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Fig. S1 Schematic of the experimental setup.



Fig. S2 Schematic of the electrode configurations and the actuation pattern. a) Star design, b) Square design and c) Circle design. For each case, the schematic (I) shows the initial position of the droplet when only the central electrode is actuated. Schematics (II) and (III) are the sequences of the droplet actuation scheme when the surrounding electrodes are turned on sequentially.



Fig. S3 Simulated domain and boundary conditions.



Fig. S4 a) Particle-focusing by actuating the surrounding electrodes in a pendulum motion. b) Actuation of a  $0.8-\mu L$  droplet. c) Particle-focusing with a  $1.2-\mu L$  droplet. For all cases the Star design is used with the Si-5 particles.



Fig. S5 Particle concentration using the Square design and Si-5 particles. The particles are concentrated on the central electrode and then the droplet is split into two daughter droplets, when the droplet on the central electrode contains 80% and the other droplet contains 20% of the particles. a) The initial mother droplet containing Si-5, b) the droplet after focusing the particles, c) droplet necking, d) splitting the droplet into two daughter droplets, e) moving the low-concentration daughter droplet away and f) moving the high- concentration daughter droplet away.



Fig. S6 Focusing the PS-5 particles by adding PS-15 particles to the sample. a) The initial droplet containing PS-5, b) no observed focused region after spinning the droplet, c) the initial droplet containing the PS-5 particles and some PS-15 particles, and d) the droplet containing the PS-5 and PS-15 particles after spinning, showing focusing behavior for the PS-5 particles.



Fig. S7 Detection of the presence of DNA in the sample with the concentration as low as  $18 \,\mu g/mL$  using the PS-15 particles: a) the focusing behavior observed after 250 s for a 1.6  $\mu L$  droplet containing the PS-15 particles with no DNA, b) the focusing behavior after 250 s for a  $1.5 \mu L$  droplet containing the PS-15 particles and DNA with a concentration of  $18 \,\mu g/mL$ .

## Calibration in the image processing technique

The calibration process is shown in Fig. S8. A droplet containing the silica particles is squeezed between two plates with the gap distance of 320 µm. The sample concentration is adjusted to ensure that the particles are not creating more than one layer (since there is enough space on the bottom plate, the particles settle down on the bottom plate rather than stacking on each other). The sample is left for 5 minutes to ensure that all the particles sediment onto the bottom plate. A top-view image of the droplet is then acquired. Four different sub-regions are considered for estimating the number of particles, and the area of each sub-region is  $0.2 \times 0.2$  mm<sup>2</sup>. Figure S8a shows a sample of these four sub-regions of a droplet that contains the 5-µm particles. Figure S9 shows one of these sections in the original image and a magnified picture of the image that is suitable for counting the particles in the image. In the next step, the images of these four sub-regions are processed by i) removing the background intensity and ii) enhancing the contrast to reduce the noise (see Fig. S8b). The images are then mapped into grayscale (see Fig. S8c), and a histogram of all the images is plotted (see Fig. S8d). Assuming that there is only a monolayer of particles in each image, the maximum intensity value (which is approximately 145 in Fig. S8d) is considered as the "high intensity threshold" of the first layer of the particles. This high intensity threshold (HIT) represents the maximum possible intensity value for a pixel in the first layer of the particles. In the next step, the pixels in the grayscale images that have the intensity value between zero and HIT are counted. These pixels represent the area with a monolayer of particles in the images. Finally, the average pixel size of a particle is calculated by dividing the number of the first layer of the pixels by the number of particles in the image. The HIT value and the average particle pixel size are then used to calculate the number of the focused particles in Figs. 4 and 5.



Fig. S8 The calibration process conducted for estimating the threshold intensity of the first layer and the average particle size. (a) Four sample images with the area of  $0.2 \times 0.2 \text{ mm}^2$  are considered. (b) The background is removed and the noise is minimized by enhancing the contrast. (c) The images are mapped into grayscale. (d) The histogram of the grayscale is obtained to find the maximum intensity of the pixels in the first layer of the particles.



Fig. S9 An area of  $0.2 \times 0.2 \text{ mm}^2$  of the sample image of the silica particles (with the average diameter of 5µm) is represented. The number of the particles counted in this area is around 114.