High-speed sorting of micro-objects in microfluidics by Yb femtosecond laser amplifier

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Archetype of reliable cell sorter had been established in 1965 by coupling capillary sheath flow [1] and electric separation [2] technologies. Recently, performance improvement toward high-speed and high-precision sorting is an important issue for the progress of single-cell analytical technologies. For example, realization of high-speed sorting for circulating-tumor cells from blood samples will highly facilitate an innovative cancer medical technology. Now we have developed a world's top high-speed cell sorting methodology by utilizing femtosecond laser (fs-laser).

When an intense fs-laser is focused into high-speed fluid in a micro-chip, a micro-explosion is induced at the focal point. We have applied such explosion as an external force to sort cells flowing in the micro-chip. Although we previously employed Ti:sapphire laser amplifier (800 nm, 100 fs, 5 kHz, 1 mJ/pulse) as the light source, in this work, Yb-laser amplifier (1040 nm, 400 fs, 1 MHz, 8 μJ/pulse) is newly incorporated to realize high-speed sorting with high repetition rate. The laser pulse from Yb-laser amplifier was focused into high-speed fluid, in which micro-objects flowed with velocity of 2 m/s, by a 20× objective lens (NA. 0.46).

The minimum pulse energy to induce micro-explosion by the Yb-laser (250 nJ/pulse) was about 4 times larger than that by the Ti:sapphire laser (70 nJ/pulse). As absorption of water exists less than 200 nm, such explosion could be induced by multi-photon absorption. Because the pulse duration and wavelength of the Yb-laser are longer than those of the Ti:sapphire laser, the threshold of the micro-explosion is considered to be larger as shown by above-mentioned result.

When the Yb-laser is focused in the vicinity of micro-object (micro-bead) in the micro-chip, stream line of the object was shifted in a direction perpendicular to the flow as shown in Fig. 1. The shift, adopted as an evaluation parameter for the performance of laser-based sorting, was increased with pulse energy and decreased with distance between the focal point and the object. The cell-sorting throughput estimated from the deflection time for each sorting event was up to 10^5 events/s which was achieved with high repetition rate of the Yb-laser. This is the highest performance in the world to our knowledge. Further, we evaluated cell viability after sorting by trypan blue exclusion test which could indicate only dead cells with damaged membrane.

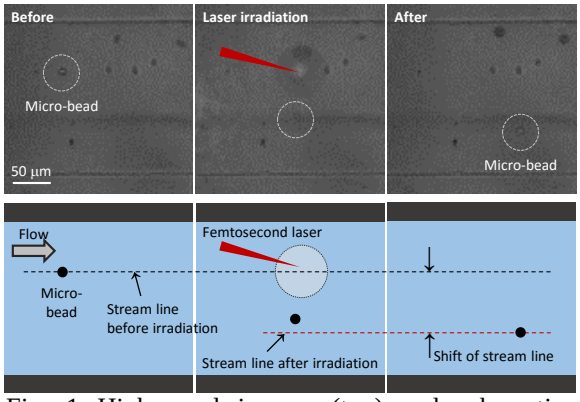

Fig. 1 High-speed images (top) and schematics (bottom) of a micro polymer bead in fluid flow before and after 1040 nm Yb fs-laser irradiation.

Reference

[1] P. J. Crosland-Taylor, et al. *Nature* **171**, 37–38 (1956)

[2] M. J. Fluwer, et al. *Science* **150**, 910–911 (1965)