CHIP BASED CRYOGENIC TIRF MICROSCOPY

Qingru Li, Christiaan N. Hulleman, Sjoerd Stallinga, Bernd Rieger
Department of Imaging Physics
Delft University of Technology
Lorentzweg 1, 2628 CJ Delft, The Netherlands
E-mail: Q.Li-9@tudelft.nl

KEY WORDS: cryogenic, fluorescence, TIRF, waveguide

Total internal reflection fluorescence (TIRF) microscopy is a powerful imaging tool for studying biological structures because of its low background level, especially for the investigation of the plasma membrane [1]. Imaging at cryogenic temperature not only enables observing structures in a near native state, but also suppressing irreversible photo-bleaching reaction rates resulting in increased photo-stability of fluorophores [2]. Here we report a photonic chip or waveguide-based TIRF microscopy (illustrated in Figure 1a) capable of working at cryogenic temperature. The sample is placed on a high refractive index waveguide and is incident to the evanescent filed.

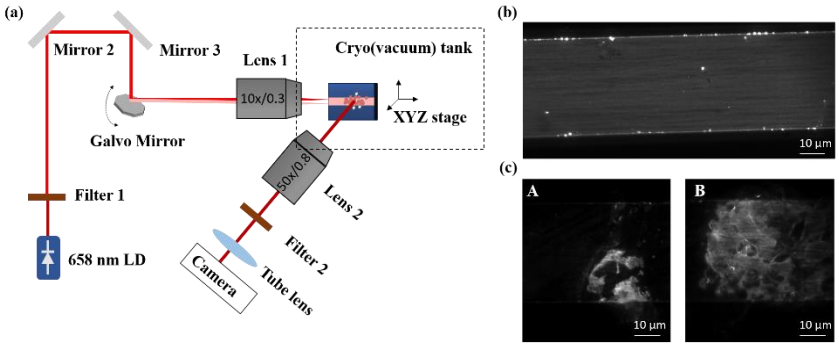

Figure 1: **(a)** Schematic diagram of the chip-based cryogenic TIRF microscopy. **(b)** Near uniform illumination on the waveguide of the chip. **(c)** TIRF images of the plasma membrane of HEK cells at cryogenic temperature.

We demonstrate that this chip-based TIRF microscopy can provide an almost uniform evanescent field in the waveguide on the chip after mode averaging. To this end we scan the focused beam over the width of the chip at high-speed (~100 Hz). Figure 1b) shows the field, with a single molecule concentration spin coated ATTO 647N sample, which was cooled to 97 K using a liquid nitrogen cryostat. The intensity modulation depth is calculated to be around 23%, comparable to the resulting illumination in [3]. Figure 1c) shows two regions of HEK cells labelled with a red plasma membrane dye illuminated by the evanescent field. The cells grew and spread on the chip, thus, only the membrane attached to the chip can be imaged. Now, we are planning to work on the observation of molecules and proteins near the plasma membrane with this chip-based TIRF microscopy.

[1] A.L. Mattheyses, S.M. Simon, J.Z. Rappoport, J Cell Sci. 123(21): 3621-3628, 2010.
[2] R. Zondervan, F. Kulzer, M.A. Kol'chenk, et al., J Phys Chem A. 108(10): 1657-1665, 2004.
[3] Ø. I. Helle, D. A. Coucheron, J.C. Tinguely, et al., Opt. express, 27(5): 6700-6710, 2019.