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ABSTRACT

In this work, we present a new confocal laser scanning microscope capable to perform sensorless wavefront optimization in real time. The device is a parallelized laser scanning microscope in which the excitation light is structured in a lattice of spots by a spatial light modulator, while a deformable mirror provides aberration correction and scanning. A binary DMD is positioned in an image plane of the detection optical path, acting as a dynamic array of reflective confocal pinholes, images by a high performance cmos camera. A second camera detects images of the light rejected by the pinholes for sensorless aberration correction.

Keywords: Digital micromirror devices, Confocal microscopy, Adaptive optics

1. INTRODUCTION

1.1 Digital micromirror device based confocal fluorescence microscopy

Digital micromirror devices can be used to produce simple, low cost confocal microscopes[1,2]. In these devices, a high power, incoherent source at the excitation wavelength is used to illuminate the surface of the micromirror device, located in the image plane of a traditional microscope. Single elements, or small clusters of elements of the micromirror device are turned "on" to generate diffraction limited excitation spots at the sample plane. Fluorescence light emitted by the sample at these spots locations is imaged by the microscope at the "on" pixels, which act as confocal pinholes, only reflecting the light on focus towards the source.

With the addition of a regular Fluorescence filters set in the path between the source and the micromirror, the surface of the micromirror device can be imaged on a camera detector. A complete confocal image of the sample plane is obtained by projecting with the DMD a sequence of patterns with diffraction limited spots in different locations, so that the sum of the full sequence illuminates the sample uniformly. If the camera exposure is equal to the total time required to project the full sequence on the DMD, the camera output is a confocal image of the sample plane.

The main advantage of a DMD confocal microscope over a traditional laser scanning configuration is given by the optical setup simplicity, with the absence of moving parts other than the micromirror device elements, and the extremely basic software required to operate the device.

1.2 Adaptive optics in DMD microscopy

DMD based microscopes, due to their peculiar optical configuration, can provide additional information on the optical properties of the sample. This is achieved due to the reflective nature of the pinhole array generated on the DMD surface.

While in a traditional laser scanning microscope all the out of focus light is absorbed or scattered by the pinhole surface, a DMD device simply reflects the rejected light in a different direction.

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This is especially useful for applications in adaptive optics. Adaptive optics is the branch of optical sciences in which adaptive phase modulators (such as deformable mirrors, or liquid crystal on silicon modulators) are employed to correct for phase aberrations generated by the unknown and inhomogeneous distribution of refractive index within a three dimensional sample. This is generally achieved by optimization of an image based metric and, therefore, limits the optimization rate to the frame rate of the microscope, which in the case of laser scanning confocal microscopes can reach a few images per seconds at best, therefore only allowing optimization times of several minutes.

In a DMD based microscope, while the exposure of the main imaging camera should be set to the duration of the full sequence of DMD patterns in order to obtain a confocal image, a second camera can be used to acquire the image of rejected light from the pinholes at each frame of the patterns sequence, and use the spatial distribution of fluorescence around the pinholes as a metric for adaptive optics optimization[2]. This has the advantage of providing the value of the metric every few milliseconds or less, and moreover provides a high quality, sample independent metric function, which is guaranteed to converge to its global maximum in a few iterations[3].

1.3 Limitations of DMD microscopes

While simple and low cost, DMD based microscopes have severe limitations which prevent their performances to reach those of a traditional laser scanning setup. The most prominent limitation is due to the low excitation efficiency, as the light source has to uniformly illuminate the full surface of the DMD, and all the light impinging on the "off pixels", which are the majority of the total, is deviated away from the optical path and never reaches the sample.

This forces DMD microscopes to be used with high power Diode sources, strongly limiting the selection of available wavelengths, and nevertheless still not proving ideal for low brightness samples.

Additionally, while in a laser scanning microscope the field of view and pixel size can be dynamically adjusted depending on the experimental conditions, the magnification, pixel size and field of view of a DMD microscope are pretty much fixed for a given experimental setup.

2. COHERENT DMD BASED ADAPTIVE MICROSCOPY

2.1 Method

In order to provide sufficient excitation power for all kinds of scanning fluorescence microscopy application, we realized a prototype DMD microscope with phase modulated coherent illumination. In this setup, a single mode laser beam is structured through a diffractive optical element in a regularly spaced lattice of excitation spots. The image of the excitation lattice is projected on the focal plane of a microscope objective through an adaptive optics microscopy system, in which phase modulation is provided by a voice coil deformable mirror, capable of enough stroke for correction of severe aberrations and simultaneous scanning of the lattice.

Fluorescence light emitted by the sample is detected in epifluorescence configuration, propagating back through the optical system, including the deformable mirror. A dichroic mirror and fluorescence filters set is positioned between the diffractive optical element and the deformable mirror.

After the dichroic mirror and emission filter, an image of the focal plane is formed on the surface of the DMD, where small clusters of pixels are used as digital pinholes. Two cameras are used to image the surface of the DMD, one imaging the pinholes surfaces, and the other collecting the rejected out-of-focus light.

In this configuration, the deformable mirror therefore performs adaptive optics correction on both excitation and fluorescence light, and provides raster scanning in a descanned configuration, so that the position of the pinholes on the DMD surface should only be selected once, and remains static during the scanning procedure.

In order to form an image, multiple images of the pinholes must be acquired during the scanning procedure. A custom computer software should isolate the intensity in each pinhole from each single image, and assign it to the correct pixel of the final confocal image.

As for adaptive aberration correction, the optimization camera can be operated independently from the scanning procedure, integrating the images of the rejected light spots with the most convenient exposure needed for a good compromise between signal to noise ratio and optimization speed.
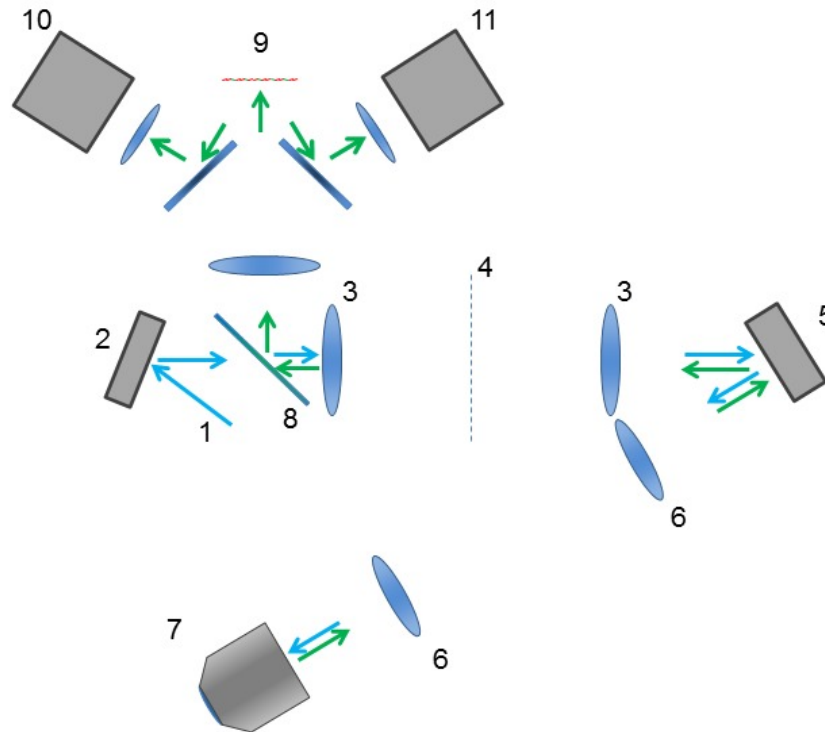

Figure 1. Schematic representation of the optical setup. A single mode, coherent beam (1) is modulated by a diffractive optical element (2), or by an SLM in the same position. The plane of the DOE is conjugated by a 4f telescope (lenses 3) to with a deformable mirror (5). An image of the lattice is visible in the mid image plane in the telescope (4). A second telescope (6) conjugates the deformable mirror with the back aperture of a microscope objective. The fluorescence light is descanned back to the dichroic mirror (8), and a flurescence image of the lattice is formed on the DMD (9) The pixels of the DMD at the lattice spots locations are turned "on", and an image of them is formed on the imaging camera (10), while the rejected light is imaged on the optimization camera (11).

2.2 Position variant adaptive optics

If in the described setup, instead of using a fixed diffractive optical element, a programmable spatial light modulator is used to generate the spots lattice, interesting new possibilities become available for adaptive optics. In particular, an advanced version of the Gerchberg-Saxton algorithm[4] allows to introduce different aberrations in each of the generated spots of the lattice. This can be used to correct, at least in the excitation light, spatially varying phase aberrations, with a resolution equal to the lattice spacing.

This sort of correction is not possible in conventional adaptive microscopy systems, and allows for correction of higher order aberrations and on wide fields of view.

While correction of the excitation light only will not provide maximum collection efficiency of light in the pinholes, the resolution of a confocal system is only dependent on the size of the excitation spot within the sample, and therefore diffraction limited resolution should be recovered.
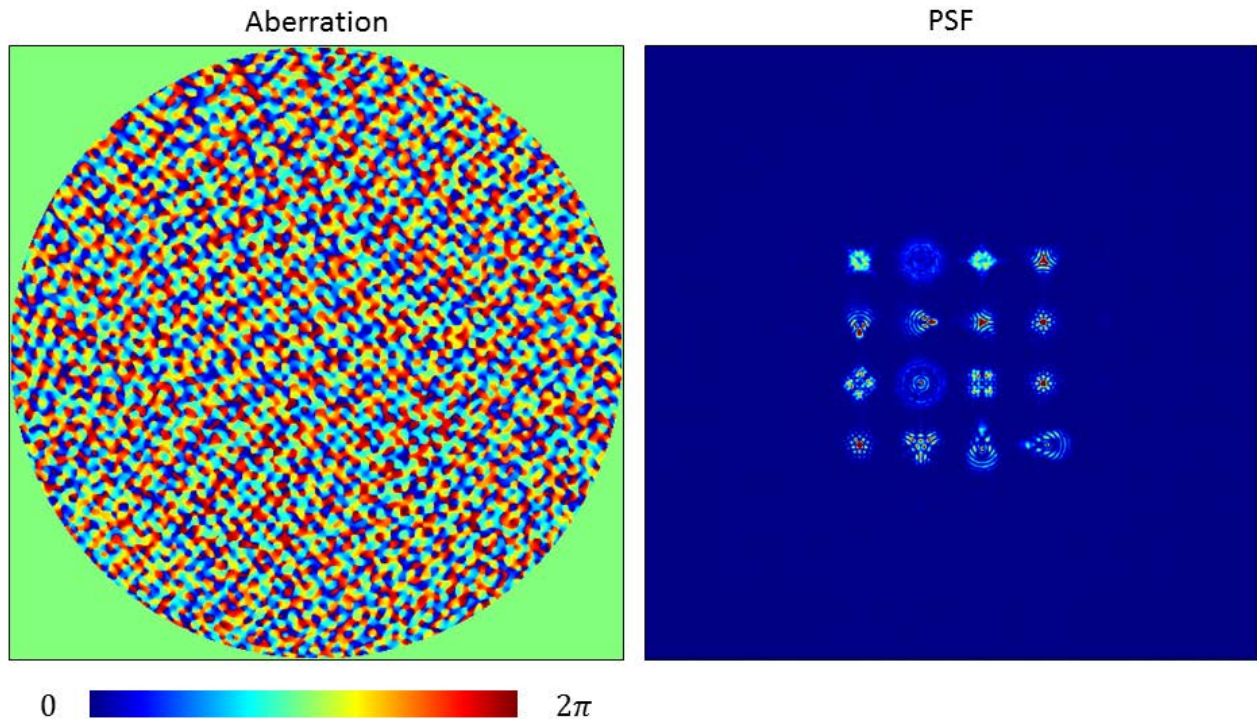

Figure 2. A diffraction pattern generating a lattice of 4 by 4 spots, each affected by a single Zernike aberration.

3. RESULTS AND CONCLUSIONS

3.1 Experimental setup

A DMD based coherent adaptive microscope was realized. For proof of principle measurements a single excitation source was employed at 488 nm (Sapphire 488 LP, Coherent, USA). A spots lattice was generated with a LCOS spatial light modulator (X13268-01, Hamamatsu, Japan). A high stroke, 69 actuator voice coil deformable mirror (DM-69, Alpao, France) was employed for both scanning and isoplanatic correction. Imaging was performed through a oil immersion objective (63X, 1.4NA, Leica, Germany). A standard set of GFP fluorescence filters (MDF-GFP, Thorlabs, USA) was used for imaging. A 1" diagonal, fullHD resolution DMD was used as the programmable pinhole array (dlplcr6500evm, Texas instruments, USA).

The two images of the DMD surface were generated on two identical sCMOS cameras (OptiMOS, QImaging, Canada). The optimization image was generated on the camera with 1x magnification through a 55mm telecentric lens (TEC-55, Computar, Japan) in Scheimpflug configuration, at a maximum speed of 100 frames per second. Light from the pinholes was collected through a standard Achromat lens with a magnification of 0.2X, in order to achieve a higher fram rate of up to 500 frames per second.

3.2 Results

The system was used to image the BODIPY stained microtubules on a standard sample BPAE cells from a commercial slide (Fluocells #2, Invitrogen, USA). A lattice of 10 by 10 spots was used for scanning, with a spacing of 5 microns

between the spots. Nyquist sampled images could be acquired with a total of 400 steps, therefore achieving a frame rate faster than 1 frame per second, comparable with that of a regular laser scanning system.

The sample used presents a relatively low fluorescence signal, which made it difficult to image on our previous DMD microscope with incoherent excitation [2]. The coherent version of the system could easily image the sample with high signal to noise ratio while using a minimal power level from the source (<1mW after the SLM).

Moreover, we could successfully perform traditional adaptive optics correction employing the deformable mirror, and space-variant correction of the excitation through the SLM. Prelimiray results are reported in Figure 3. While still preliminary these results show how a coherent DMD based microscope could provide the first ever reported fully anisoplanatic aberration correction.
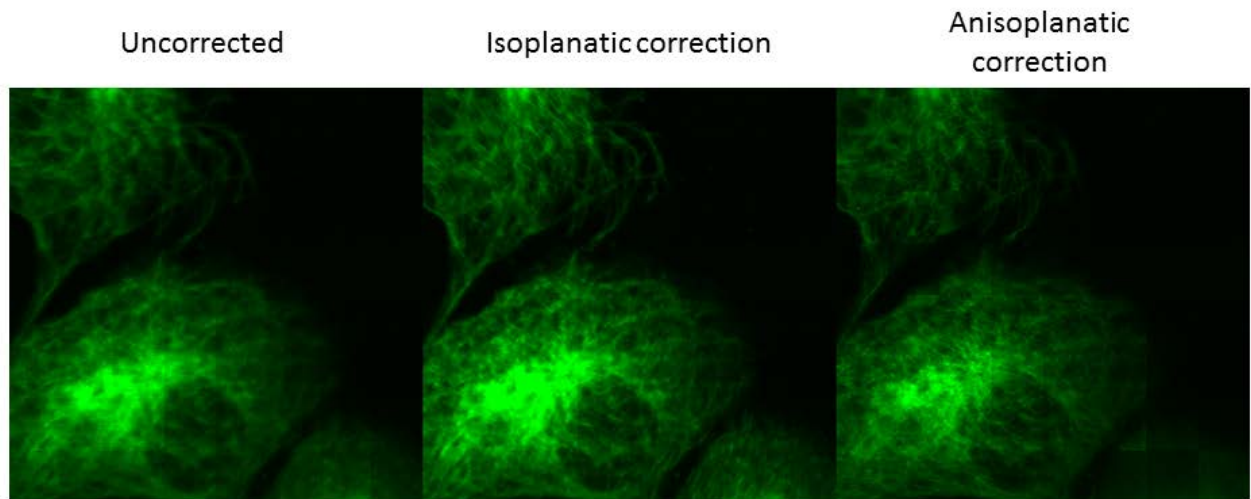

Figure 3. Example of adaptive optics correction with isoplanatic and anisoplanatic method. The isoplanatic correction was achieved only using the deformable mirror, correcting the same aberration in every point of the field of view. Anisoplanatic correction was performed with the SLM, independently for each of the spots of the lattice. While in mostly dark areas of the sample the anisoplanatic correction still shows some problem, the resolution achieved is visibly better in most of the field of view.

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