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# Micro-particle sorting by Newton-ring device

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***Received (in Cambridge, UK) 4th August 2004, Accepted 3rd September 2004***
***First published as an Advance Article on the web 11th October 2004***

**A Newton-ring micro-particle sorter was constructed from a small convex lens and a flat glass. The sub-micrometer gap between them was controlled by a piezo-actuator and utilized for the fractionation of very small amounts of micro-particles in liquid.**

Analytical methods for the separation of small molecules and ions have been extensively developed so far.[1,2] For example, HPLC and capillary electrophoresis[3] have been widely used in various fields, including environmental chemistry, pharmaceutical chemistry, forensic chemistry and bio-technology. Gel electrophoresis[4–6] has been applied for the separation of ionic biomolecules such as lysed DNA and protein, but it is difficult to separate DNA which is larger than several tens kbp. Field alternation gel electrophoresis[7] can separate these large DNA, but the method needs a long separation time. Usually, such large molecules have to be decomposed prior to the analysis with ordinary analytical separation methods.[8–10]

In biological or environmental systems, almost all molecules are functioning as various kinds of aggregates, *e.g.*, genes, nuclei, vesicles and cells. Moreover, these micro-particles are working as individual particles. Therefore, in order to know the real function of intact bio-particles in liquid, some new methods that can separate rapidly and characterize the properties of these particles at the single-particle level are required.

In the present study, a simple and innovative method for the size measurement and the size fractionation of micro-particles in liquid was invented. The nano-gap was precisely determined by the interference moiré pattern of light, so called Newton ring.[11] This technique is versatile and can be used for the sorting of any kind of micro-particles in liquid.

Fig. 1 shows a schematic illustration of a nano-gap Newton-ring device. The nano-gap was built by combining a cover glass and a plano-convex lens. The cover glasses were purchased from Matsunami Glass Ind. (24 × 32 mm, thickness 0.12–0.17 mm), and the lenses were purchased from Edmund Optics Japan Co. (3 mm in diameter, 7.78 mm in radius of curvature). The lens was fixed to the piezo-electric element. The distance between the lens and the cover glass was roughly controlled by a manual *z*-axis stage and finely controlled by a piezo-electric element to the nanometer order. The piezo-electric element can be stretched 70 nm per V. The gap distance was accurately determined by observing the interference moiré pattern of light (Fig. 2), with a fluorescence microscope (BX51WI, Olympus Co.) equipped with a cooled CCD camera (ImagePoint, Photometorics). The microscope was also used to observe the sorted pattern and to determine the trapped positions of the fluorescent microparticles. All experiments were carried out in a thermostatted room at 25 ± 1 °C.

Fluorescent polystyrene carboxylate microparticles (Abs. 530 nm, Em. 590 nm) (Fluoresbrite microspheres 1.7 μm (1.614 ± 0.064 μm) and 3.0 μm (3.015 ± 0.138 μm) in diameter) were purchased from Funakoshi Co., Japan. The polystyrene particles were suspended with the concentration of $5 \times 10^{-3}$ wt% in 0.01 wt% Triton X-100 aqueous solution.

The sample solution was injected into the gap between cover glass and lens with a micro-syringe and spread by capillary action. With the evaporation of the solvent water, the sample particles were drawn toward the center of the nano-gap by the meniscus force of water and trapped at the position where the particle diameter was equal to the gap distance. The gap distance at any position was accurately determined by the interference moiré pattern of reflected light. In this case, the interference occurred between the reflected light at the upper surface of the lens and that at the bottom surface of the cover glass. Therefore, the conditions for the enhanced and weakened periodic Newton rings were

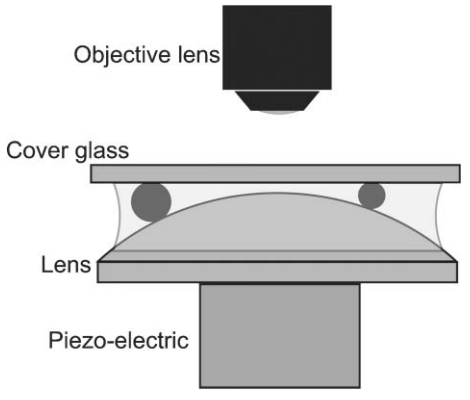


**Fig. 1** Nano-gap produced betwen a flat cover glass (upper) and a plano-convex lens (lower). Larger particles are trapped outside, but smaller particles are drawn closer to the center of the nano-space. The height of the lens is controlled by a piezo-electric element.

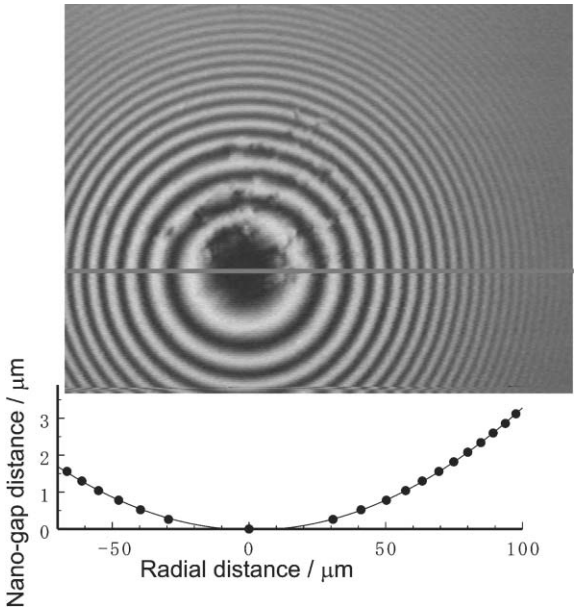


**Fig. 2** Relationship between the nano-gap distance and the radial distance from the center. This graph was plotted along the line in the photograph. These two figures are in the same scale for the radial distance.

DOI: 10.1039/b411995a

 

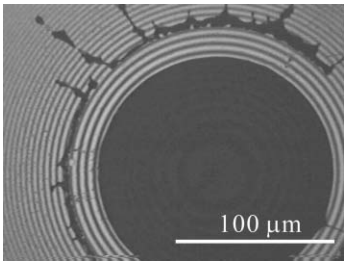


**Fig. 3** A photograph of trapped polystyrene particles with a diameter of 3 μm. Size measurement can be simply performed by counting the number of the stripes of the Newton ring.

represented by eqn. 1 and eqn. 2, respectively:

$$2h = \lambda/2 \times (2m + 1) \quad (1)$$

$$2h = \lambda/2 \times 2m \quad (2)$$

where $h$ is the vertical distance of the nano-gap, and $m$ is an integer.

The nano-gap distance $h$ can be correlated with the radial distance from the center of the lens $r$ by counting the number of these interference fringes from the center by eqn. 3:

$$h = R - \sqrt{R^2 - r^2} \quad (3)$$

where $R$ is the curvature radius of the lens. Fig. 2 shows the relationship between the nano-gap distance and the radial distance. Then, when particles are trapped at any position of the gap, their diameters can be calculated from the radial distance from the center.

The trapped profile of the particles was observed using the CCD fluorescence microscope. The radial distribution of the averaged fluorescence intensity, $I(r)$, per unit length of the circumference of the ring with the radius $r$ was obtained by eqn. 4:

$$I(r) = \Delta t \Sigma I_p(r)/2\pi r \quad (4)$$

where $I_p(r)$ is the fluorescence intensity per pixel at $r$, and $\Delta t$ is the length per pixel (1.27 μm/pix). The summation covers all pixels on the whole circumference of $2\pi r$. The intensity $I(r)$ was plotted against the radius $r$. The Newton-ring micro-sorting diagram was obtained by plotting the normalized fluorescence intensity $I(r)$ against the nano-gap distance $h$. Then, the plots were fitted with Gauss functions.

First, the size measurement of the polystyrene reference particles with a diameter of 3 μm was carried out. They moved toward the center of the lens and were trapped in the shape of a ring as shown in Fig. 3, after the evaporation of water. In the observed micro-sorting diagram, the peak position was at 2.926 μm with the peak width of 0.149 μm, so agreed well with the certified particle diameter.

The size-separation ability of this method was demonstrated by using a mixture of polystyrene particles of 1.7 and 3 μm diameters. For this mixture, the observed size fraction diagram is shown in Fig. 4. Two peaks are clearly observed at 1.664 μm and 2.863 μm, and their widths were 0.098 μm and 0.220 μm, respectively. This result definitely indicates the innovative potential of this new separation method.

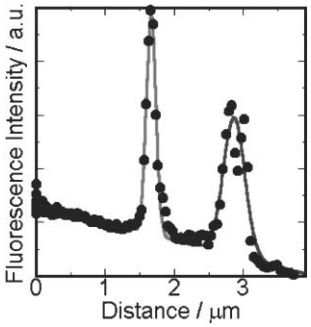


**Fig. 4** Size chromatographic diagram of the mixture of the polystyrene reference particles with diameters of 1.7 and 3 μm. In this chromatogram, the peak positions were at 1.664 μm and 2.863 μm and their widths were 0.098 μm and 0.220 μm, respectively.

Furthermore, we examined the applicability of this method to real biological micro-particles. For example, salmon sperm DNA (*ca.* 40 kbp) was trapped at the Newton-ring radus corresponding to the particle radius of 1.1 μm.

In summary, a novel micro-Newton-ring device with a nano-gap was invented for micro-particle sorting by using a lens and a glass plate. Nano-gap width was precisely determined from the analysis of the Newton-ring pattern. The amount of sample required for sorting is very small (below 1 μl), and the sorting can be performed in a rather short time (less than ten minutes). The high sensitivity, simplicity and rapidity are definite advantages of this method over the conventional FFF and cell sorter. The present report has proposed a new concept and technique for the sorting of micro to sub-micro particles in liquid. This sorting method will be highly useful for the analysis of a small amount of blood cells and whole chromosomes in a single cell.

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