WAVEGUIDE-PAINT: AN OPEN PLATFORM FOR LARGE FIELD-OF-VIEW SUPER-RESOLUTION IMAGING

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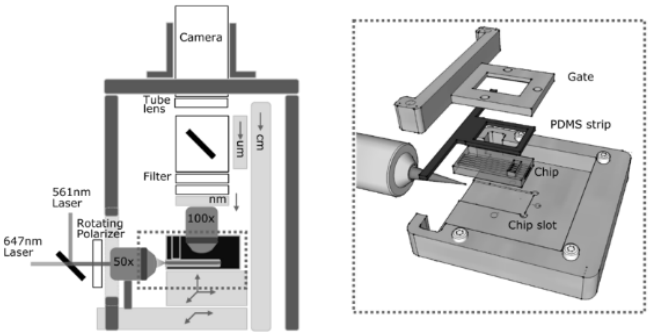
ABSTRACT

Single molecule localization microscopies have been widely adopted due to their demonstrated performance and their accessibility resulting from simple hardware and open software. Moreover, fluorophores have become highly optimized in their targeting, photostability, and photoswitching, so that is routinely possible to access nanometric localization precision. Yet, in localization microscopy, resolution depends not only on localization precision, but also on the density of localizations. This practical limitation of stochastic photoswitching is circumvented by methods that instead use binding and dissociation of fluorescent probes, such as 'points accumulation in nanoscale topography' (PAINT) and extensions thereof which include complementation between target and imager DNA strands in DNA-PAINT [1].
In this work, we extend the waveguide TIRF approach [2] to enable increased throughput and data quality for PAINT, by generating a highly uniform ~100×2000 μm^2 area evanescent field for TIRF illumination. To achieve this, we designed and fabricated waveguides optimized for efficient light coupling and propagation, incorporating a carefully engineered input facet and taper. We also developed a stable, low-cost microscope and 3D-printable waveguide chip holder for easy alignment and imaging. We demonstrate the capabilities of our open platform by using DNA-PAINT to image multiple whole cells or hundreds of origami structures in a single field of view.

FIGURE

Waveguide chip holder and upright microsope for waveguide-PAINT. The waveguide holder enables a free space coupling through a XYZ nanometric stage. The holder design presents a precision slot to position and orient the waveguide chip properly with respect to the laser line, and a sealing gate to hold the imaging buffer and to shield scattered light.



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