25 Plane Multifocus Microscopy with Camera Array

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Abstract

We here report an optical system for live 3D microscopy that we call the Multifocus 25-camera microscope (M25). M25 can capture 25 simultaneous focal planes at >100 Hz. This design takes advantage of the latest generation of small, fast and sensitive CMOS cameras to simultaneously but separately capture 25 focal planes on individual camera sensors, arranged in a 5×5 array **(Fig. 4)**. Aberration-corrected multifocus microscopy (MFM) is a simultaneous 3D imaging technique based on diffractive Fourier optics that allows fast live imaging of biological samples [1]. Classically, MFM has been applied in an optical layout where the entire stack of focal planes is recorded on a single camera. Our new design—that we here demonstrate for the first time—simplifies the multifocus optical layout so that it can be built from affordable off-the-shelf components and a set of custom manufactured diffractive gratings. (We made the M25 diffractive optics in the UCSB NanoFab, an open access facility). We are currently launching M25 in biological research projects to study neural circuit function in model organisms such as drosophila and fish

Introduction

One modern biological imaging and research challenge is retrieving live-three-dimensional (3D) information rapidly. The movement of biomolecules, organelles, or structures moving in living organisms introduces motion artifacts, and the acquisition speed of 3D imaging methods can be slow for fast changing events. Hence, a solution is the aberration-corrected multifocus microscopy (MFM) technique, which eliminates the need for scanning in z by simultaneously recording individual focal planes into one camera. This wide-field technique allows live-3D diffraction-limit imaging without loss of resolution.

MFM technique uses custom Fourier diffractive optics to focus-shift images and refocus images aberration-free. The custom diffractive optics for the MFM are the diffractive-multifocus grating (MFG) ,which splits the fluorescence emission light from sample into 5x5 array, and the chromatic correction grating (CCG).

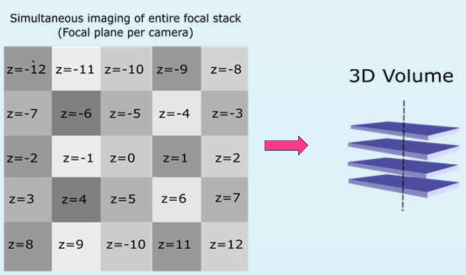


Figure 1 : Using the M25 grating function and custom optics, an instant 3D image is simultaneously acquired from the 25-plane focal stack. The tile pattern shows each individual focal plane capture by individual cameras.

Custom Diffractive Optics

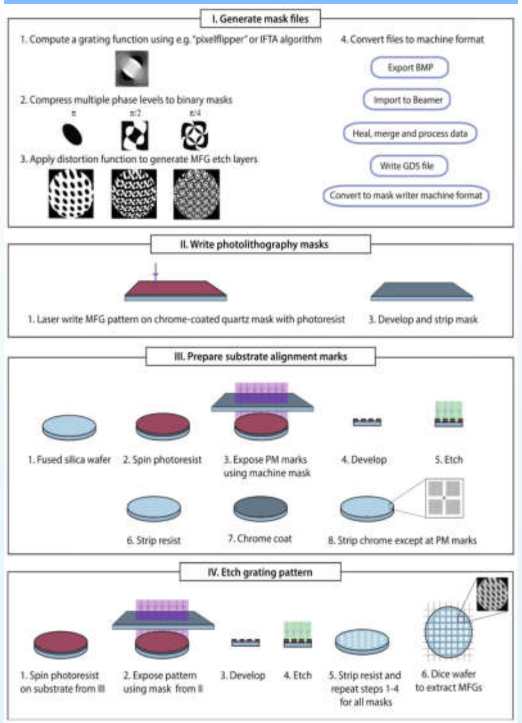


Figure 2: Fabrication of custom Fourier diffractive optics for the 25 multifocus microscope. Process applies for generating the multifocus grating (MFG) and color correcting grating (CCG) on fused silica wafers. Glass wafers are processes with tools in nanofabrication facility to ensure high light efficiency and optical performance. Code for generating the grating functions and distortion pattern for MFM available [2].

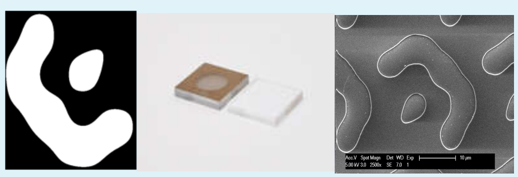


Figure 3 : Multifocus 25 plane grating function. (Left) Grating function acquired through 'pixelFlipper.m' algorithm for 5x5=25 plane. Black and white region correspond to zero and π phase steps. (Center) Mask pieces with diffraction grating pattern. (Right) SEM image of the grating function optimized for 500-550nm green light with pi phase step.

M25 Design

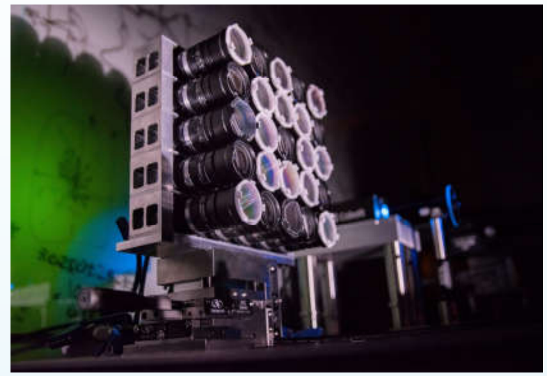
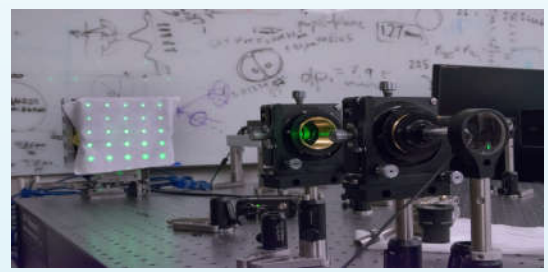


Figure 4: Multifocus 25 plane camera array with 3D printed holders for diffractive optics. High speed, sensitive, and small CMOS cameras in a 5x5 array. Each camera captures individual focal planes.



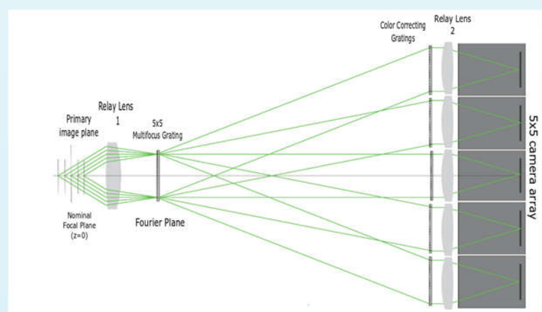


Figure 5 : Ray tracing diagram for the 25 multifocus microscope (M25) with chromatic correction. The M25 uses off-the-shelf components and custom diffractive optics that can be made in nanofabrication facilities. The MFG is placed in the Fourier plane to form the multifocus image.

Conclusion

The 25-plane multifocus microscope (M25) wide-field microscopy technique with a camera array allows simultaneous live-3D imaging of biological. Instead of refocusing the focal planes into one sensor, our new design uses a 5x5 camera array, which simplifies the classical MFM by using affordable off-the-shelf components and custom diffractive optics. Using the latest fast, sensitive, and small CMOS cameras, we are able to achieve acquisition speeds of whole volumes of >100Hz. The MFG photon count efficiency of ≈86% allows faster imaging of small and dim fluorescently tagged specimens. The M25 high acquisition speed solve for motion artifacts from imaging live specimens by imaging the entire volume simultaneously, and image contrast can be increased through deconvolution.

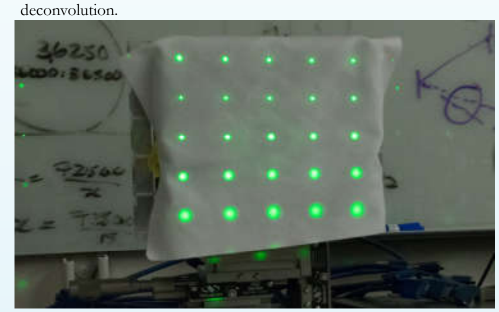


Figure 6: Green laser through MFG shows the phase shift in z from +12 to -12.

Future Work

The M25 provides truly simultaneous 3D imaging of large volumes at >100Hz with diffraction-limited resolution. This technology hopes to advance the field of biology by allowing live imaging on optically transparent organisms. The fast and simultaneous live-3D imaging has the potential to record rapid changing events such as embryo development and neural activity. We are currently deploying this technology in fish and drosophila

Acknowledgements

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References

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