Photonic-chip: a multimodal super-resolution imaging tool for histopathology

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Histopathological assessment involves the observation of tissue samples over large section areas for the identification of diseases. While a vast majority of pathologies are diagnosed with conventional optical microscopes, some syndromes require ultrastructural analysis beyond the resolution capabilities of these instruments. Although electron microscopy (EM) provides sufficient resolution in such cases, both the lengthy sample preparation and the small field-of-view (FOV) offered by this technique severely limit its adoption in routine histopathological examination. In the past two decades, the advent of diverse fluorescent optical super-resolution microscopy techniques (SRM), commonly referred to as optical nanoscopy, bridged the resolution gap between conventional microscopes and EM, promising significant life-science breakthroughs and advances in clinical diagnosis [1]. However, the practical implementation of these novel techniques in histopathological laboratories remains far from the reality due to multiple constraints. These include complex and expensive equipment, the need for highly specialized operators, and a limited FOV that results insufficient to fulfill the throughput requirements of routine histological analyses. Hence, a platform capable of high-throughput and high-resolution imaging would prove advantageous for the implementation of SRM in histopathology.

In this study, we propose the photonic-chip as a multimodal imaging platform for histopathological assessment, allowing large fields-of-view across diverse microscopy methods including total internal reflection fluorescence (TIRF) and single-molecule localization. To ensure optimum ultrastructural preservation and antigenicity, we employed cryo-preserved Tokuyasu sections as the main sample model for the study. In this talk, we will discuss the labeling and the imaging protocols necessary for conducting chip-based microscopy of thin cryo-sections and provide an insight into the challenges and the opportunities offered by a photonic-chip for histopathology imaging (Figure 1).

Photonic chip-based illumination provides several advantages that can be exploited for super-resolution imaging of histopathology samples such as:

a) Decoupling of the excitation and the emission light paths, which translates into high-contrast images with improved imaging throughput.
b) A multi-mode interference pattern illumination that assists in generating the necessary emission sparsity for diverse super-resolution fluorescent optical microscopy methods namely on-chip TIRF[2], on-chip intensity fluctuation-based optical nanoscopy (IFON), on-chip single-molecule localization microscopy (SMLM)[3] and on-chip structured illumination microscopy (SIM)[4].

c) Correlative imaging with other established methods including EM and quantitative phase microscopy can be seamlessly implemented on the photonic chip[5].

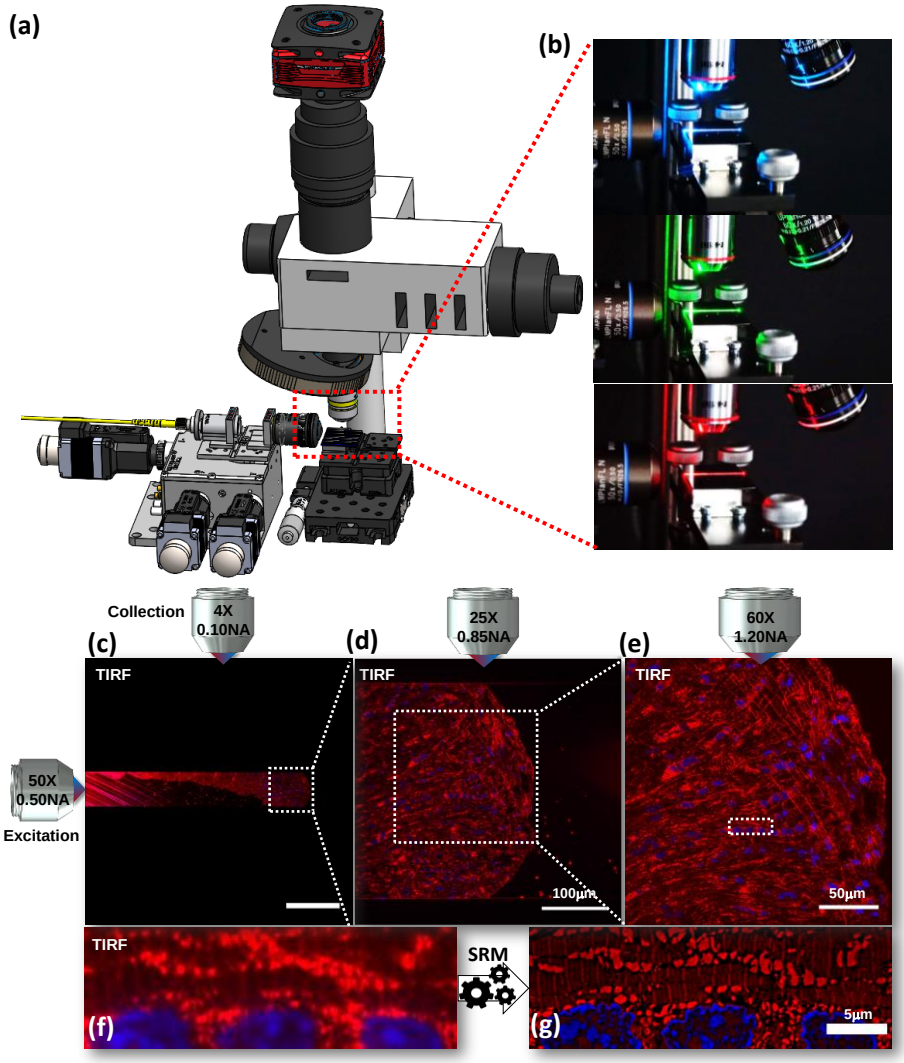

Figure 1. Schematic representation of the chip-TIRFM setup. (a) The chip-TIRFM setup is composed of a custom-made photonic chip module and a commercially available upright collection module. (b) The photonic chip allows decoupling of the excitation and the collection light paths, enabling TIRFM imaging using conventional microscope objectives. Different wavelengths propagating on the waveguide core allow for multicolor TIRFM imaging. (c-e) TIRFM images of a 100 nm thick pig heart cryosection imaged on a photonic chip through different magnification. Membranes in red and nuclei in blue. (f) Magnified view of the diffraction-limited TIRFM image acquired with a 60X/1.20NA water immersion microscope objective. (g) Subsequent post-processing of the raw data enables super-resolution microscopy (SRM), allowing the visualization of structures beyond the diffraction limit of conventional optical microscopy.

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