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.

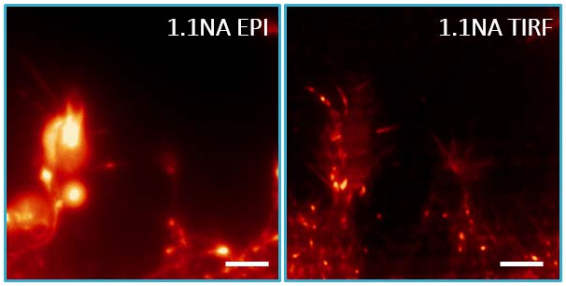

Figure 1: Axonal growth cones (from Xenopus laevis) can be studied with advantage using TIRF illumination (right image) compared to episcopic illumination (left). The scale bars are 10 µm.

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

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