SIMFLUX - EFFICIENT AND FAST STRUCTURED ILLUMINATION FOR SINGLE-MOLECULE LOCALIZATION

Taylor Hinsdale, Jelmer Cnossen, Rasmus Thorsen, Carlas Smith, Bernd Rieger, and Sjoerd Stallinga
Delft University of Technology

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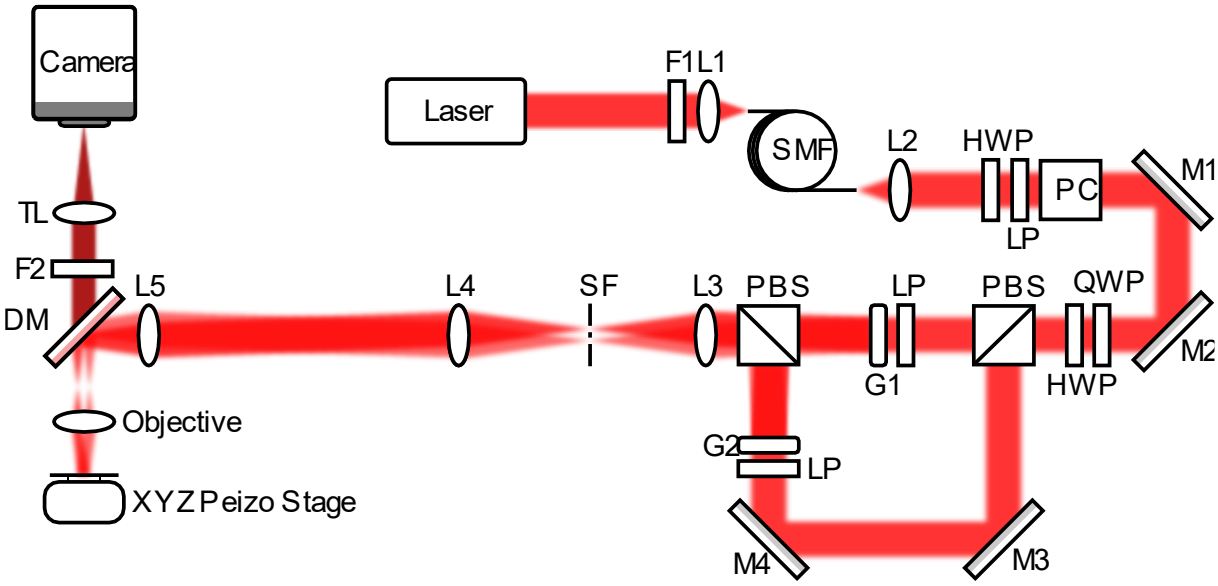
We have developed a novel widefield localization microscopy platform that enhances localization precision, on average, by a factor of two. SIMFLUX utilizes structured light to enhance the information content within the sample, resulting in more precise localizations [1]. To accomplish this, two orthogonal binary phase gratings were illuminated with a laser source and relayed to the sample plane to form a standing wave interference pattern. Two orientations suffice for an isotropic localization precision, as opposed to the customary three or five orientations for Structured Illumination Microscopy (SIM). Three $\pi/3$ phase shifted patterns were then imaged from each grating by combining fast, 3 ms step and settle, piezoelectric stages for phase shifting and a fast switching Pockels cell to alternate between the two grating beam paths. We are currently limited by laser power, 200 mW, to imaging at 70 frames per second, or 11.6 frames per second for a full six image series, but improvements up to several 100 Hz are feasible. We demonstrate our method on GATTAQUANT nano rulers with 80 nm separations between a series of three binding sites. Our results show a factor of two improvement in localization precision of the binding site point clouds when compared to Gaussian widefield localization.



SIMFLUX microscope setup. Laser 640 nm 200 mW, F1 excitation filter, L1 fiber coupling lens, SMF polarization maintaining single mode fiber, L2 – fiber collimation lens, HWP zero order half wave plate 633 nm, QWP zero order quarter waveplate 633 nm, LP glan-laser linear polarizer, PC Pockels cell, M1-4 aluminum steering mirrors, PBS polarizing beam splitter, G1,2 binary phase gratings mounted on piezo stages, L3 75 mm relay lens, SF spatial filter, L4 350 mm relay lens, L5 180 mm relay lens, Objective 1.49 NA TIRF, XYZ Piezo Stage 100x100x100 µm travel range piezo stage, DM dichroic long pass mirror, F2 emission filter, TL tube lens, Camera sCMOS Hamamatsu Orca Flash 4.0 V2

[1] Balzarotti, Francisco, et al. "Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes." *Science* 355.6325 (2017): 606-612.