Microfluidic Platform for Pathogen Load Monitoring

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This paper presents a microfluidic diagnostic platform for pathogen load monitoring which is compact and can be operated by primary care personnel. The platform combines magnetic separation and optical detection in order to rapidly detect the presence of pathogens and provide additional clinical information (the pathogen load) in a simple, quick and compact manner without flow. One of the methods used to monitor and initiate treatment of several diseases (e.g. HIV or Influenza) is a pathogen (e.g. viral) load count of patients. Knowing the number of pathogens per unit volume of blood is essential in order to determine the initiation and dosage of therapeutic medication and thus increase the survival rate.

Our microfluidic platform consists of a separation channel, the detection chamber (chamber A) and the reference chamber (chamber B) as shown in Fig. 1. The platform was fabricated using a dry photoresist thin film (Ordyl) and standard photolithography process. Fig. 2 shows a photo of the device. Magnetic nano-particles (MPs) functionalized with ligands (e.g. antibodies) of specific affinity to the pathogens to be detected are mixed with the fluid under investigation. If pathogens are present, they will bind to most of the functionalized MPs. The resulting fluid, containing the magnetically labeled pathogens (called loaded magnetic particles - LMPs) and the remaining MPs that did not attach to any pathogens, is inserted in the microfluidic platform. The fundamental idea behind the magnetic separation method is that the induced velocity of MPs in suspension, while imposed to a magnetic field gradient, is inversely proportional to their volume; the volumetric increase of the MPs due to binding of pathogens onto their surface, changes consequently their velocity[1-2]. Thus, the LMPs need more time to travel the same distance compared to plain functionalized MPs (smaller). This way, the LMPs (slower) can be separated from the plain MPs (faster) without flow and simply by being imposed to a magnetic field gradient, generated by integrated current carrying conductors (Group A) as shown in Fig. 1. The faster MPs are directed to the reference chamber by sequentially applying current to the Group C conductors. The slower LMPs are then directed to the detection chamber by sequentially applying current to the Group B conductors. For the pathogen quantification inside the chip only a conventional optical microscope equipped with a camera and a PC with the relevant software are required. Within the detection chamber the LMP size is determined by conventional or laser light scattering and subsequent tracking of the Brownian motion without flow[3]. The size of the plain MPs inside the reference chamber is also determined for comparison. This way, the amount of attached pathogens to the surface of the MPs is calculated from the volumetric increase of the plain MPs.

In order to prove the concept a chamber was filled with commercially available Nanomag-D nanoparticles suspended in water (nominal diameter ~250 nm) composed of iron oxide nanoparticles encapsulated into a dextran matrix with protruding amino groups (-NH$_2$). The sample chamber was directly illuminated with light in a shallow angle resulting in a dark field arrangement. The scattered light was observed with a microscope and a camera. A sequence of images taken with the digital camera was analyzed with MATLAB. Software scripts were used to determine the centre of the scattering particle image in every frame and link these positions. This way a certain number of particle displacements due to Brownian motion were measured and subsequently used for size calculation. Fig. 3 shows the measured diameter values proving that our method can be used to reliably determine the size of the nanoparticles. Fig. 4 illustrates the absence of convective drift motion of particles in any direction during tracking measurements. Experiments with magnetically labeled Feline Herpesvirus 1, which is non-pathogenic to humans, are planned to be conducted.

References

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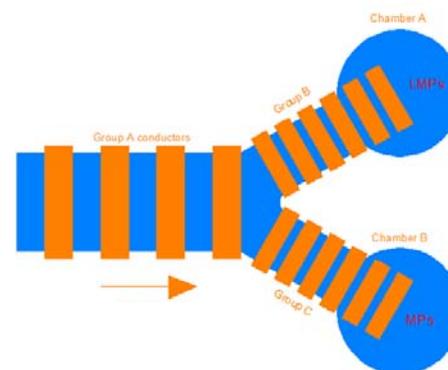
Figure 1. Schematic of the microfluidic platform; it consists of a separation channel, a detection chamber (chamber A) and a reference chamber (chamber B).
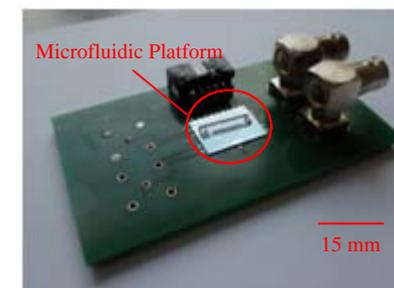

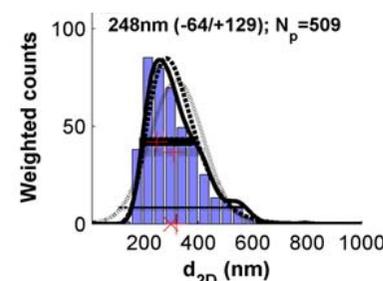Figure 2. Photo of the device and the BNC connectors.

Figure 3. Particle size distribution of magnetic Nanomag-D particles determined by tracking of their Brownian motion. The histogram fit yields the modal diameter, which agrees very well with the specifications.
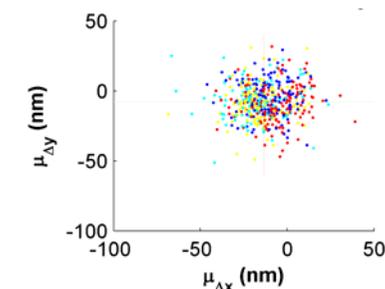

Figure 4. Scatter plot of the mean particle displacements in x- and y-direction between subsequent frames shows that there was no significant drift motion that would deteriorate the sizing procedure.