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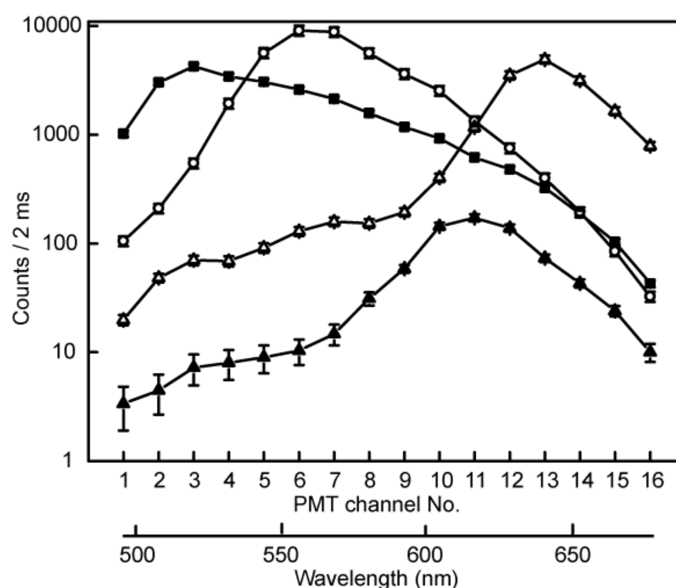
Supplementary Information for ***On-chip microfluidic sorting with fluorescence spectrum detection and multiway separation*** by Hirokazu Sugino, Kazuto Ozaki, Yoshitaka Shirasaki, Takahiro Arakawa, Shuichi Shoji and Takashi Funatsu.

1 Spectrum measurement

To examine whether fluorescence spectra were measured with the optical system shown in Fig. 3, four different fluorescent microspheres were introduced into a multichannel chip. First, Yellow-green microspheres suspended in 10% (w/v) TGP solution were introduced from Inlet 2 using compressed air, while buffer solution containing 10% (w/v) TGP was introduced from Inlet 1. The beads were excited by a blue laser, and the fluorescence spectrum was measured for one minute with this optical system. Subsequently, fluorescent spectra of the other three microspheres, namely Orange, Red, and Crimson microspheres, were also measured in a similar way. The fluorescence emission spectra of four different microspheres measured by the 1x16 arrayed PMT are shown in Supplemental Fig.1. The emission maxima of Yellow-green, Orange, Red, and Crimson microspheres were observed at channel Nos. 3, 6, 11, and 13, respectively. By comparing these spectra with those obtained by a fluorescence spectrophotometer (data not shown), we determined that the wavelength range was about 495-685 nm and the bandwidth per channel was about 12 nm.



Supplemental Fig.1

The fluorescence emission spectra of *E. coli* cells expressing three different fluorescent proteins were also measured by the 1x16 arrayed PMT (Supplemental Fig. 2).

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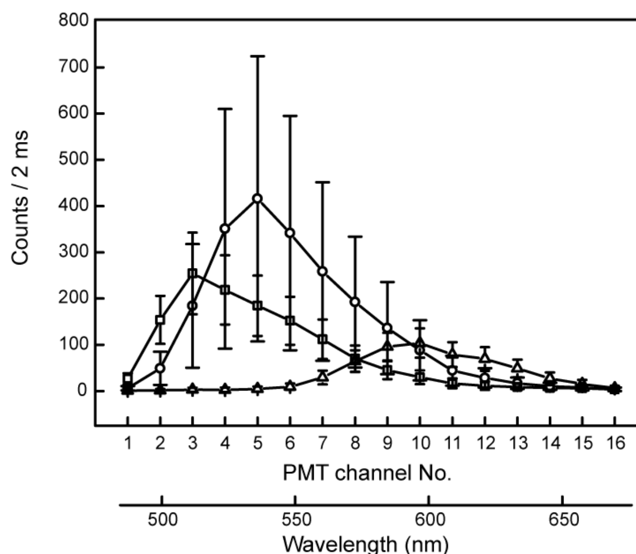


Supplemental Fig.2

2 Identification of fluorescent microspheres and *E. coli* cells expressing fluorescent proteins

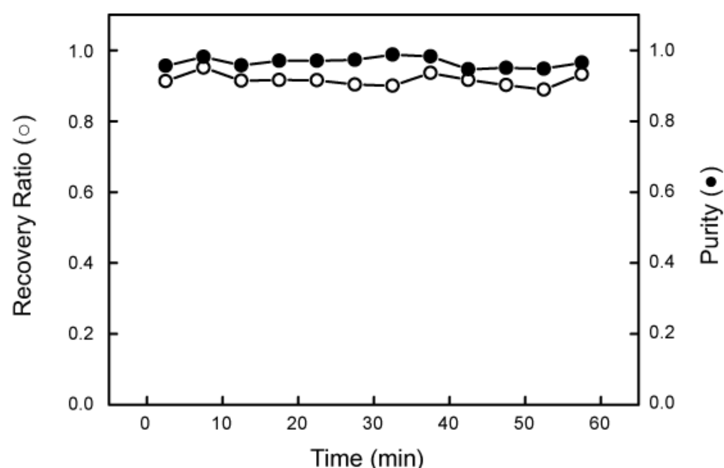
We evaluated how accurately the software distinguished between four types of microspheres. The algorithm for identifying microspheres was as follows. In the case of Yellow-green microspheres, for example, a lower limit was set on channel No. 3 of the 1x16 arrayed PMT, where the emission maximum of Yellow-green microspheres was observed. Then, higher limits were set on channel Nos. 6, 11, and 13, where the emission maxima of other beads were observed. When the fluorescence signal from channel No. 3 exceeded the predefined lower limit and the fluorescence signals from channel Nos. 6, 11, 13 did not exceed the higher limits, the software judged that the microsphere was Yellow-green. To evaluate the accuracy of identification, a mixture of Yellow-green, Orange, Red, and Crimson microspheres was introduced in a chip and the program identified the kinds of individual microspheres passing through the detection area at a rate of 3260 times per hour. Whether the identification by the software was correct or not was confirmed by comparing the identification results to each spectrum. The identification accuracies of Yellow-green, Orange, Red, and Crimson microspheres, defined as the number of correctly identified microspheres divided by the total number of detected microspheres, were 99.1, 99.7, 99.8, and 99.8%, respectively. In spite of the lower fluorescence intensity of Red microspheres (Fig. 4d-1 and Supplemental Fig. 1), they could be identified by the software with similar accuracy to other beads.

Next, we evaluated how accurately a computer program distinguished between three

types of *E. coli* cells expressing different fluorescent proteins. Since the intensities of fluorescence spectra were different from cell to cell (Supplemental Fig. 2), they were normalized before being identified by the computer program. The identification accuracy of fluorescent proteins was 99.5 % (659/662). In spite of the lower fluorescence intensity of *E. coli* cells expressing DsRed, they could be identified by the software with similar accuracy to other cells.

3 Stability of the separation

Finally, to evaluate the stability of sorting under a laser irradiation time of 60 ms, we sorted four different microspheres for one hour. The final concentration of total microspheres introduced from Inlet 2 was 7 x 10^6 particles/ml and throughput was 0.8 particles/s. Supplemental Fig.3 shows the time course of recovery ratio and purity. The accuracy of identifying microspheres was 99.6%. The average recovery ratio and purity were 91.3 and 96.4%, respectively. This shows that microspheres can be sorted stably for at least one hour.



Supplemental Fig.3

Supplementary Figure Captions

Supplemental Fig. S1 Fluorescence spectra of microspheres in a microchannel taken by the 1x16 arrayed PMT. Microspheres of Yellow-green, Orange, Red, and Crimson were illuminated by a 488-nm laser. Photon counts taken every 2 ms are shown as a function of channel number of 1x16 arrayed PMT or wavelength. Mean values ± SD of photon currents from Yellow-green (closed squares, n = 76), Orange (open squares, n = 210), Red (closed triangles, n = 123), or Crimson microspheres (open triangles, n = 110) are shown.

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Supplemental Fig. S2 Fluorescence spectra of *E. coli* cells expressing fluorescent proteins in a microchannel taken by the 1x16 arrayed PMT. *E. coli* cells expressing GFP, Phi-Yellow and DsRed were illuminated by a 488-nm laser. Photon counts taken every 2 ms are shown as a function of channel number of the 1x16 arrayed PMT or wavelength. Mean values ± SD of photon currents from *E. coli* cells expressing GFP (open squares, n = 208), Phi-Yellow (open circles, n = 208), and DsRed (open triangles, n = 208) are shown.

Supplemental Fig. S3 Time-course of the recovery ratio and the purity calculated every 5 min. A mixture of Yellow-green, Orange, Red, and Crimson microsphere was separated for 1 h by an IR laser with a power of 34 mW and an irradiation duration of 60 ms.