. Through C, the light beam is collimated and illuminates the sample. The CCD captures a second hologram from which the distorted complex amplitude at CCD plane can be retrieved. The full field image of the sample can be obtained from this distorted complex amplitude. Finally, the phase aberration in this distorted complex amplitude can be removed through multiplying it by the complex conjugate of the phase aberration data stored in the first step. This way, the aberration compensated image can be obtained.



**Fig.1** The schematic diagram of the DHAO system. L1-L3: lens, BS1-BS2: Beam splitter, BC: Beam combiner, R: biological samples, C: lens, A: aberrator, C and A simulate a high aberration lens. H: the plane conjugate with CCD.

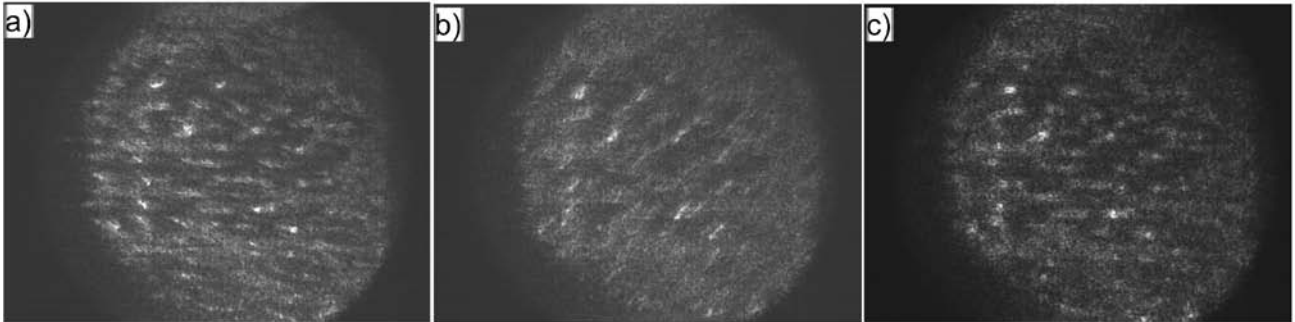
### 3. Experimental Results



**Fig.2** Full field imaging and phase aberration sensing of DHAO. a) the hologram of the full field imaging. b) and c) the amplitude and the phase map extracted from a). d) the hologram of phase aberration. e) and f) the amplitude and the phase map extracted from d). The phase map ranges from  $-\pi$  to  $\pi$  in red-blue representation.

A layer of onion tissue is used as the biological sample. A piece of broken glass is used to generate the phase aberration of the lens C. The full field hologram is shown in Fig.2 a), from which the amplitude and the phase map can be extracted

as shown in Fig.2 b) and c) respectively. This phase map has been distorted by the phase aberration introduced by aberrator A. The phase aberration is obtained from the hologram, Fig.2 d) that is obtained in the phase sensing process. From this hologram, the complex amplitude of the distorted plane wave can be obtained. The resulting amplitude and the phase aberration are shown in Fig.2 e) and f) respectively. As a baseline, the holographic full field image of the sample, without the aberrator in place, is shown in Fig.3 a). The field of view is  $2519 \times 1889 \mu\text{m}^2$  with  $1024 \times 768$  pixels. Fig. 3 b) shows the image affected by the phase aberration. Compared to the baseline image Fig. 2 a), the aberration greatly reduces the quality of the image as shown by Fig.3 b). To rid the aberration of the distorted image, the aberration sensing is performed. The distorted image is corrected by the complex amplitude, as shown in Fig.2 e) and f). The corrected image, as shown in Fig.3 c), is much better than the uncorrected image, Fig.3 b).



**Fig.3** image correction by DHAO. a) the baseline image without aberration in place. b) the image distorted by phase aberration. c) the corrected image by DHAO.

#### 4. Conclusions

The DHAO compensated image shows great improvement compared to the uncorrected images. The experimental results imply the potential application of DHAO in biological imaging.

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