SUPPORTING INFORMATION FOR

Multiplexed spectral signature detection for microfluidic color-coded bioparticle flow

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This supporting information includes the following sections:

I. Detail microdevice information of MMFC setup

II. The definition of Peak waveleength, peak intensity and linear coefficient

III. Comparison of commercial flow cytometry

IV. Statistical analysis of HeyA8-Gfp and Heya8-calcein AM groups

I. DETAIL MICRODEVICE INFORMATION OF MMFC SETUP

The grating microdevice used in our study consists of electrostatic microelectromechanical systems (MEMS) actuators and a tunable polymer diffraction grating made from polydimethylsiloxane (PDMS) (Figure S-1A). The PDMS microbridge containing a grating pattern on the top surface is attached to a suspended silicon shuttle beam containing a series of comb-shaped electrodes while the other end is fixed onto a silicon substrate. Electrical wires are bonded onto on-chip contact pads to connect the device to a power supply generating a sinusoidal voltage signal at 1KHz. The resulting electric fields between the adjacent comb-shaped electrodes generate actuation force driving the oscillatory motion of the shuttle beam. The shuttle beam motion repeatedly stretches and contracts the PDMS grating microbridge in response to the actuation voltage signal. This enables high-speed wavelength tuning capability with the microdevice. The MMFC setup incorporates this device in the spectroscopy system coupled with the optofluidic chamber via an optical fiber waveguide (Figure S-1B). The diffraction of light by the grating images a single wavelength or a narrow wavelength band onto a photodetector surface. Fluorescence emission light is collected from a particle flowing in a microfluidic channel and transmitted to the spectroscopy system.

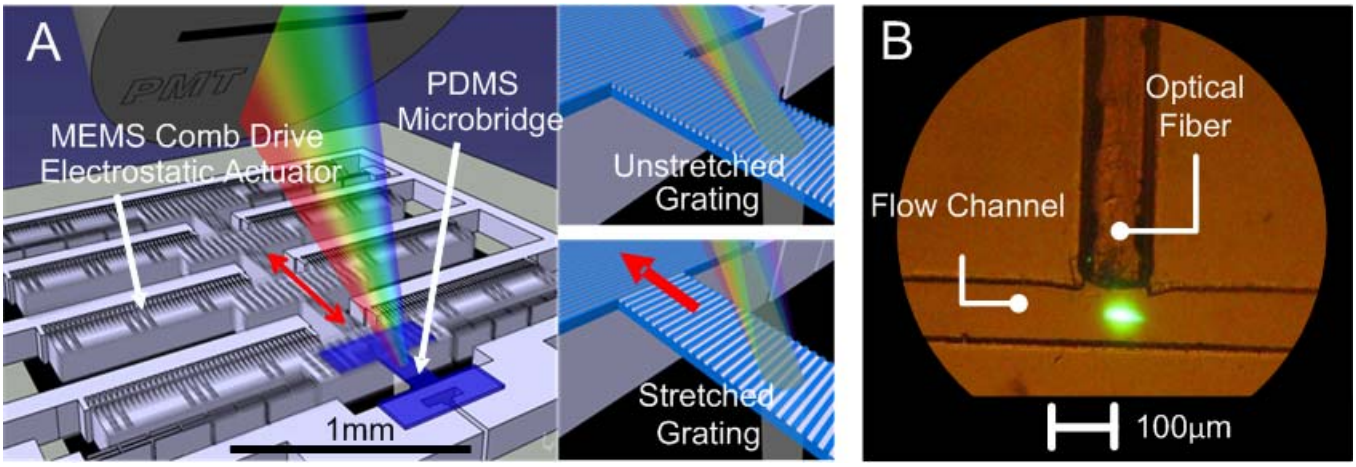

Figure S-1. (A) 3D schematic drawing of the entire structure of the grating microdevice. The strain-tuning of the grating profile varies the diffraction angle of transmitting light. The grating microbridge is 300μm in length, 300μm in width, wide, and 20μm in thickness and has a surface pattern with a 700 nm nominal pitch. (B) The optical microscope image of the microfluidic channel shows the snap shot of a hydrodynamically focused particle flow in front of the optical fiber probe.

II. THE DEFINITION OF PEAK WAVELEENGTH, PEAK INTENSITY AND LINEAR COEFFICIENT

In conventional fluorescent microscopy or flow cytometry, the definition of "fluorescence intensity" is total radiant energy emitted by fluorophores over a certain period of time. In practice, it is measured by a photodetector in a specific wavelength band determined by an emission filter. In this case, the "fluorescence intensity" is an integration of the spectrum intensity (i.e., radiant energy emitted at a given wavelength) of fluorophores over the wavelength band. In contrast, our study identifies the "peak intensity" and "peak wavelength" from continuous spectral profile measurements. Here, the "peak intensity" refers to the intensity value of maximum emission, and "peak wavelength" refers to the wavelength value of maximum emission (Figure S-2A). We first resolve the spectral profiles of different cell groups with a monochromator system consisting of a tunable grating microdevice and a PMT detector (Figure S-2A). By identifying the intensity and wavelength values of maximum emission, we construct a two-dimensional plot of peak wavelength vs. peak intensity (Figure S-2B).

To quantitatively identify the spectral characteristics of the emission of different cells or microspheres, we develop a curve fitting scheme based on a Matlab program. To simplify the analysis process, we use a quadratic function to approximate the shape of a MMFC spectral plot a

$$\boldsymbol{y = \alpha x^2 + \beta x + \gamma} \qquad \text{(Eq. 1)},$$

where x represents the wavelength, and y represents the spectrum intensity. Here, α, β and γ are called the quadratic coefficient, the linear coefficient, and the constant term, respectively. The values of α, β and γ mathematically define the spectral shape of the MMFC plot in a given wavelength range.

The graph of a quadratic function forms a parabola, where the positive or negative value of the quadratic coefficient α determines whether the parabola opens upward or downward. The value of α also governs the rate of increase (or decrease) of the quadratic function graph from the vertex; a larger positive value of α makes the function increase faster and the graph appear more closed. The linear coefficient β alone is the declivity of the parabola in a given wavelength range. With a larger value of β, the shape of the spectrum becomes sharper. The constant term γ represents the offset of the spectral curve. To make the comparison clear, the value of γ is adjusted to make each spectrum curve with same peak intensity. The quadratic coefficient α and the linear coefficient β together determine the peak wavelength, which is given by -β/2α. Here, we already have peak wavelength as one of the characteristic parameters. As a result, only one of the values of α and β is additionally needed to uniquely obtain the full mathematical description of the spectral shape since the other value can be uniquely determined from the peak wavelength value.

To give an example of how curve fitting method works, we choose one of HeyA8-Calcein AM spectrum data plot (shown as the dotted line in Figure S-2) obtained by the MMFC measurement to show how these characteristic parameters are extracted. With the known peak wavelength value (–β/2α), we only vary the value of the linear coefficient. Here, the theoretical curve becomes sharper with the increasing value of β. The least

square curve fitting method is applied to obtain the optimal value of β = 51.17, which yields the theoretical curve best fitted to the experimental HeyA8-Calcein AM spectrum data plot (the dot line in Figure S-1). By comparing the linear coefficient of the quadratic function, we can quantitatively differentiate the spectrum difference of fluorescent labeled cells or microspheres with similar spectra.

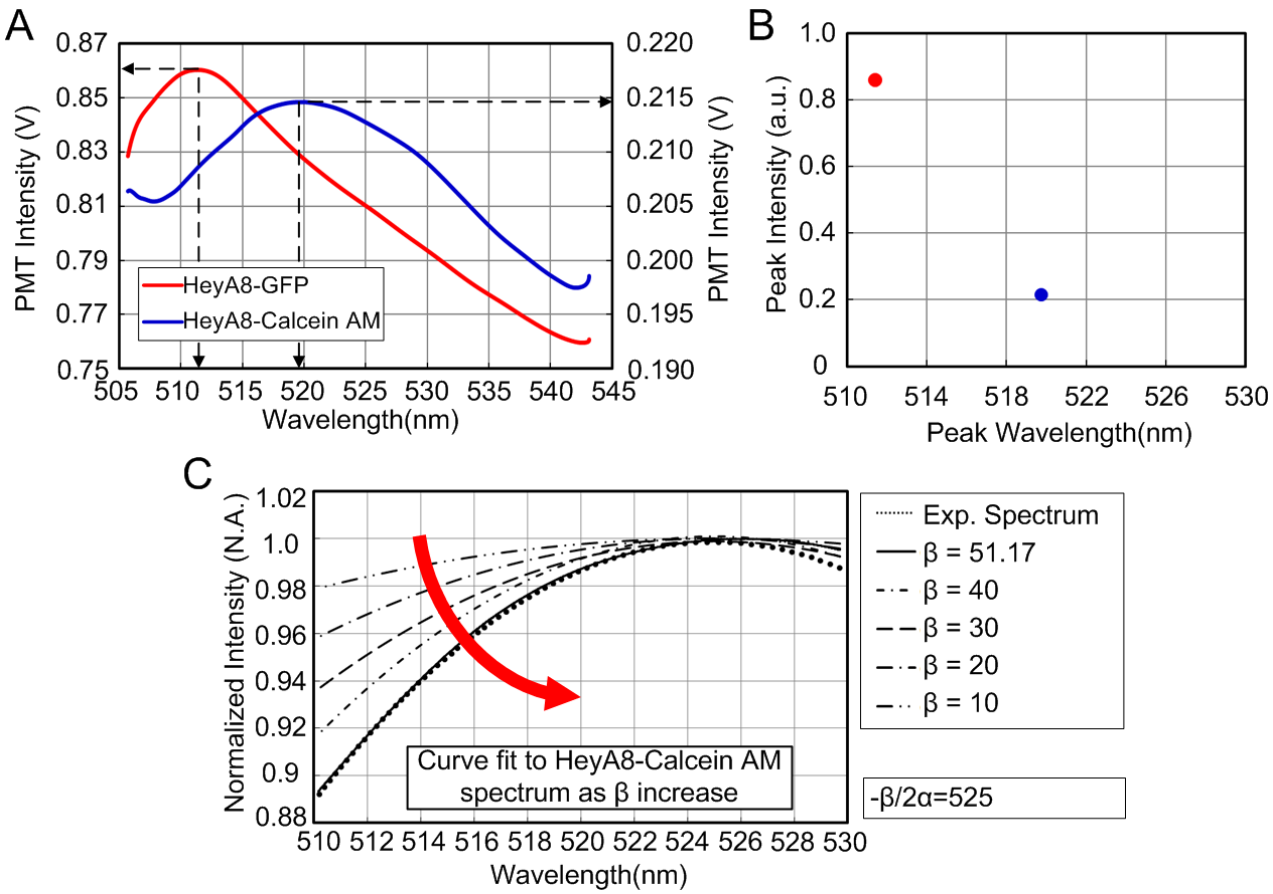

Figure S-2. Definition of peak wavelength, peak intensity and linear coefficient (A) Spectrum plots of HeyA8-GFP and HeyA8-Calcein AM (B) By finding the intensity and wavelength value of maximum emission, a two-dimensional plot of peak wavelength vs. peak intensity can be generated (C) Effect of the linear coefficient β on the spectrum shape. For known values of the peak wavelength, which is determined by –β/2α, and the constant term γ, the quadratic function plot becomes sharper with the increasing linear coefficient value. By adjusting the linear coefficient, we can fit the theoretical quadratic function curve to the experimental HeyA8-Calcein AM spectrum data plot (dotted line).

III. COMPARISON WITH COMMERCIAL FLOW CYTOMETRY

To compare our technique with conventional flow cytometry, we flow HeyA8 cell groups with three different labels: (1) HeyA8-GFP cells, (2) HeyA8-Calcein AM cells and (3) unstained HeyA8 cells in a commercial flow cytometer (BD LSR II, BD bioscience). The sample of the unstained HeyA8 cells is used as a control. The system uses blue octagon (488nm) laser excitation and FITC (500nm long pass dichroic mirror with 530/30nm bandpass filter) and PE (550nm long pass dichroic mirror with 575/26nm bandpass filter) channels. We obtain a univariate histogram of FITC fluorescence (Figure S-3A), an overlay bivriate histogram of FITC fluorescence versus PE fluorescence (Figure S-3B) and a univariate histogram of PE fluorescence (Figure S-3C) for the three sample groups. The significant spectral overlap between GFP and Calcein AM prohibits us to statistically distinguish the two sample groups by the two-parameter flow cytometry setup.

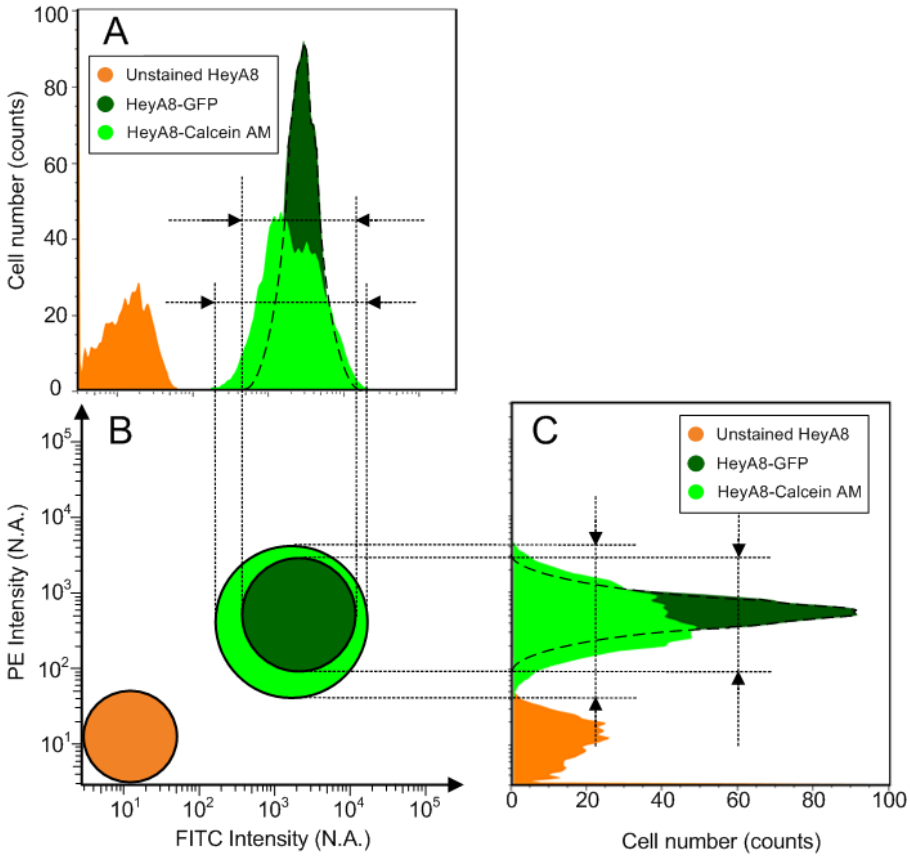

Figure S-3. HeyA8 cell analysis by the commercial flow cytometry (BD LSR II, BD bioscience). (A) A univariate histogram of FITC fluorescnece. (B) A bivariate histogram of FITC fluorescence versus PE fluorescence. (C) A univariate histogram of PE fluorescence. In this plot, 97% of the HeyA8-GFP cell population overlaps with 86% of the HeyA8-Calcein AM cell population, which makes the discrimination of these cell groups very difficult.

IV. STATISTICAL ANALYSIS OF HEYA8-GFP AND HEYA8-CALCEIN AM GROUPS

		Mean	Standard deviation
Peak wavelength of	GFP	511.98 (nm)	3.44 (nm)
	Calcien AM	519.04 (nm)	4.19 (nm)
Peak intensity of	GFP	0.78 (N.A.)	0.43 (N.A.)
	Calcien AM	0.39 (N.A.)	0.28 (N.A.)
Linear coefficient of	GFP	20.82 (N.A.)	19.29 (N.A.)
	Calcien AM	43.65 (N.A.)	22.30 (N.A.)

Table S-1. Table showing the mean value and standard deviation of the peak wavelength, peak intensity and linear coefficient of the HeyA8-GFP and HeyA8-Calcein AM cell groups.