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Adaptive optics-enabled lattice light sheet microscope for imaging organ-on-chip systems

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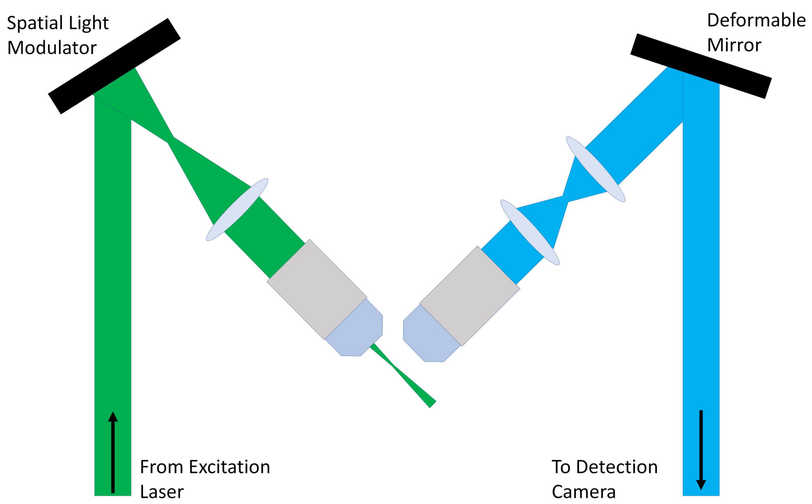
Introduction

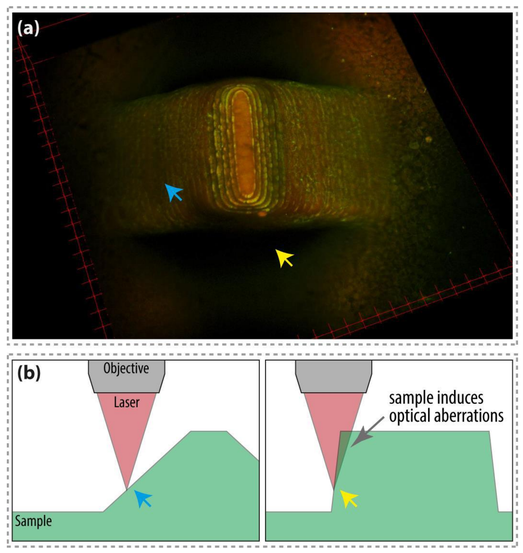
The symbiosis between the development of exotic biomaterials and invention of imaging techniques has kept both fields advancing. Our goal at the CitBIO consortium is to improve the way of the delivery of biopharmaceuticals - and while a branch of the consortium develops biomaterials that mimic the morphological and functional properties of the intestines provide a supervised arena to test these molecules [1], our own group implements a lattice light sheet microscope with adaptive optics capability to be able to see interaction and the transport of them. The lattice light sheet, which is a thin laser beam pattern formed by the interference of multiple Bessel beams, is scanned through the volume of the sample to generate a live 3-dimensional visualization of cellular motion [2]. Additionally, the system also has a deformable mirror that can compensate for the aberration due to sample that allows for the acquisition of high-resolution images deep in the sample [2].



The lattice light sheet is a thin laser beam pattern formed by the interference of multiple Bessel beams using a spatial light modulator. It is scanned through the volume of the sample to generate a live 3-dimensional visualization of cellular motion.. A deformable mirror in the detection path compensates for the sample-induced aberrations, thus enabling of high-resolution imaging deep in the sample.

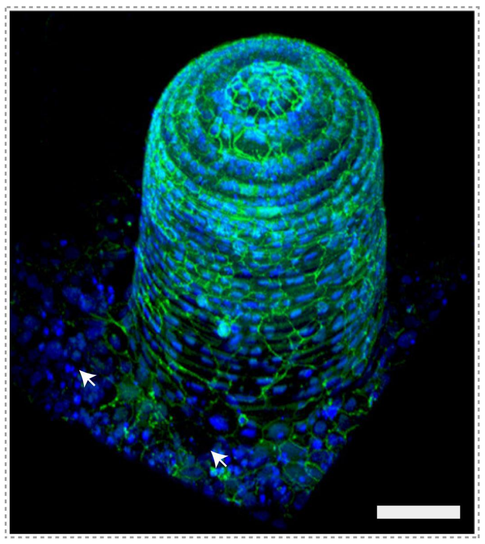
Challenges with two-photon microscopy

a) Pyramid-shaped 3D-printed test sample that has two sides with steep inclination (yellow arrow) and two side with low inclination (blue arrow). On the steep side, the fluorescence signal is lost fast as imaging depth.



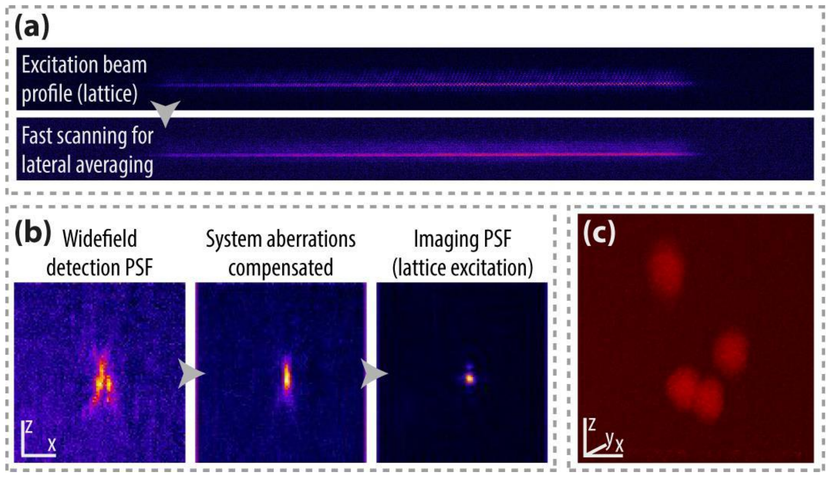
b) The loss of signals comes from sample-induced aberrations which distorts the laser focus and reduces the peak-intensity for two-photon excitation.

3D representation of image-stack capturing a 3D printed hydrogel mimicking a villus in the small intestine. Fluorescence-labeled Caco-2 cells cover the< surface of the hydrogel (nucleic acid = blue, ZO-1 = green).



White arrows indicate regions where ZO-1 (tight junctions) is either absent or not captured due to sample-induced aberrations. Two-photon excitation, NA = 1.0, scalebar = 100 µm

Implementing an adaptive optics enabled lattice light sheet microscope



(a) Excitation beam profile without (top) and with (bottom) fast scanning for lateral averaging of the profile. Wavelength = 488 nm.

(b) PSFs acquired by scanning a fluorescent bead (100 nm) along the optical axis of the detection objective (z-axis). Insets from left to right: (i) Maximum-intensity projection of 3D image stack capturing the fluorescent bead under widefield illumination prior to aberration correction, (ii) Same bead imaged after aberration correction (only system-aberrations), and (iii) Same bead imaged with lattice light-sheet illumination. The final inset (iii) represents the imaging PSF. Scalebars = 2 µm.

(c) 3D representation of image-stack: Very large fluorescent beads (2 µm) embedded in a hydrogel.

The imaging system used here was built following schematics from the Betzig Lab (Janelia Research Campus, VA, USA).

References

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