Microscopic PSF estimation and resolution enhancement by speckle pattern illumination

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Abstract: A method for estimating the PSF of an imaging system by projecting a speckle pattern onto the imaged object is presented. This provides a critical measure of the imaging performance and of the presence of aberrations.

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In wide field imaging, the diffraction properties of the electromagnetic waves limit the image resolution to the wavelength scale. However, in cases of irregularities of the imaging medium or imperfection of the optics, phase deformations occur and the diffraction limited resolution deteriorates. For instance, in microscopic imaging, the objective lens is usually optimized to homogeneous watery medium; however biological samples are usually inhomogeneous when imaged on the visible spectrum. To improve the resolution usually two general approaches are used: deconvolution and adaptive optics (AO). Yet, deconvolution is in general sensitive to the initial guess of the point spread function (PSF) [1], while AO, which yields superior results in astronomical [2] and retinal imaging [3], fails to obtain diffraction limited resolution in cases were the reference laser is also deformed by aberrations or in scenarios of significant and fast changing aberrations.

In this talk, I will present a new approach termed PEPSI (PSF Estimation by Projection of Speckle Illumination) for estimating the transverse PSF when imaging through phase deforming media [4], and its application to microscopic imaging. The method is based on measuring the deformation of a speckle pattern illuminating a fluorescent object formulating an inverse problem that estimates the PSF. The unique advantage of the speckle here arises from their random phase distribution which forms a projected pattern that is not affected by aberrations and thus enables an objective measure of the imaging aberrations irrespective of the illumination path's optical aberrations. PEPSI generally estimates the average PSF of the entire field of view from a *single* pattern projection, and is thus suitable for dealing with dynamic phase aberrations; in cases where the aberrations are non-isoplanatic, the local PSF for selected areas in the field of view can be obtained by the same analysis on those areas.

To obtain a practical PSF estimation technique, we derive our solution using Wiener-type minimization, leading to a Wiener-type deconvolution algorithm for the PSF estimation (requiring only a change in the input functions) that we validate using both simulations and experiments. Finally, to apply PEPSI to improve the resolution of the (phase-deformed) images we use a common iterative maximum likelihood-based image reconstruction algorithm.

Figure 1(a) shows the optical setup that was built to validate the method and demonstrate resolution improvement. Both speckle projection and imaging were performed through a phase deforming plate. Since the numerical aperture of setup was too small for collecting sufficient light to directly measure the PSF, PEPESI's accuracy was tested by indirect two-step method. In the first step, we estimated the PSF by projecting speckle onto a thin fluorescent dye film (Figs. 1 (b)-(d)). In the second step, we took two images of a fluorescent bead, one with the same aberrations as in the PSF estimation process and one clear image of the bead (without aberrations). By convolving the clear image of the bead with the estimated PSF (Fig. 1(e)), we obtained a degraded image (Fig. 1 (f)) that can be compared with the optically acquired degraded image acquired in the second step (Fig. 1 (g)). Comparison between the degraded bead image formed by convolution with the estimated PSF (Fig. 1(f)) and the optically acquired bead across four different examples (Fig. 1(g) and 1(h)) shows a very good agreement.

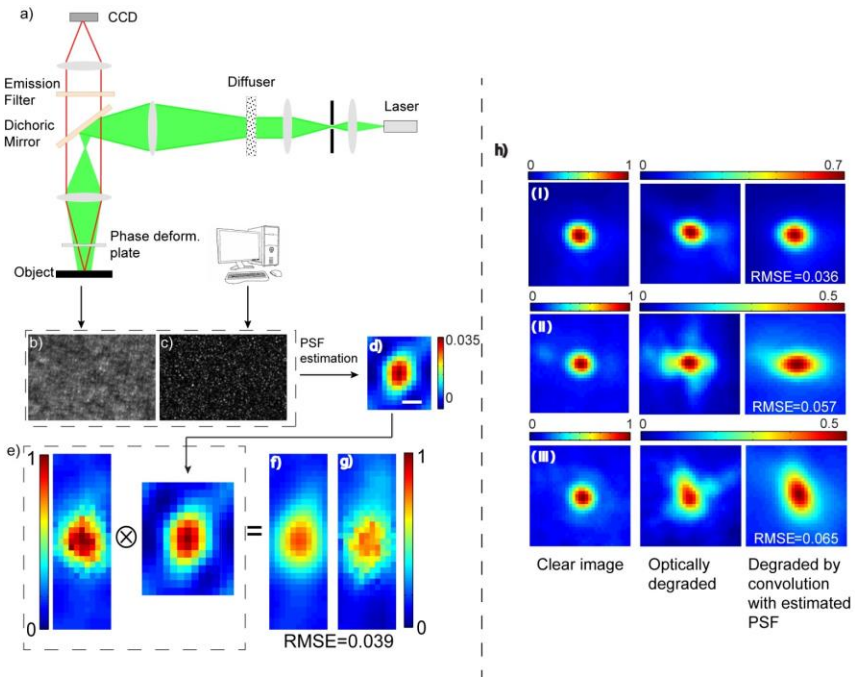

Figure 1. Experimental validation of PEPSI. (a) Experimental setup used for speckle pattern projection and imaging. The phase deformation was introduced by a phase deforming plate, and the speckle pattern was formed by a diffuser and projected onto a thin film of fluorescent dye. (b)-(d) the degraded speckle pattern, a computer generated speckle pattern and the estimated PSF (scale bar: 3.6 μm) respectively. (e) By convolving the 'clear' image of a 4μm diameter fluorescent bead (diffuser and phase plate removed) with the estimated PSF, a degraded image is obtained (f) that can be compared to its optically acquired degraded image (g): the RMS error between the degraded bead images is only 0.039, showing their good agreement. To avoid discrepancy due to noise, we applied an adaptive noise removal filter to the bead's clear (e, left) and optically acquired degraded (g) images, which mostly removed noise in the bead's surroundings without significantly changing it. (h) Three more cases validating the estimated PSF's accuracy: the optically acquired degraded images of 6 μm beads (central column) closely match images obtained by convolving their clear bead images (left column) by the estimated PSF (right column, also lists the images' RMSE). Noise was reduced by averaging 50 frames (here without a noise removal filter).

As an application for PEPSI, we applied the estimated PSF to enhance the resolution of aberration-degraded microscopy images using both simulated and experimental data. The image reconstruction was based on an iterative maximum likelihood (ML) algorithm that uses PEPSI-estimated PSF as an initial input. Figure 2 shows an example for the resolution improvement of image taken by inverted microscope

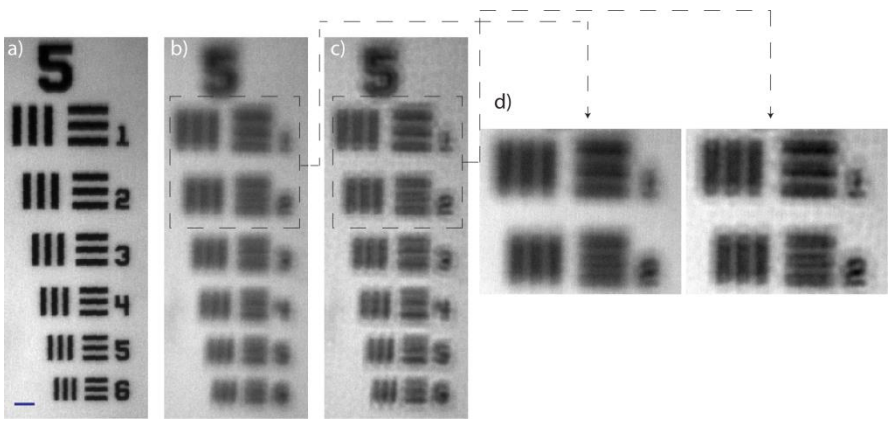

Figure 2. Applying PEPSI in a microscopic images. (a) Clear image of a fluorescently dyed section of a USAF resolution target, obtained by a Nikon microscope and 10× objective. Scale bar: 40 μm (b) Degraded image of the target following introduction of an aberrating agar drop. (c) PEPSI-based reconstructed image. (d) Enlarged regions of the deformed and reconstructed images.

(Nikon TE2000-U). In this experiment the sample (USAF resolution target) was stained by a fluorescent dye and imaged through a 10x objective (Fig 2(a)). To deteriorate the image we used a drop of agar on the target (Fig. 2(b)). The results of the PEPSI-based image reconstruction clearly demonstrate a dramatic resolution improvement (Fig. 2(c, d)).

One of the strengths of PEPSI is its ability to estimate the entire field of view (or parts of it) using only one projection of speckle pattern. This makes it highly suitable for *in-vivo* retinal micro-imaging, which suffers from random and dynamic phase aberrations that are caused by the ocular optics. Figure 3 shows our initial results in fluorescent stained mouse retina. This *in-vivo* image was obtained by funduscope based retinal camera and the estimated PSF (Fig. 3 inset) qualitatively matches the dimensions of PSF in this system as inferred using other methods [6].

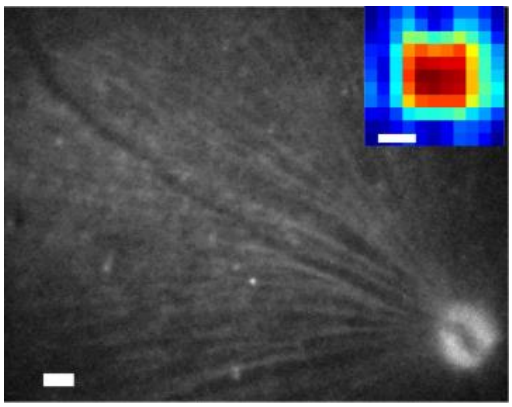

Figure 3. Applying PEPSI in *in-vivo* microscopic retinal imaging. The image was obtained by funduscope based retinal camera (scale bar: 30 μm). The PEPSI estimated PSF (inset) matches the dimension of the system (scale bar: 3 μm).

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