Photonic-chip: a multimodal imaging tool for histopathology

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Abstract: 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 and single-molecule localization.

Super-resolution microscopy in histopathology: 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.

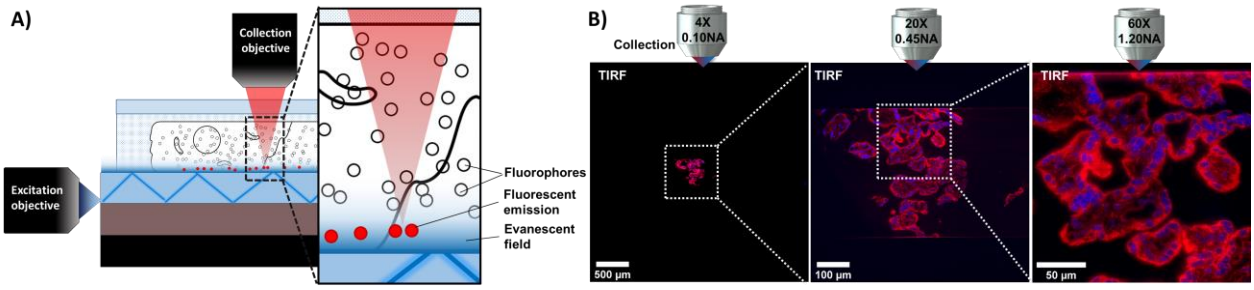

Fig. 1. Schematic representation of the chip-based TIRFM. A) Working principle: upon coupling onto the input facet, the incident light propagates through the waveguide core material via total internal reflection. A thin evanescent field of approx. 150 nm height excites the fluorescent dyes in the vicinity of the waveguide surface, allowing for TIRFM imaging. B) Large field-of-view TIRF micrographs of 400 nm thick human chorionic villi cryosection imaged on a photonic-chip with standard objectives at different magnifications. Lipid membranes in red and nuclei in blue.

Large FOV multimodal imaging through a photonic-chip: Recently, the photonic-chip has been proposed as a promising tool for bioimaging applications, enabling SRM over large FOVs [2-6]. However, existing chip-based microscopy studies have focused solely on fixed [7, 8] and live cells [9, 10], leaving the histological analysis relatively unexplored. Here we interrogate the photonic-chip as a potential imaging platform for morphological assessment of tissues. To ensure optimum ultrastructural preservation and antigenicity, we employed cryo-preserved *Tokuyasu* sections [11] 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 (see Figure 1A) for histopathology imaging. Photonic-chip enables multimodal observation of tissues using diverse microscopy techniques including total

internal reflection fluorescence microscopy (TIRFM) (see Figure 1B), intensity fluctuation-based optical nanoscopy (IFON) [12], and single-molecule localization microscopy (SMLM).

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