

PARALLELIZED 3D LIGHT-SHEET MICROSCOPY BASED ON ILLUMINATION ENCODING

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A challenging task in biomedical research is to image large samples at high rates and sub-cellular resolution while avoiding photodamage. Recently, Light-Sheet Microscopy (LSM) has gained a prominent role in 3D characterization methods thanks to its intrinsic optical sectioning combined with low levels of phototoxicity, high Signal-to-Noise Ratio (SNR) and fast 2D widefield acquisition scheme [1]. However, in traditional LSM the presence of moving parts limits the volumetric acquisition speed. Extending the depth of field with spherical aberration or varifocal lenses is the simplest way to increase acquisition speed; however, such approaches result in reduced SNR. To overcome the speed/SNR trade-off, we implemented an encoding/decoding scheme based on simultaneously illuminating the sample with multiple light-sheets. As shown in Figure 1, we used a fast solid-state scanner, consisting of two acousto-optic deflectors, to rapidly create Hadamard-encoded illumination patterns [2]. Following a decoding step, information from individual planes can be restored, with an increase in SNR that scales with the square root of the number of light sheets [3]. We demonstrate the feasibility of our system by imaging phantoms and biological samples. Our results are in perfect agreement with theory.

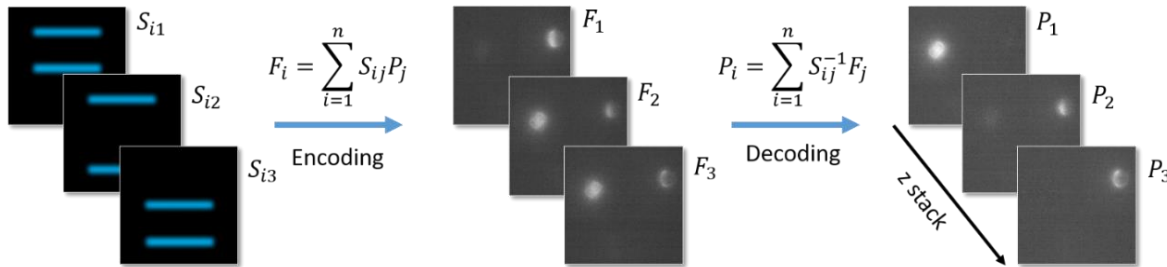


Figure 1. The process of encoding and decoding demonstrated with a pollen grain sample. S_{ij} is a 3-light-sheet illumination sequence, F_i are the raw frames containing a superposition of the decoded planes P_i .

[1] J. Huisken, J. Swoger, F. Del Bene, J. Wittbrodt, and E. H. Stelzer, “Optical sectioning deep inside live embryos by selective plane illumination microscopy”, *Sci.* 305, 1007–1009 (2004).

[2] M. Duocastella, G. Sancataldo, P. Saggau, P. Ramoino, P. Bianchini, and A. Diaspro, “Fast Inertia-Free Volumetric Light-Sheet Microscope”, *ACS Photonics* 4, 1797–1804 (2017).

[3] A. Diaspro “Taking Three-Dimensional Two-Photon Excitation Microscopy Further: Encoding the Light for decoding the brain”, *Microsc Res Tech* 76, 985-987 (2013).