

DEEP WIDE-FIELD MULTIPHOTON IMAGING (TRAFIX)

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Fluorescence microscopes have been adapted to the needs of the researchers, from super-resolution to fast volumetric imaging. However, moving on from superficial imaging to deep functional imaging is still a remaining challenge to date. Several approaches have been made to tackle this challenge, including aberration correction or characterisation of the complex media. Although they are remarkable, they are usually very complex and time-consuming.

Our new microscope [1], termed TRAFIX, uses temporal focusing illumination [2] to project light patterns through turbid media onto a fluorescent structure. A portion of the fluorescent light emitted by the sample is then collected via the same objective and is measured with a single-pixel detector [3]. The use of temporal focusing extends the penetration depth of the projected patterns through scattering media compared to traditional methods, and the single-pixel detection eliminates the need to resolve spatial frequency in the detection system in a wide-field imaging approach. Spatial information is retrieved by virtue of using patterned illumination. We demonstrate the performance of TRAFIX by imaging various fluorescent samples including microspheres and human embryonic kidney cells through rat brain and human colon tissues at depths up to 7 scattering mean-free-path lengths. We show that it achieves a fivefold increase in signal-to-background ratio with respect to standard point-scanning two-photon microscopy as well as an important reduction in photobleaching. Finally, we discuss the outlook for the technique in terms of imaging speed, higher order multiphoton processes and novel image reconstruction algorithms with compressive sensing.

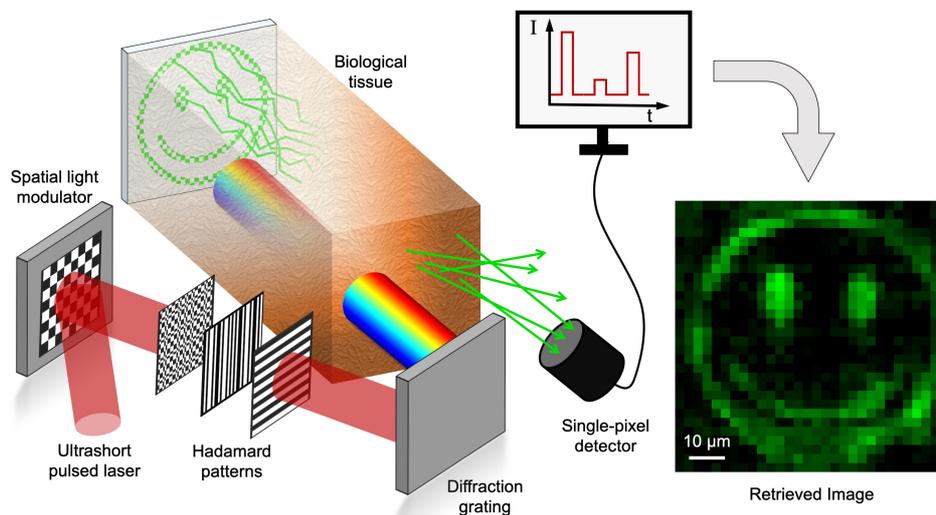


Figure 1: Diagram of TRAFIX and retrieved image through 200 μm of human colon tissue.

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