Microparticle Image Velocimetry for Improving Dielectrophoretic Concentrator Using the LabSmith LabPackage

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Sample preparation is a bottleneck for protein, cell and nucleic acid analysis, regardless of the analytical technique used.

An important, and tedious, preparation step is selective concentration of the target of interest, particularly when target abundance is low. One approach is insulator-based dielectrophoresis, or iDEP, in which insulators, often etched in glass or stamped in plastic, serve as in-line, on-chip concentrators.

In order to optimize the performance of in-line, on-chip iDEP, microparticle image velocimetry (µPIV) has been used to characterize the electrokinetic flow under operating conditions. Successful µPIV experimentation requires precise control of the electoosmotic flow, the relative conductivity and the applied electric field. A high quality camera, microscope, and software for measuring the particles in motion are also necessary.

LabSmith's LabPackage is the first complete solution for controlling this experiment. Using a LabSmith high voltage sequencer and synchronized video microscope, Dr. Blanca H. Lapizco-Encinas and her team create and test predictive theory for in-line iDEP concentration of particles on-a-chip (Figure 1).

Described below is a guide to implementing Lapizco *et al.'s* application, first published in 2009. (*Anal Bioanal Chem* (2009) 394:293-302.)

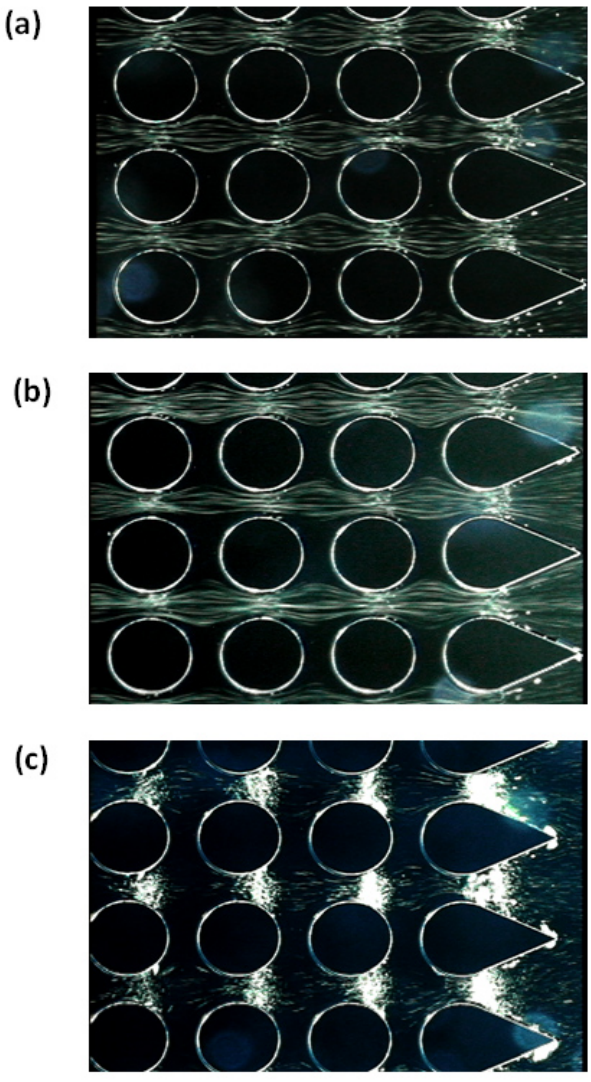

Figure 1. iDEP trapping of particles of electroosmotic velocity and mobility under varying pH, conductivity, and applied electric fields, as determined by µPIV measurements: (a) pH 6 and 25µS 800 V, (b) pH 9 100µS 800 V, and (c) pH6 100µS 800 V (optimal conditions).

Find out more about the Microscale Bioseparations Lab.
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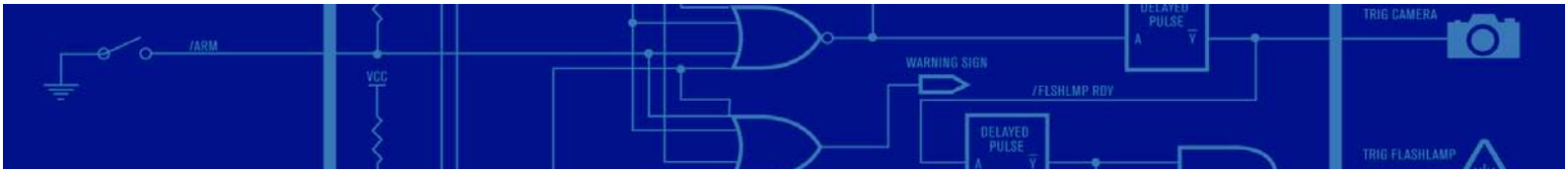

Introduction

Insulator-based dielectrophoresis uses two electrodes to apply a DC electric field across an insulating structure array. This array creates an electric field gradient with regions of high and low electric field strength. Depending on the relative conductivity of the particles to the suspending medium, the particles can be trapped as the dielectrophoretic force overcomes electroosmosis and electrokinesis. This has been demonstrated in microfluidic structures to successfully and selectively concentrate cells, particles, and proteins *(1- 3)*.

The utility of this technique is increased by optimization and predictability of the dielectrophoretic trapping voltage and the insulator array. In particular, the utility of iDEP as a selective concentrator or filter for microsystems depends on accurately predicting the conditions under which iDEP trapping will occur. iDEP trapping occurs when:

$$[\mu_{DEP}\text{grad}E^2]\cdot E \geq [\mu_{EK}\text{grad}E^2]\cdot E \qquad \text{(Eq.1)}$$

where μ_{DEP} is the dielectrophoretic mobility, E is the electric field, and μ_{EK} is the electrokinetic mobility. Per Equation 1, dielectrophoretic trapping only occurs when dielectrophoretic mobility is greater than the electrokinetic mobility. Therefore, the dielectrophoretic force acting on the particles must exceed the electrokinetic force acting on the same particles.

The electrokinetic velocity is given by the following equation:

$$v_{EK} = \mu_{EK}E = (\mu_{EO} - \mu_{EP})E \qquad \text{(Eq. 2)}$$

For a channel etched in glass, the electrokinetic velocity, v_{EK}, is obtained from the electrokinetic mobility, μ_{EK}, as a function of applied electric field, E, as shown in Equation 2 *(5)*. It follows that the electroosmotic and electrophoretic velocities follow the same relationship as a function of mobility and applied electric field:

$$v_{EO} = \mu_{EO}E \qquad \text{(Eq. 3)}$$

$$v_{EP} = \mu_{EP}E \qquad \text{(Eq. 4)}$$

The assumptions are as follows:

1. Particles are 1 µm diameter with a surface charge of 20 mC/m^2. μ_{EP} is negligible and goes to zero *(5)*.
2. $v_{EK} \approx v_{EO}$ and $\mu_{EK} \approx \mu_{EO}$.

The electroosmotic mobility is given by

$$\mu_{EO} = \zeta\varepsilon_m\varepsilon_\infty/\eta \qquad \text{(Eq.5)}$$

where ζ is the zeta potential, ε_∞ is the permittivity of free space and ε_m is the permittivity of the suspending media. The zeta potential depends on the thickness of the electrical double layer, which is dependent on the wall charge (governed by the pH of the suspending media) and the conductivity (ions in solution). By controlling the applied electric field, suspending media conductivity and pH, the conditions were found where:

$\mu_{DEP} \geq \mu_{EO}$

and the optimum dielectrophoretic mobility could be found using micro particle velocimetry (µPIV) to measure the electroosmotic velocity.

Careful velocity measurement was ensured using the LabSmith SVM340 Synchronized Video Microscope with µScope software, and with the LabSmith HVS448 High Voltage Sequencer controlling the applied electric field. From this measurement the electroosmotic mobility and the zeta potential could be calculated to optimize iDEP *(4)*. Multiphysics software (COMSOL) was used to predict the dielectrophoretic and electrokinetic forces exerted on the particles, and the modeling results were confirmed by the experimental findings of the optimal conditions for iDEP using µPIV. These results provided optimization guidleines for iDEP separations and were verified experimentally using the SVM340 to collect images of the conditions where iDEP trapping of 1 µm beads occurred.

Electrokinetic Velocity Measurements

µPIV Measurements

To obtain the electrokinetic velocity the researchers used a microfluidic chip with a single channel wet-etched in two Schott 263D glass wafers (Howard glass, Worcester, MA, USA). The channel was 30 mm long, 1 mm wide and 10 µm deep, as in Figure 2(a). The channel was cleaned with bidistillated water prior to each measurement. The suspending medium was introduced into the channel, followed by 80 µL of a fluorescent microsphere solution (7.639 x 10^{10} microspheres/mL). Fluorescent, 1 µm polystyrene beads with excitation/emission wavelengths of 505/515 nm were used (Invitrogen, Carlsbad, CA). A variety of suspending media was prepared from potassium phosphate buffer at pH 6, 7, 8 and 9, with conductivities of 25, 50 and 100 µS/cm. The LabSmith SVM340 performed the image and video capturing for the µPIV experiment, with microscope control and PIV measurements provided by the µScope software. The elecric field was applied and controlled using the LabSmith HVS448 in manual mode.

For each suspending medium four electric fields were applied: 50, 100, 20, and 300 V/cm. Each experiment was performed twice. The measurements were made by taking videos of 90 frames, recorded at 30 frames per second at a central position in the channel with a 256 x 256 pixel interrogation region using a cross-correlation algorithm, as in Figure 2(b).

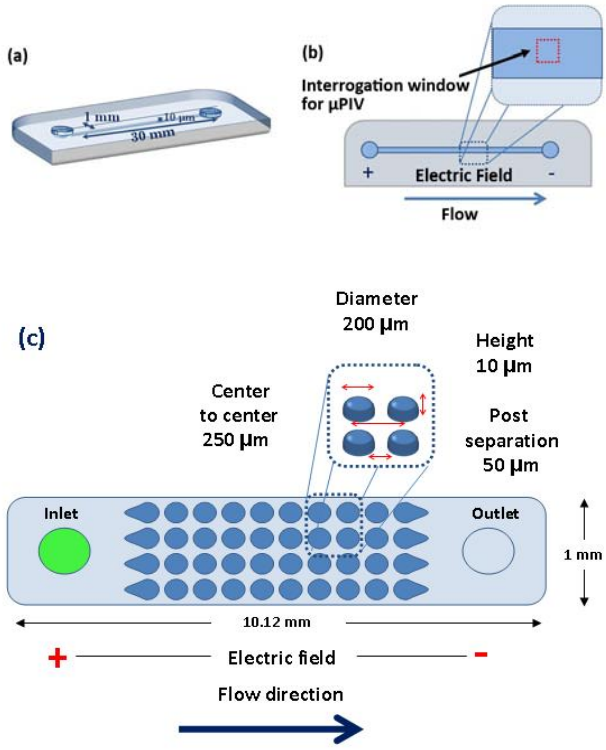

Figure 2. Microfluidic chips for µPIV and iDEP measurements: a) single channel for µPIV, b) region of EK velocity µPIV measurement, and c) iDEP chip with post region enlarged.

Create a µPIV Probe Using µScope™

To create a probe:

1. Click the **PIV** toolbar button  .

2. Double-click in the center of the video image to create a new Probe.

3. Choose **File >Measurement File Naming** to open the **Naming Settings for Measurements** dialog box. Here you can choose where measurement data files will be saved. You can also choose to **Auto-name Files**, and select whether the date, time and/or a serial number will be automatically appended to each file name. An example of the format appears at the bottom of the dialog box.

4. To begin recording choose **File >Record**, or click the **Start/Stop** toolbar button  . If Autonaming is selected, recording will begin immediately. Otherwise, recording will begin after you name the file and click **OK**.

5. To end recording, choose **File > Record** or click the **Start/Stop** button again. The PIV output file will include four columns for each probe: the X and Y locations of its centroid, measured from the upper left of the window, and the X and Y velocity at each point in time. The X/Y location columns will only have entries in the first row. An example of a saved PIV probe used in the experiments to measure electrokinetic velocity is shown in Figure 3.

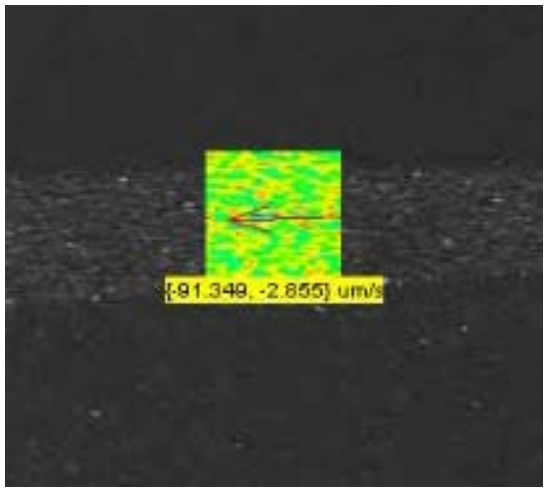

Figure 3. µScope PIV probe used to conduct µPIV measurements.

Insulator-Based DEP Measurements

iDEP experiments were made in a microfluidic chip with a microfluidic chip consisting of a channel that is 10.12 mm long, 1 mm wide and 10 µm deep, with an array of 8 columns x 4 rows of cylindrical insulating posts 200 µm in diameter with 250 µm spacing center-to-center (Figure 2c). Using the same procedure as for the PIV experiments, dielecrophoretic response of the particles was recorded employing the HVS448 and the SVM340.

Results

Figure 4 shows the electrokinetic velocity results as a function of pH and conductivity. As predicted, higher electric field strengths result in higher overall velocity surfaces. The velocity surface shape is a function of pH and conductivity, with the lowest electroosmotic velocities, and therefore mobilities, occurring for high conductivity, low pH suspending media. This suggests that this is the best condition to obtain dielectrophoretic trapping.

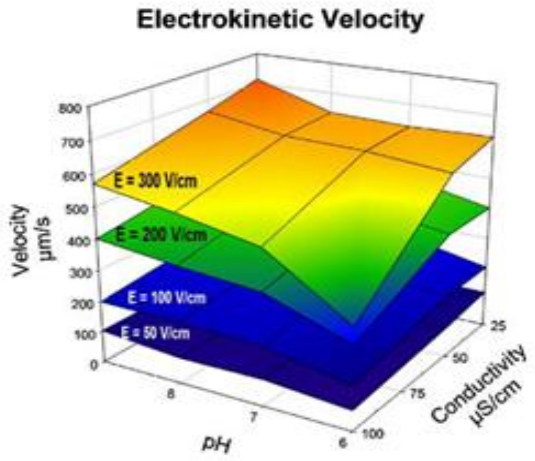

Figure 4. µPIV results showing the measured electrokinetic velocities as a function of pH and conductivity. Velocity surfaces at varying electric field strengths are graphed.

Conclusions

The challenges of this application require the precise, accurate, and reproducible control of electrical fields at high voltages, and the accurate capture and imaging of 1 micron fluorescent beads moving in a flow field. The LabSmith LabPackage, consisting of the HVS448 sequencer, SVM340 microscope and µScope software with its PIV probe application, allowed the research team to easily measure distances traveled and thus velocities for electroosmotic flow. These measurements were integral in developing models and experimental conditions for the iDEP trapping of the same particles that were measured using the LabSmith SVM340.

References

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(5) Green, N.G.,Morgan, H., J. Phys. Chem. B, 1999, 103:41-50.

LabSmith Products Used for µPIV Application

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