Characterizing electrokinetic mobility of microparticles using a transparent platform and in-line holographic microscopy

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Abstract—Traditionally, electrokinetic phenomena are demonstrated under a microscope. Since an ordinary light microscope has a short depth of focus and in many cases the electrokinetic phenomenon may take place in a relatively large volume, it is essential to use a microscopic method which has a long depth of focus and adequate depth resolution. The advantage of using a transparent platform and in-line holographic imaging for particle tracking is shown. Dielectrophoretic (DEP) particle motion is demonstrated on a fully transparent platform with 2D indium-tin-oxide (ITO) electrodes, and images of the whole sample volume are reconstructed using the digital in-line holographic method (DIHM) in order to find the 3D positions and speeds of the particles influenced by the DEP force and other electrokinetic forces. DIHM imaging proved to be a practical tool for analyzing particle motion due to multiple, simultaneously acting, electrokinetic forces.

Electrokinetics includes a number of phenomena and forces which could mobilize a particle: dielectrophoresis, electro-osmosis, diffusiphoresis, capillary osmosis, Brownian motion, gravitation, buoyancy, etc. In the present case we have used dielectrophoresis to mobilize microparticles. Dielectrophoresis, as a phenomenon, has been used in characterization of fractioning and manipulating colloidal microscale particles in an aqueous media [1-3]. It is a deterministic and easily controlled phenomenon.

Digital in-line holographic microscopy (DIHM) has useful features in 3D and 4D imaging not offered by traditional microscopy. The main advantage of DIHM is the possibility to reconstruct focused images within a large depth range from a single hologram [4, 5].

The phenomenon of dielectrophoresis can take place in a relatively large sample volume. Usually, it is demonstrated using a traditional microscope in which the depth of focus is relatively short, and therefore particle tracking at different depths of the sample volume is not possible. There is a substantial need to track particles and their route in the whole volume since particle mobilization happens at all depths of the volume and since the magnitude of the electric field moving the particle varies according to the distance from the electrode plane.

For the experiments, hyperbolic quadrupole electrode geometry, with indium-tin-oxide (ITO) electrodes, was manufactured. ITO is electrically conductive but transparent to light, which offers possibility to visualize particle mobility also on top of electrodes, and therefore possibility to see any hydrodynamic or electrokinetic phenomena during the experiments. Hyperbolic electrode geometry produces a linear electric gradient within the active region of the platform. One hundred and fifty micrometer glass was attached on both sides of the platform to support a cover plate. This was done to achieve constant sample volume and to prevent possible optical errors due to the droplet shape of the sample fluid without a cover plate.

Polystyrene-carboxylate particles of 5.98μm in diameter were used as a target and 0.1mM potassium-chloride as a carrier dilution. The conductivity of the carrier dilution was measured to be 1.44mS/m. Sinusoidal signals of $5V_{p\text{-}p}$ and frequencies of 10kHz and 15MHz were used for the electric field generation to demonstrate the positive (pDEP) and negative (nDEP) dielectrophoresis, respectively.

The DEP particle motion is based on the differences of polarizability of the particle and carrier dilution. When the dielectric particle is set under the influence of an external electric field, it becomes polarized [6-8] and if the size of the field non-uniformities is larger than the particle, it forms a dipole. In a non-uniform electric field the forces affecting the negative and positive elements of the dipole are unequal to the sum of these forces being the dielectrophoretic force which is mobilizing the particle. Usually, the bigger the particle the higher the DEP force, since the potential difference of the electric field gradient is bigger than with the smaller particles. For spherical particles the dielectrophoretic force is:

$$\overline{F}_{DEP} = 2\pi\varepsilon_m r^3 (\mathrm{Re}[f_{CM}])\nabla E^2_{RMS}, \quad (1)$$

where ε_m is the absolute permittivity of the medium, r is the radius of the particle and ∇E^2_{rms} defines the properties of the applied electric field. The $\mathrm{Re}[f_{CM}]$ represents the real part of the Clausius-Mossotti factor which defines the direction of the DEP force: whether it is a repulsion or attraction force of the higher electric field. For the whole

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frequency range the real part of the Clausius-Mossotti factor:

$$\mathrm{Re}[f_{CM}] = \frac{(\varepsilon_p - \varepsilon_m)\omega^2\tau^2}{(\varepsilon_p + 2\varepsilon_m)(1+\omega^2\tau^2)} + \frac{(\sigma_p - \sigma_m)}{(\sigma_p + 2\sigma_m)(1+\omega^2\tau^2)}, \quad (2)$$

where ε_p and ε_m define the permittivity of the particle and the carrier fluid ω corresponds to the angular frequency of the electric field and τ the relaxation time for the Maxwell-Wagner interfacial polarization. With defining the sign of the force this factor also affects the magnitude, together with the properties of the electric field and particle size. In principle, when a particle's polarizability is smaller than the carrier dilutions, it undergoes a repulsion force, and when larger it experiences an attraction force. These phenomena are called negative (nDEP) and positive (pDEP) dielectrophoresis [9].

Digital in-line holographic microscopy (DIHM) is a sophisticated imaging method which can provide information about the particle shape, place and mobility in a large sample volume. In the experiments we used DIHM equipment integrated into an inverted microscope. The light source of the DIHM consists of a 405nm diode laser and a round pinhole of 500nm in diameter. The holograms were captured with a 2048×2048 pixel CMOS camera. The imaging system is lens-less, and therefore mechanically simple. Figure 1 illustrates the in-line DIHM set-up and its operating principle.

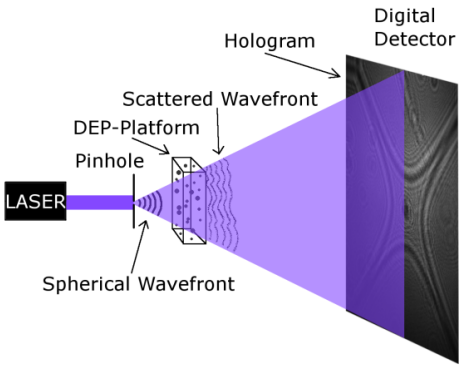

Fig. 1. DIHM set-up.

The measurement equipment consisted of an inverted compound microscope, a DIHM system (Resolution Optics Inc.), a function generator, and a PC for data acquisition. The carrier dilution with the particles was dropped on the DEP platform, the cover plate was set on top of the supporting glass rods, and hologram capturing started simultaneously with turning the voltage on.

Figure 2 demonstrates particle motion caused by the nDEP and pDEP forces. The images are reconstructions from captured holograms and composed of multiple superimposed images, each providing the sharpest possible image of the particle in each depth position. 3D visualizations of the same phenomena are provided in Figs. 3 and 4.

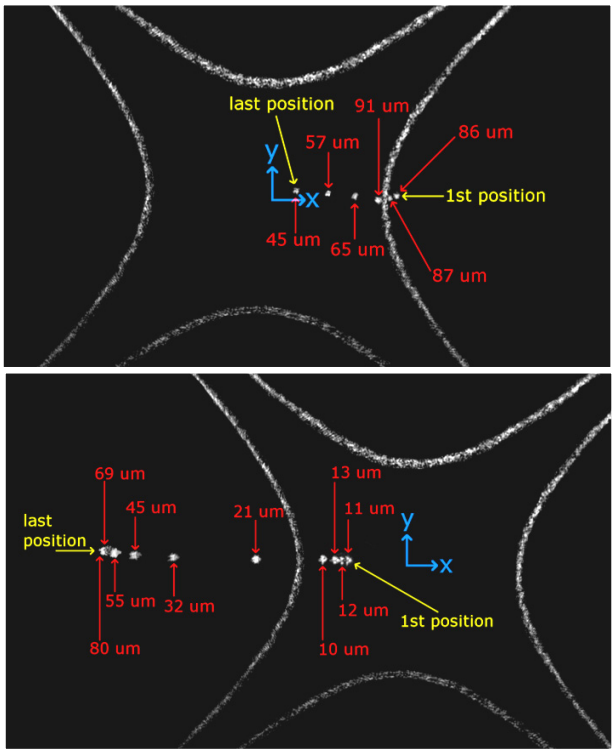

Fig. 2. Platform and measured particle depth coordinates in nDEP (above) and pDEP (below). The values indicated with red are the distances from the electrode plane to the focus level at each tracked position of the particle.

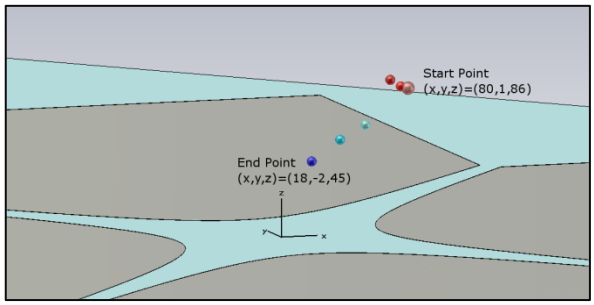

Fig. 3. Particle track in the nDEP experiment.

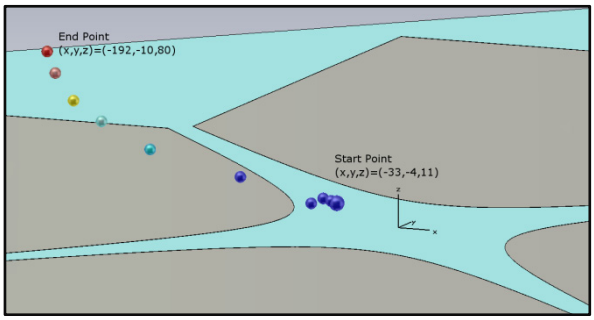

Fig. 4. Particle track in the pDEP experiment.

The particle position in the beginning is indicated by a large dot and successive positions are determined using constant time intervals. The origin of the coordinate system is set to the center point of electrode geometry on the electrode plane. Figures show that particles influenced by the electric field of the planar electrode geometry move not only in the direction of the electrode plane but also in the depth direction. In the pDEP experiment a particle moves away from the center toward the electrode edge and is finally pushed above the electrode. The particle should be trapped by the high electric field at the electrode edge, but due to the electro-osmotic fluid flow the particle is pushed over the edge of the electrode and simultaneously thrown upwards (away from the electrode plane) when traversing above the electrode.

Figures 5 and 6 show particle speed, with blue bars indicating speed in the direction of mobility and red bars in the direction of the electrode plane. In the nDEP experiment particles tend to have a relatively low speed (maximum 1.45µm/s). Also in the beginning of the pDEP experiment particle motion is relatively slow but is finally accelerated to the speed of 52.5µm/s. This indicates that particle velocity due to DEP force is much smaller than that caused by electro-osmotic fluid flow.

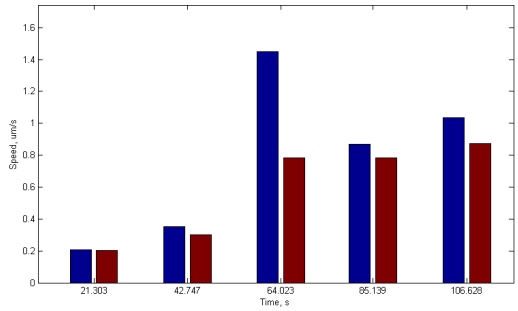

Fig. 5. Particle speed in nDEP. The blue bars indicate speed in the direction of mobility and the red bars indicate speed in the direction of the electrode plane.

Table 1 contains determined particle speeds in both nDEP and pDEP experiments. Maximum and average speeds are presented separately for the active region of the electrode geometry since pure DEP happens only within the active region.

The DEP platforms with transparent ITO-electrodes were manufactured and tested. The platform enables the use of DIHM imaging within the whole sample volume. Thereby it is possible to create 3D and 4D images of particle behavior under the influence of an applied electric field. Here particles influenced by a DEP force were tracked in a large sample volume. The main advantage of the DIHM imaging, namely the long depth of focus as compared to ordinary microscopy, proved to be useful when analyzing particle motion under the influence of various electrokinetic forces. Also, since DEP forces produced by 2D electrode geometries have effects not only on the planar direction but also in the depth direction, DIHM can be used very effectively to characterize the DEP platforms by measuring a particle's actual 3D path and velocity instead of measuring just one 2D projection of its route using an ordinary microscope.

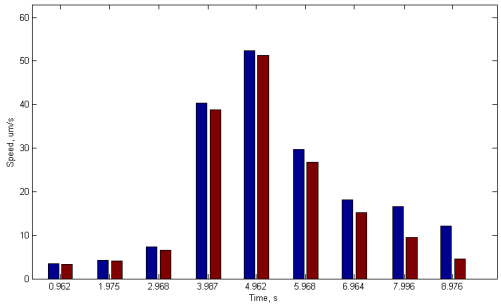

Fig. 6. Particle speed in pDEP. The blue bars indicate speed in the direction of mobility and the red bars indicate speed in the direction of the electrode plane.

Table 1. Particle speed.

	nDEP [µm/s]	**pDEP [µm/s]**
Max. Speed	1.45	52.5
Avg. Speed	0.78	20.5
Max. Speed, act.reg.	1.03	7.29
Avg. Speed, act.reg.	0.95	5.01

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