

A Nano Grating Tunable MEMS Optical Filter for High-Speed On-Chip Multispectral Fluorescent Detection

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Abstract— We report a microelectromechanical (MEMS) tunable optical filter and its integration in a fluorescence microscope for high speed on-chip spectral measurements. This integration allows for measurements of any fluorescence sample placed onto the microscope stage. We demonstrate the system capabilities by taking spectral measurements of multicolor fluorescent beads and fluorescently labeled cells passing through a microfluidic cytometer. The system has applications in biological studies where the measurement of multiple fluorescent peaks is restricted by the detection method’s speed and sensitivity.

I. INTRODUCTION

Light emitted from fluorescent proteins and nanomaterials provides important information for biologists to elucidate genetic transcription in cells [1-3], protein bindings at cell membranes [4], and molecular-level physiological properties of tissue [5]. A technological breakthrough in biophotonics and molecular imaging significantly advances scientific knowledge leading to drug discovery and disease prevention.

We have recently developed a novel on-chip high-speed optical microdevice, namely “nano grating tunable filter (Nano-GTF),” using polymer-silicon hybrid nanophotonic MEMS technology, as shown in Fig. 1(a). The device consists of an elastic microbridge with a nanoimprinted [6-8] grating pattern on the top surface. The microbridge is fabricated from an optically transparent (more than 90% transmission for $\lambda = 350 - 1200$ nm) polymer, polydimethylsiloxane (PDMS), and attached to MEMS silicon comb drive electrostatic actuators. Applying a voltage difference across the comb drive generates a force that mechanically stretches the microbridge, altering the grating spacing on the top surface. The tuning of the nanoscale (~ 350 nm) surface grating feature dynamically changes an optical wavelength diffracted at a particular angle, allowing for selectively capturing a single narrow-bandpass wavelength at a photodetector as illustrated in Fig. 1(b). The nanoscale grating feature simultaneously permits the high spectral resolution and the device miniaturization yielding high dynamic bandwidth. The entire grating size limited to $400 \mu\text{m} \times 400 \mu\text{m}$ in area can achieve high-speed (> 2 kHz)

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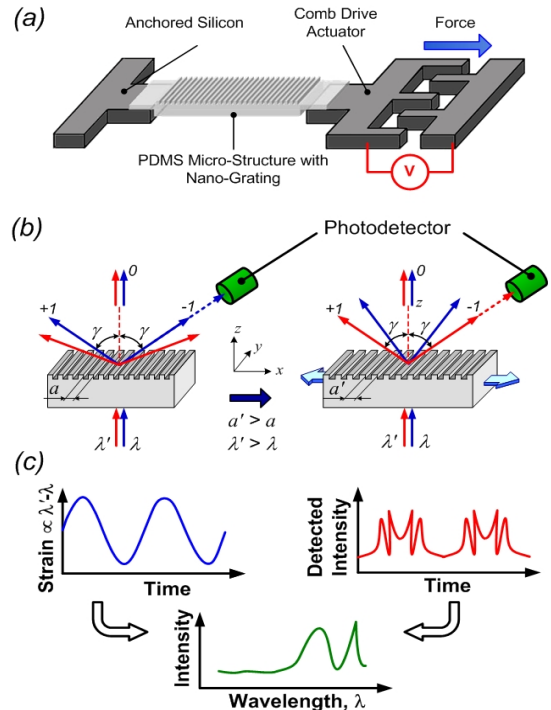


Fig. 1. Polymer-silicon hybrid nanophotonic MEMS for spectral analysis. (a) Schematic of on-chip strain-tunable soft polymer nano-grating optical MEMS filter (Nano-GTF). (b) PDMS microbridge of Nano-GTF with grating imprint at initial state and stretched state. The light dispersing characteristic of the microbridge varies with the applied strain. (c) Spectrum data acquisition from the correlation between the PDMS strain and the detector signal.

wavelength tuning. The total device area is $4 \text{ mm} \times 2.5 \text{ mm}$. The device was fabricated by a process named the “soft-lithographic lift-off and grafting process” that we developed for the construction of its unique polymer-silicon hybrid structure [9-11].

This paper demonstrates multispectral flow cytometry using a microfluidic device optically coupled with a Nano-GTF integrated monochromator. This approach can also be extended to a wide variety of dynamic photospectral measurements accompanying time-varying multiplexed fluorescent emissions.

II. METHODOLOGY

Implementation of the Nano-GTF monochromator for spectroscopy measurements permits the photodetector to be a single photomultiplier tube (PMT) capable of higher speeds and greater sensitivity compared to other spectroscopy measurement techniques. Here, we integrate the MEMS

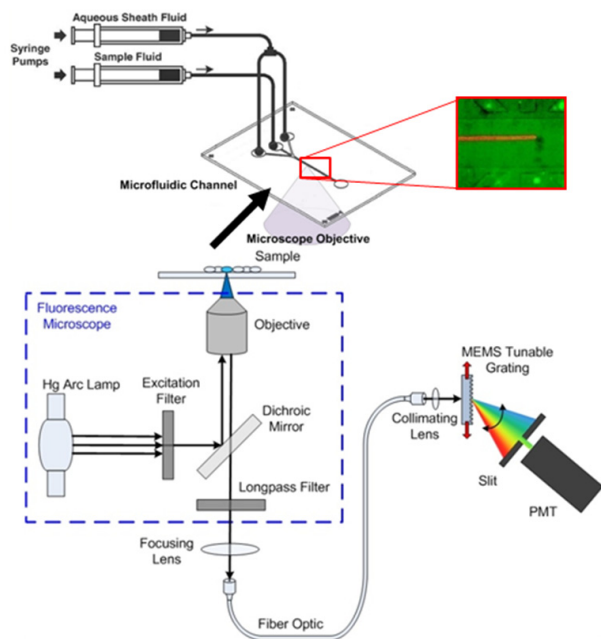


Fig. 2. Schematic of the MEMS-integrated optical system used for spectral measurements of samples placed on the stage of a fluorescence microscope. A PDMS microfluidic device is placed on the microscope stage for spectral cytometry measurements.

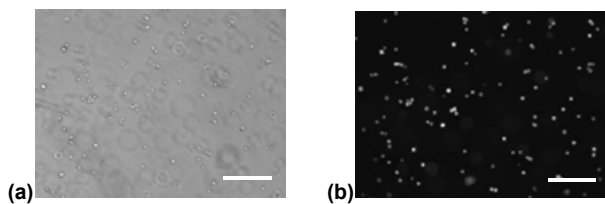


Fig. 3. (a) Bright field image and (b) fluorescent microscopy image of Calcein AM-stained PC-3 cell lines. Scale bar is 200 μm .

monochromator setup with a fluorescence microscope and implement a microfluidic cytometer that we developed in our previous work [12], as illustrated in Fig. 2. The fluidic sample contains two different fluorescent beads, i.e., green fluorescent beads 35-50 μm in diameter (35-8, Duke Scientific Co.) and red fluorescent beads 20-30 μm in diameter (36-6, Duke Scientific Co.). According to the specifications, the green and red beads have emission peaks at 530 nm and 610 nm, respectively. The microfluidic channel files the beads past the focal point of the microscope objective. A mercury arc lamp excites the passing beads, and fluorescence emission is collected through the same objective. The light signal is then directed onto the MEMS grating, and the diffracted signal is detected by the PMT. The MEMS device is simultaneously actuated with a 200 Hz voltage sine wave. The voltage actuation and prior MEMS characterization determine the continuously changing wavelength directed towards the PMT. Multiple spectral sweeps are taken as the beads pass through the 300 μm detection zone in approximately 100 ms.

To validate the system's compatibility with biological particles, we also prepare fluorescently labeled PC-3 prostate cancer cells. These cells, originally isolated from vertebral

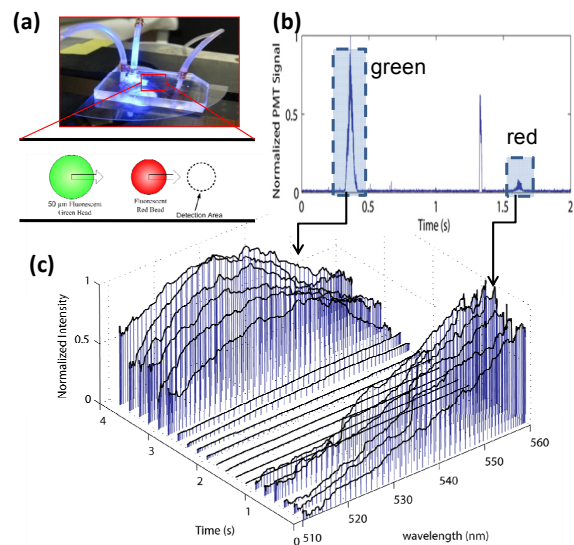


Fig. 4. Results of spectral cytometry measurements taken with the microfluidic cytometer, microscope setup, and MEMS device. (a) Fluorescent beads forming a single profile reach the detection zone of the microfluidic channel. (b) PMT signal as several fluorescent beads pass through the microfluidic channel. (c) Real-time spectral measurements of a red (or green) fluorescent bead passing through the microfluidic detection zone. Each plane is a spectral sweep taken with the MEMS device. The spectral intensities are normalized by the maximum PMT signal found in each detection.

metastases in prostate cancer patients, are obtained from ATCC (Rockville, MD). The label used here is calcein acetoxymethylester (Calcein AM) (BD Sciences, CA). Calcein AM is a fluorescent dye with excitation and emission wavelengths of 494/517, respectively, and is commonly used in biology. The dye molecule can be transported through the cellular membrane into live cells, which makes it useful for testing of cell viability based on cells' cytoplasmic membrane integrity. The PC-3 cell lines are labeled *in situ* using 1 μM Calcein-AM in HBSS/0.1% BSA for 60 minutes at 37 $^{\circ}\text{C}$, washed with saline solution, and resuspended in buffer, following the manufacturer's protocol (Fig. 3). The dynamic spectral acquisition is repeated for the cells flowing through the microfluidic channel.

III. RESULTS AND DISCUSSIONS

Measurements in Fig. 4 show the spectral sweeps taken of a red bead and a green bead, respectively. The spectral data correctly shows a peak for the green bead near 530 nm and a peak for the red bead near 610 nm. The data also correctly measures the difference in intensity between the two types of beads, with the green beads over 10 times brighter than red. The results prove our device's capability of tuning the bandpass optical wavelength at high speed. Figure 5 shows results taken for the Calcein AM-stained PC-3 prostate cancer cells. The data were taken at a sampling rate of 50 cells per second. The maximum emission peaks measured for these cells consistently appear at 510-520 nm, which well matches with the data provided by the manufacturer.

In our previous study, we demonstrated optical spectroscopy for $\lambda = 550 - 620$ nm with high-speed (spectral acquisition time of ~ 250 μ s) and high sensitivity capable of detecting a few tens picowatt (10^{-12} watt) optical power through coupling the developed device with a single point PMT detector (H5784-20, Hamamatsu Photonics) [13]. Our long-term operation test over 500 million cycles proved the excellent structural reliability of our device [10].

The sensitivity of the current measurements is primarily limited by optical loss at the fiber couplings. By improving the optics of our setting, we may further increase the spectral acquisition speed by a factor of 10.

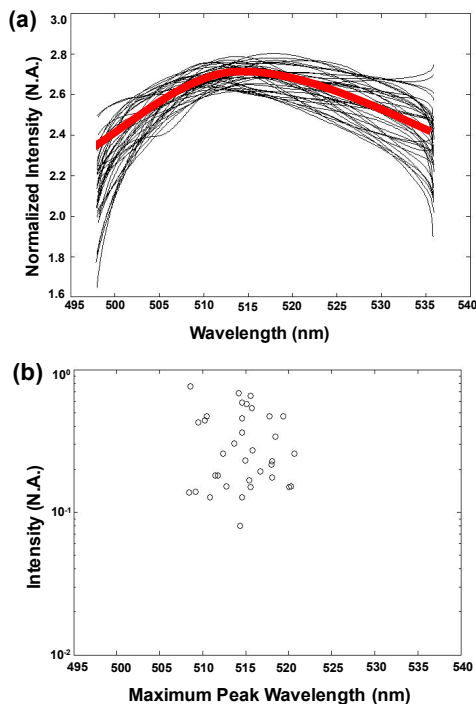


Fig. 5. Results from emission spectrum measurements for Calcein AM-stained PC-3 cells. (a) Plots showing spectral sweeps taken of multiple in-flow PC-3 cells. The signal intensity is normalized by the total intensity integrated over the spectral detection range (i.e., the area defined by the intensity-wavelength curve for each spectral sweep). (b) Distribution of intensity-maximum peak wavelength combination for cell samples ($N = 35$). The variance in the maximum peak wavelength data is as small as ± 5 nm with a mean value of 515 nm.

IV. CONCLUSION

We have successfully demonstrated microfluidic multispectral flow cytometry with a fluorescence microscope incorporating a nanograting MEMS tunable optical filter. The presented results prove the setup's usefulness for measuring weak fluorescent signals at a rapid pace. As the beads and cells pass by the detection region, multiple spectral sweeps are taken, and the rise and fall of the particles' signals passing through the interrogation zone can be seen. In particular, the spectral measurements are able to distinguish the type of bead by its wavelength peak regardless of its relative intensity. Future measurements will demonstrate high sensitivity

spectral sweeps within 1 ms for single cells encoded by multiple fluorescent dyes of different colors. With cytometry improvements, cells could flow over 100 cells per second, and a spectral sweep could be taken of each cell.

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