

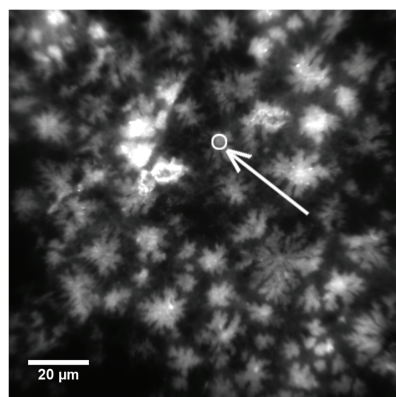
TWO-MODE TOTAL INTERNAL REFLECTION FLUORESCENCE HYPERSPECTRAL MICROSCOPY

Jianping Li and Robert K. Y. Chan

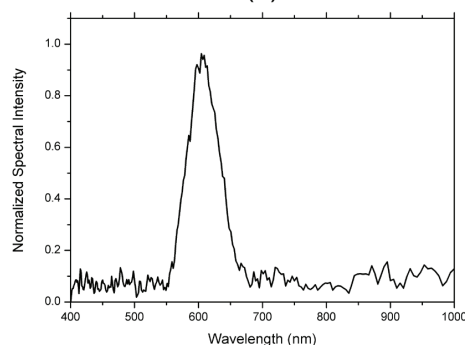
Department of Physics, Hong Kong Baptist University,
224 Waterloo Road, Hong Kong, China

Email address: rchan@hkbu.edu.hk

KEY WORDS: TIRFM, hyperspectral imaging, fluorescence imaging spectroscopy.



(a)



(b)

Figure 1: (a) TIRFM micrograph of quantum dot cluster samples. (b) Fluorescence spectrum obtained at the pixel pointed by the arrow in (a).

Total internal reflection fluorescence microscopy (TIRFM) selectively excites fluorophores in a thin layer ($\sim 100\text{nm}$) of specimen near the interface between solution and substrate to avoid background fluorescence due to the excitation of much larger population of non-bound molecules in conventional fluorescence microscopy. Hence the better SNR performance gained by TIRFM has greatly improved the studies of a large number of molecular events in cellular surfaces and made it possible to observe single molecules fluorescence [1]. While on the other dimension, fluorescence spectroscopy (FS) has long been a well-established noninvasive technique for biology as biochemical information can often be extracted prior to any visual change could be discerned from imaging methods. [2].

We combine both TIRFM and FS technologies into a single hyperspectral microscopic system with two working modes. Under the imaging mode, it works in the same principle as TIRFM which can acquire high resolution fluorescence micrographs; while its hyperspectral mode can simultaneously record lower resolution 2D imaging as well as 1D spectroscopic fluorescence information in one measurement. The spectral resolving ability of the hyperspectral mode is achieved by Fourier transform interferometric method instead of using filters or dispersive prisms and gratings. The advantage of interferometric method is that it is very flexible to achieve high and tunable spectral resolution within very broad spectral range. A simple whole

optical sampling method was devised to extend the system's spectral coverage down to visible wavelengths with low-cost and good reliability [3]. Preliminary measurements demonstrate that, under imaging mode, the TIRFM hyperspectral microscope can achieve a maximum field-of-view (FOV) of $160\times 160\mu\text{m}$ and resolution as high as $1\text{k}\times 1\text{k}$ pixels with a $50\times$, $\text{NA}=0.55$ objective; under the hyperspectral mode, similar FOV with lower spatial resolution up to 300×300 pixels and tunable spectral resolution of about $9.78\text{cm}^{-1}\sim 246.9\text{cm}^{-1}$ ($0.3\text{nm}\sim 7.5\text{nm}@550\text{nm}$) can be selectively obtained at the same time.

References

1. Axelrod, D., Biophysical Tools for Biologists, (Elsevier Academic Press Inc, San Diego, 2008).
2. Lakowicz, J.R., Principles of Fluorescence Spectroscopy, (Springer Science, Boston, 2006).
3. Li, J., R.K.Y. Chan, and X. Wang, "Tests of a practical visible-NIR imaging Fourier transform spectrometer for biological and chemical fluorescence emission measurements." *Opt. Express*, **17**, 21083-21090 (2009).