Addressable multiregional and multifocal multiphoton microscopy based on a spatial light modulator

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Abstract. Through a combination of a deflective phase-only diffractive spatial light modulator (SLM) and galvo scanners, an addressable multiregional and multifocal multiphoton microscope (AM-MMM) is developed. The SLM shapes an incoming mode-locked, near-infrared Ti:sapphire laser beam into multiple beamlet arrays with addressable shapes and sizes that match the regions of interest on the sample. Compared with conventional multifocal multiphoton microscope (MMM), AM-MMM achieves the effective use of the laser power with an increase of imaging rate and a decrease of photodamage without sacrifice of resolution.

Keywords: multiphoton microscopy; multifocus; spatial light modulator; addressable scanning; dynamic imaging.

1 Introduction

In biomedical research, multiphoton microscopy is a powerful tool capable of deep penetration into strongly scattering specimens and 3D imaging of living cells at high resolution without use of confocal aperture. Multiphoton fluorescence can be generated only at very high peak intensity, which can be achieved by focusing a femtosecond (fs) laser beam to its diffraction limit; such intensity cannot be achieved by illuminating the full field, even with laser power that approaches the photodamage threshold. In addition, the power required to generate the peak intensity is relatively low and cannot be so high as to cause photodamage. Thus, in conventional multiphoton microscopy, only a very small portion of the laser power is useful, and point-by-point scanning of the entire field of view (FOV) gives a very low imaging rate. To make full use of the laser’s power and simultaneously increase the imaging rate, multifocal multiphoton microscopy (MMM) has been developed: The laser’s full power is distributed into multiple beamlets and thus the image rate. In some applications, however, only a small area of the FOV contains the features that must be rapidly imaged with high resolution. For example, when studying a sarcomere’s contraction, we first locate a target muscle cell in the FOV. We then focus the image system into this cell body to acquire high resolution images of the sarcomere at a very high rate that freezes the contraction. In such cases, a small number of beamlets can be used to scan the FOV with low speed and resolution and then scan the area(s) of interest selected rapidly with high resolution. A SLM can be used to realize this addressable multifocal imaging concept. Scanless microscopy has been developed using a SLM, which distributes the illumination light dynamically into multiple areas of interest. In scanless SLM microscopy, however, a lack of the confocal gate for 3D sectioning and multiphoton excitation occurs when an entire area of interest is simultaneously illuminated.

Here, we report the design of an addressable multiregional MMM (AM-MMM) that comprises of SLM and galvo scanners to produce multiple focal arrays. The SLM we used is based on reflective liquid crystal on silicon, which modulates an incident light beam spatially in phase to generate dynamic beamlets according to the displayed phase image that is transferred by a computer interface. By altering the phase image, the orientation of the beamlets can be changed, and thus multibeam scanning can be achieved. However, the transfer rate of the phase image for a commercially available SLM is too slow (less than 100 Hz) to achieve high-speed imaging. Consequently, we should use the SLM to achieve only beam splitting (without scanning), and use a pair of galvo scanners to realize image scanning. For this purpose, the SLM can be modeled as a DOE coupled with a reflecting mirror: The DOE feature will...
allow the SLM to split the incident beam into multiple beamlets, and the mirror feature will permit the SLM to reflect the incident beam with various incident angles into the conjugated angle according to Snell’s Law, thus achieving beam scanning. Consider a traveling plane wave: \[ E_{\text{in}} = \exp[i(kx \cos \alpha + ky \cos \beta)] \] at \( z = 0 \), where \( E_{\text{in}} \) is the optical field, \( k \) is the wave vector, \( x \) and \( y \) are the coordinates in the \( x-y \) plane parallel to the SLM surface, \( \cos \alpha \) and \( \cos \beta \) are the direction cosines, and \( \alpha \) and \( \beta \) are functions of the incident angle \( \theta \) (an angle between the incident wave and the SLM surface’s normal). A phase-modulated function of the phase-only SLM can be expressed as: \[ \Phi(x, y) = \exp[\Phi(x, y)] \] in which \( \Phi \) represents the phase-recovery function corresponding to the pattern of the focus array. Accordingly, the electronic field of the SLM-reflected wave can be expressed as: \[ E_{\text{ref}} = E_{\text{in}} \cdot E_{\text{mod}}. \] The optical field at the focal plane of the Fourier lens will be given by:

\[
G(u, v) = F(u, v) * \delta \left( u - \frac{\cos \alpha}{\lambda}, v - \frac{\cos \beta}{\lambda} \right) = F \left( u - \frac{\cos \alpha}{\lambda}, v - \frac{\cos \beta}{\lambda} \right),
\]

where \( F(u, v) \) is the Fourier transform of \( E_{\text{mod}} \), \( u = x/f/\lambda \), \( v = y/f/\lambda \) and \( u \) and \( v \) are the coordinates on the Fourier plane. According to Eq. (1), when the incident angle changes, the focus array will be only translated in the \( u, v \) plane based on the relationship of \( \Delta u = \cos \alpha/\lambda \) and \( \Delta v = \cos \beta/\lambda \); the other features of the focus array, such as the shape and the number of foci, will not change.

Especially when \( \theta \) is very small, \( \Delta u = \theta_{1x}/\lambda \) and \( \Delta v = \theta_{1y}/\lambda \), which means that the translation of the focus array is proportional to the change of the incidence angle. Therefore, a scanning focus array can be generated with a pair of galvo scanners by scanning the single beam \( \theta_{1x}, \theta_{1y} \) in \( x \) and \( y \) direction that is incident to the SLM.

Multiple arrays of beamlets can be generated. The location and orientation of the beamlets and the beamlet number in each array are based on the location and shape of the corresponding area of interest to be illuminated. At the beginning of each imaging process, the entire FOV was scanned as in conventional MMM, with a focal array (e.g., \( 10 \times 10 \)) generated by the SLM. Using the image thus obtained, we selected the areas of interest by a two-mirror galvo scanner (Cambridge Tech) driven by a line-by-line or a normalized white-noise waveform generated by a NI-PCI6115 DAQ card. The fluorescence signal is separated from the NIR excitation light by a dichroic mirror (Semrock, FF665) and imaged by an Andor cooling CCD (DU-888E-C00-#DZ).

The flexibility and resolution of the AM-MMM was examined using a 760 nm laser beam with an average power of 2 mW for each beamlet. Figure 2(a) is a plot of the fluorescence-intensity profile along the center line of a 0.5 \( \mu \)m fluorescent bead. Figure 2(b) shows the distribution of the fluorescence intensity along z-axial in the beam center obtained by scanning the same 0.5-\( \mu \)m fluorescent bead. In terms of these data, the lateral and axial resolutions are estimated with a deconvolution procedure to be 0.46 and 1.26 \( \mu \)m.

Figure 3 shows a two-photon image of three adult cardiomyocytes, which are fluorescence-labeled for \( \alpha \)-actinin, in three regions of the FOV. The phase pattern for generating the three focal arrays is produced based on the location and the shape of the tracer cells and then uploaded to the SLM. The pattern of the three arrays, which comprises 43 foci in total and is
produced by imaging a Rhodamine-6G film on coverglass, is shown in Fig. 3(a). The sarcomere structures are shown in Fig. 3(b). The laser power of 1 to 3 W from a typical fs laser can be used effectively in our MMM system. To rapidly image an FOV of 200 × 200 μm², we can use a dense foci array such as 30 × 30 with an interfocal distance of 6.5 μm. The max power per focal point would be approximately 0.3 to 1 mW with the typical laser-to-focal-point delivery efficiency of 30%. This low power may not be sufficient for some applications such as imaging deep tissues. Using our MMM, we can increase power at the focal points by producing the desired foci pattern based on the imaging area of interest. For example, to image an adult cardiomyocyte in the FOV, we may use a focal array of 4 × 25 that is oriented along the cell axis. The laser power at each focal point can be adjusted to a typical value of 2 to 4 mW. By doing so, the laser power will be used effectively, and the temporal resolution will be high because a scan is unnecessary.

In conclusion, we have demonstrated an addressable AM-MMM, which can rapidly scan multiple regions in a sample according to the areas of interest. It improves the efficiency of use of the laser power while maintaining high spatial and temporal resolution and decreases photodamage to samples.

Acknowledgments
This work has been partially supported by NIH (SC COBRE P20RR021949 and Career Award 1k25hl088262-01), NSF (MRI CBET-0923311 and SC EPSCoR RII EPS-0903795 through SC GEAR program), The National Natural Science Foundation of China (31171372), Guangdong Province Science and Technology Project (10B060300002), Shenzhen University Application Technology Development Project (201136), and the Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province (Shenzhen University).

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