PHOTONIC CHIP-BASED PLATFORM FOR LARGE AREA TIRFM FOR LIVE-CELL SUPER-RESOLUTION NEUROIMAGING

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ABSTRACT: Total internal reflection fluorescence microscopy (TIRFM) has the advantage of providing a highly specific illumination area (e.g. 100-200 nm from the substrate) providing sharp contrast optimal for e.g. protein analysis or super-resolution microscopy and a gentle illumination mode suitable for live-cell imaging of even sensitive cell types like primary neurons. Drawbacks in typical objective-based TIRFM are the complexity of the optical set-up, and a non-flexible choice of imaging objective and a small, non-uniform excitation pattern, limiting the field-of-view, throughput and feasibility of many applications [1].

An alternative approach for TIRFM, is to use a photonic waveguide chip to form the TIRF excitation pattern, circumventing the previous drawbacks by allowing essentially any objective to be used for capturing the TIRF image. The chip-based TIRF image can in a straight-forward manner be combined with other microscopy modalities like episcopic or brightfield microscopy, capturing also cellular features above the substrate layer [2]. Additionally, when using multimode waveguides and scanning of the laser coupling along the input facet, the individual mode patterns can be imaged and exploited in super-resolution algorithms relying on fluorescence fluctuations like e.g. SOFI, ESI or MUSICAL.

The chip-based TIRF imaging approach also carries some new challenges in the fabrication of photonic waveguides and in adapting cell-culture and live-cell imaging on-top of photonic chips instead of in conventional cell-culture dishes.

To demonstrate the feasibility of our chip-based TIRF imaging approach for demanding but realistic biology applications in need of large fields-of-view, we show live-cell imaging of primary hippocampal neurons and retinal ganglion cells, both cultured on photonic chips after explantation. We also demonstrate the exploitation of waveguide mode patterns in live-cell super-resolution image reconstruction using MUSICAL reconstruction [3].

REFERENCES