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Image Processing, Adaptive Gridding, and Optimal Nonlinear Filtering Techniques for Particle-Image Velocimetry

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Image processing, adaptive gridding, and optimal nonlinear filtering
techniques for particle-image velocimetry

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We have developed a methodology for extracting accurate, high-spatial-resolution measurements from large ensembles of particle-image pairs of a stationary flow. Processing of the subimages and their correlations produces an estimate of the velocity probability density function that improves with ensemble averaging. An optimal nonlinear filter precisely extracts flow measurements from the measured velocity distribution. In a mixed pressure-driven and electrokinetic flow in a microscopic flow cell, the filter was able to dissect the pressure-driven component from the electrokinetic component of particle motion using a customized nonlinear filter. Efficient adaptive-gridding, processing software based upon this methodology demonstrated velocity measurements with 2×2 -pixel spatial resolution from an ensemble of 50 interlaced video images.

1 Introduction

Particle Image Velocimetry (PIV) is a standard technique for quantifying flow fields in macroscopic flows. The technique typically works by illuminating a measurement plane in the flow using a sheet of pulsed laser light and imaging the scattered light at an angle (usually normal to the sheet) onto an array detector or photographic film. Small, reflective particles seeded into the flow produce a random particle image on the detector. A comparison of images of the particles at two times provides flow velocity information within the light sheet.

The comparison of particle locations between images is performed either directly by tracking individual particles or statistically by correlating pairs of subimages of the particle images. The former, often called particle-tracking velocimetry (PTV) works best when the particle seeding density is low enough that it is straightforward to identify particles between frames and few particles move in and out of the measurement plane between images. In microscopic PIV, the technique is complicated by the presence of a Brownian component of the particle velocity.

Image correlation algorithms work best at high

particle seeding densities. These algorithms divide image sets into a number of subimage pairs. The location of the peak in the correlation between pairs of subimages provides the Cartesian displacement of the second subimage with respect to the first that maximizes the similarity of the particle subimages. Hence the subimage correlation provides a statistical measure of the displacement field or velocity of the particles.

If the particle seeding density is insufficient, spurious peaks of the subimage correlation can overwhelm or obscure the main peak. For stationary flows, one may avoid this difficulty while not increasing the particle-seeding density excessively by recording multiple frame pairs and temporally averaging the subimage correlation [Santiago et al. 1998]. Because secondary peaks are generally uncorrelated between different frame pairs, the ratio of their magnitude with respect to the correlated main peak decreases as the square root of the number of frame pairs. While spatial ensemble averaging can distort velocity and derived fields [Luff et al. 1999], temporal averaging does not distort these fields for steady flows. We demonstrate 2×2 -pixel spatial resolution and ~ 0.2 -

pixel uncertainty of particle-displacement measurements from an ensemble of 50 interlaced video images.

Here we also present a method by which the probability density function (PDF) of particle velocities may be inferred from subimage correlations and used to obtain more information than the maximum-likelihood velocity. This approach applies to unsteady, stationary flows, e.g., turbulent jets, etc., as well as steady flows. The method has been used to measure the mean velocity and viscosity as well as the electrokinetic and pressure-driven components of the mean velocity in mixed electroosmotic and pressure-driven flows in microscopic geometries.

The particle images used in this analysis have no depth-wise resolution. The velocity PDF measured at each point contains contributions from each plane in the unresolved direction. The velocity PDF of the parabolic profile of pressure-driven flow is distinctly different from that of the uniform or linear profile of electroosmotic flow, allowing the simultaneous, independent measurement of both contributions to the mean flow. In many practical microfluidic devices, it is difficult to resolve only particles in a layer thin enough that the possibility of depth-wise velocity gradients can be ignored. While it is usually impractical or impossible to form a suitably thin illumination sheet over the measurement region in microflows, large-numerical-aperture, high-magnification objectives may provide a suitably thin depth of focus [Santiago et al. 1998] in special cases.

The approach outlined in this paper takes advantage of depth-wise gradients to extract additional information from primarily planar flows. Furthermore, it does not suffer from particles entering and leaving the detection layer, requires low particle-seeding density, and provides information about the variation of velocity in the depth-wise direction in an experimentally and computationally efficient manner.

The particle-image-processing algorithm employs iterative grid refinement and continuous offset between subimage pairs that is designed to ensure that the peak of the inferred velocity PDF is centered in the correlation. Hence the size of the subimage that is required to capture the velocity PDF depends only on the width of the velocity PDF, not the location of the peak.

The image-processing procedure extracts a statistical estimate of the particle-velocity PDF from an ensemble of subimage-pair correlations. First, background subtraction and flat-field normalization of the particle images removes sources of correlation bias.

Then, a deconvolution removes imperfect imaging, finite-particle-size, streaking, particle-arrangement, and detection-system contributions from each correlation. Finally, a normalization removes the combinatorial skew from the resulting correlation, producing an estimate of the particle-displacement PDF inside each subimage. While random spurious peaks from noise and finite particle statistics distort each PDF, these peaks are uncorrelated between independent subimages, hence the temporal ensemble average of each measured displacement PDF approaches the true particle-displacement PDF from which the fluid velocity PDF may be inferred.

The second step in the procedure is the application of an optimal nonlinear filter to the measured particle-displacement PDF to extract best estimates of the parameters to be measured. The optimal filter consists of a nonlinear least-squares fit of the measured PDF to a theoretical velocity PDF based upon flow, particle, and experimental properties. In the filter, the parameters to be measured are adjusted to minimize the sum of the square of the deviation between experiment and theory at each pixel of the PDF. This process can produce the best possible estimate of the adjustable parameters provided that the theoretical PDF accurately represents reality. Optimal nonlinear filtering produces better measurements than linear peak-finding techniques because it makes optimal use of oversampled information and whatever additional information is utilized in the model, e.g., the distribution of particle sizes, mobilities, etc. However, incompleteness or inaccuracy in the theoretical signal can skew results and otherwise invalidate the advantages of nonlinear filtering.

The results that we present are derived from standard (NTSC) videos of fluorescent-particle motion viewed through an epifluorescence microscope. These videos were digitized and recorded as thirty 8-bit $\times$ 480 $\times$ 640-pixel interlaced frames per second directly to a computer disk. The analysis treats each interlaced image as two spatially and temporally offset 240 $\times$ 640-pixel images. We present mean velocity profiles with 2 $\times$ 2-pixel spatial resolution and measurements of electrokinetic and pressure-driven components of the velocity field inferred with 4 $\times$ 8-pixel resolution.

2 Image Processing

Two different strategies may be employed for optimal nonlinear filtering to extract flow- and fluid-property measurements. One approach is to derive the theoret-

ical image correlation that would be produced with particular flow, fluid, and experimental parameters accounting for all imaging, particle-arrangement, and combinatorial defects. This theoretical signal would then be fit to the unprocessed ensemble-averaged image correlation. This procedure has the advantage of requiring only convolution operations which do not produce excessive spurious noise spikes. Two disadvantages are that the particle-velocity PDF is never directly obtained, and that computationally inefficient convolutions are required during each calculation of the theoretical correlation.

The second strategy involves deconvolving the imaging and particle defects and normalizing the combinatorial defect from the experimental subimage correlations to obtain best estimates of the true particle-velocity PDF, i.e.,

$$\begin{aligned} \Xi\{\Delta x, \Delta y|\delta x_0, \delta y_0\} &\rightarrow \Xi_p\{\Delta x, \Delta y|\delta x_0, \delta y_0\} \\ &\rightarrow \mathcal{P}_p\{\Delta x, \Delta y|\delta x_0, \delta y_0\} \rightarrow \mathcal{P}_p\{u, v\}, \end{aligned} \quad (1)$$

where $\Xi\{\Delta x, \Delta y|\delta x_0, \delta y_0\}$ is the correlation at the discrete point $\{\Delta x, \Delta y\}$ of the subimage pair shifted relative to each other continuously through $\{\delta x_0, \delta y_0\}$, Ξ_p is the displacement correlation of the subimages, obtained by deconvolving imaging- and particle-related defects, $\mathcal{P}_p\{\Delta x, \Delta y|\delta x_0, \delta y_0\}$ is the particle displacement probability density function within the subimage, obtained by normalizing the combinatorial bias, and $\mathcal{P}_p\{u, v\}$ is the particle-velocity probability density function.

Fitting the theoretical particle velocity PDF to the deconvolved estimate of the experimental PDF is computationally efficient, but suffers from spurious peaks produced in the deconvolution step. The image-processing software described in Section 4 allows either approach. For brevity we explicitly discuss only the latter, faster procedure.

2.1 Suppressing Static Correlations

The first step in this algorithm optionally involves removing potential correlation biases caused by static image information. This information includes images of solid surfaces, non-uniform illumination, non-uniform seeding, etc. Both background suppression and flat-field correction should be applied to particle images by respectively subtracting the background and dividing the intensity of the images by the illumination/seeding field. Both fields may optionally be synthesized by forming an image of the ensemble mean intensity and ensemble root-mean-square

(RMS) deviation from the mean intensity. In a suitably large ensemble, Brownian motion and convection smears the images of individual particles in a mean-intensity image into a background image. Subtracting this synthetic background from each individual image removes correlation biases introduced by static image elements, such as boundaries. The image of the RMS deviation from the mean provides a measure of the statistical weight of each region of the image. Normalizing by this image removes biases in correlations toward regions with higher statistical weighting, such as areas with high particle-seeding density. If an insufficiently large ensemble of images is used to derive the synthetic fields, this procedure itself can introduce biases.

2.2 Correlating Subimages

For computational speed, subimage correlations employ fast Fourier transforms via the Fourier convolution theorem, using in addition the Hermitean symmetry of the transforms of real-valued functions, i.e.,

$$\begin{aligned} \Xi\{\Delta x, \Delta y|\delta x_0, \delta y_0\} &= \\ &\mathcal{FT}_{\{\Delta x, \Delta y\}}^{-1}\{\tilde{A}^*(p, q)\tilde{B}(p, q)\}, \\ \tilde{A}(p, q) &= \mathcal{FT}_{\{p, q\}}\{A(\Delta x, \Delta y|\delta x_0, \delta y_0)\}, \\ \tilde{B}(p, q) &= \mathcal{FT}_{\{p, q\}}\{B(\Delta x, \Delta y)\}, \end{aligned} \quad (2)$$

where A and B are the first and second subimage arrays, respectively, $0 \leq x < n$, $0 \leq y < m$. For efficiency, continuous shifts $\{\delta x_0, \delta y_0\}$ are applied via interpolation to the first subimage, while integral shifts are applied to the second subimage. The operator $\mathcal{FT}_{\{p, q\}}\{\}$ denotes the two-dimensional finite discrete Fourier transform with independent variable $\{p, q\}$. By Hermitean symmetry, $0 \leq p < n/2$ and $0 \leq q < m$.

Because the Fourier transform of a finite data set implicitly treats the data as periodic, the correlation from displacements larger than half the subimage size “wrap” into the opposite side of the correlation function Ξ_p .

Wrapping effects are eliminated by padding the first subimage with null elements to at least twice its original size. This padded subimage is correlated with a second subimage of the same size. The size of the resulting correlation is the same as the padded subimage. The interrogation region remains the unpadded subimage, so padding does not affect spatial resolution.

2.3 Suppressing Imaging Defects

The widths of the correlation fields in PIV are proportional to the imaged size of the particles, a convolution of the point spread function of the imaging apparatus with the particle geometry. Blurring from particle motion also broadens the correlation function. Furthermore, spurious correlations naturally appear because of the finite number of particles in a subimage. These non-idealities are treated by deconvolving the cross-correlation of two subimages with the average of the autocorrelation of the subimages on a frame-by-frame basis, i.e.,

$$\Xi_p = 2FT^{-1} \left\{ \tilde{A}^* \tilde{B} \frac{\tilde{H}}{\tilde{A}^* \tilde{A} + \tilde{B}^* \tilde{B}} \right\}, \quad (3)$$

where $\tilde{H}$ is a calibration function that is needed to account for additional nonidealities in the camera and video digitizing system. For an ideal system, this function is unity. However, we have observed spatially repeatable measurements of displacements in image sets with no convective velocity. When cross-correlating even-odd and odd-even frames of the interlaced video, these offsets are typically 0.1 pixel per frame with a peak of about 0.35 pixel per frame. When cross-correlating even-even and odd-odd frames these offsets are typically an order of magnitude smaller. They may be effectively nulled by inferring $\tilde{H}$ from known- or no-displacement measurements. The results presented in Section 5 do not use a calibration.

Equation (3) is successful at narrowing the correlation peak nearly to the limit imposed by Brownian motion. However, it introduces spurious correlation spikes that do not average away quickly. Because of our limited ensemble, we replaced $\tilde{A}^* \tilde{A} + \tilde{B}^* \tilde{B}$ with the temporal ensemble-averaged values of the autocorrelation to reduce noise in the results presented in Section 5. This procedure fails to remove spurious correlations on an individual frame-pair basis, however these spurious correlations are naturally mitigated by ensemble averaging. Deconvolution stiffens the nonlinear fitting problem. The alternative approach discussed earlier and applied in Section 5 is to convolve the averaged autocorrelations with the theoretical signals to recreate the imaging non-idealities in the theoretical signal rather than to remove the non-idealities from the experimental signals.

2.4 Normalizing Combinatorial Bias

The particle displacement correlation Ξ_p is not a probability density function of particle displacement

because it bears a combinatorial bias toward low displacements relative to the shifted subimage. The chance that a pixel corresponding to a fluid element that appears in the first subimage also appears in the second (shifted) subimage and contributes to the correlation decreases with the displacement between the images. For a displacement of $\{\Delta x, \Delta y\}$ pixels using a pair of $n \times m$ -pixel subimages with the first subimage padded with zeros to twice its height and width and shifted by $\{-\delta x_0, -\delta y_0\}$, the combinatorial probability is

$$C\{\Delta x, \Delta y | \delta x_0, \delta y_0\} = \frac{wh}{mn}, \quad (4)$$

where

$$w = \begin{cases} n & \text{if } |\Delta x - \delta x_0| < \frac{n}{4}, \\ \frac{3n}{2} - 2|\Delta x - \delta x_0| & \text{if } \frac{n}{4} \leq |\Delta x - \delta x_0| < \frac{3n}{4}, \\ 0 & \text{otherwise,} \end{cases} \quad (5)$$

and

$$h = \begin{cases} m & \text{if } |\Delta y - \delta y_0| < \frac{m}{4}, \\ \frac{3m}{2} - 2|\Delta y - \delta y_0| & \text{if } \frac{m}{4} \leq |\Delta y - \delta y_0| < \frac{3m}{4}, \\ 0 & \text{otherwise.} \end{cases} \quad (6)$$

Correlations are combinatorially biased toward small displacements relative to the shifted subimages. The probability density function of finite particle displacements is obtained by normalizing the particle displacement correlation:

$$\mathcal{P}_p\{\Delta x, \Delta y | \delta x_0, \delta y_0\} = \Xi_p\{\Delta x, \Delta y | \delta x_0, \delta y_0\} / C\{\Delta x, \Delta y | \delta x_0, \delta y_0\}. \quad (7)$$

The PDF of particle velocities, $\mathcal{P}_p\{u, v\}$, is obtained from $\mathcal{P}_p\{\Delta x, \Delta y | \delta x_0, \delta y_0\}$ by substituting

$$u = \frac{\Delta x + \delta x_0}{\Delta t}, \quad \text{and} \quad v = \frac{\Delta y + \delta y_0}{\Delta t}, \quad (8)$$

where Δt is the time interval between the recording of the image pair. This simple differencing scheme is only appropriate when velocity gradients are negligible along the particles' paths between successive frames.

If the velocity varies significantly across the subimage, Ξ_p is the integral over $\{x, y\}$ across the shifted subimages of the product of the combinatorial probability $C\{\delta x, \delta y\}(x, y)$ of detecting a displacement $\{\delta x, \delta y\}$ at a subimage location $\{x, y\}$ and

$\mathcal{P}_p\{\delta x, \delta y\}(x, y)$, i.e.

$$\Xi_p = \frac{1}{nm} \int_{x_0}^{x_0+n} \int_{y_0}^{y_0+m} C(x, y) \mathcal{P}_p(x, y) dy dx, \quad (9)$$

which is generally complicated to invert. The present analysis assumes that variations across the subimage are negligible. For this and a variety of other reasons including spatial resolution and processing time, it is desirable to use the smallest practical subimage size for the correlations. The use of an excessively large subimage produces a systematic error in the analysis that can skew measurements.

2.5 Ensemble Averaging

The final step in the subimage processing is superimposing an ensemble of measurements of $\mathcal{P}_p\{u, v\}$ from an array of images into a high-quality mean PDF. Ensemble averaging reduces noise levels and spurious peaks produced in the image processing step, but obviously cannot remove static or systematic errors from the correlation, e.g., background, illumination uniformity, camera system non-ideality, etc. The previous steps in the processing are designed to minimize residual systematic errors in $\mathcal{P}_p\{u, v\}$. In addition to improving accuracy, ensemble averaging allows the experimenter to trade time resolution for spatial resolution (and vice-versa). For example, a 32×32 -pixel subimage correlation from a spatially uniform flow has the same signal to noise and accuracy as a 256-ensemble-averaged 2×2 -pixel subimage correlation from a stationary flow with similar seeding density.

3 Optimal Filtering

Filtering is a means of extracting desired information from a signal with embedded interfering information. Conventional linear (frequency) filters utilize, at best, a knowledge of the expected frequency distribution of the desired and interfering information. For a real signal sampled at N discrete points, there are N discrete independent frequency bins and hence N -independent degrees of freedom to the signal. Linear filtering removes one degree of freedom for every frequency bin that is effectively removed. Hence, the number, M , of degrees of freedom remaining in the filtered vector is proportional to the spectral width of the desired information. Usually this reduced vector is further processed to obtain useful measurements. In contrast, the output of an optimal nonlinear fil-

ter¹ is a vector with one degree of freedom for each measurement extracted from the filter. Hence the optimal nonlinear filter eliminates the post-filter processing step and maximizes the over-sampling ratio, N/M , a measure of the noise immunity of the measurements.

The filter works by adjusting the output-vector parameters to minimize the error between the experimental and theoretical signal. Our implementation of the filter uses a nonlinear least-squares fitting routine based upon the Levenson-Marquardt method, which is a combination of Newton's method and the method of steepest descents. This routine requires a function that supplies an array bearing the theoretical signal given an array of parameters.

3.1 The Theoretical Signal

The molecular velocity PDF at each point in the measurement region, $\mathcal{P}_m(\mathbf{u} - \bar{\mathbf{u}})$ is assumed to be Gaussian. This PDF is peaked near the mean local flow velocity, $\bar{\mathbf{u}}$. The width of the peak depends upon the local temperature and viscosity of the fluid. The flow tracers have a particle-convection-velocity PDF $\mathcal{P}_{pc}(\mathbf{u} - \bar{\mathbf{u}})$ with the same mean velocity but a narrower Gaussian distribution width. After a time interval Δt , the probability that $\{\delta x, \delta y\}$ are the x - and y -components of the displacement vector of a particle is

$$\mathcal{P}\{\delta x, \delta y | \Delta t\} = \frac{1}{\pi D \Delta t} \exp \left(- \frac{(\delta x - \bar{u} \Delta t)^2 + (\delta y - \bar{v} \Delta t)^2}{D \Delta t} \right). \quad (10)$$

The symbols $\bar{u}$ and $\bar{v}$ denote the x - and y -components of $\bar{\mathbf{u}}$, respectively. For spherical particles of diameter d that is large enough that the immersion fluid at temperature T , with dynamic viscosity μ is effectively continuous,

$$D = \frac{kT}{3\pi\mu d}. \quad (11)$$

The broadening of the distribution is a manifestation of Brownian motion of the particles. Assuming that the size of the particles is known, the viscosity or temperature may be inferred from a measurement of the magnitude of this broadening. A number of competing effects also broaden the measured velocity

¹The term "nonlinear" is used loosely here to distinguish this optimal filter from a conventional optimal linear filter, i.e., a Wiener filter. The dependence of the output vector components on the input vector need not be nonlinear.

PDF, however, increasing the difficulty of viscosity measurement.

An important complication appears if the particle motion has an electrophoretic component. There is an asymmetrical broadening of the distribution caused by the variation in surface charge density of the particles. For particles whose diameters are much larger than the Debye length of the immersion fluid, the mean electrophoretic velocity $\mathbf{u}_{ep}$ of the particles may be written

$$\mathbf{u}_{ep} = \frac{\epsilon \zeta_p}{\mu} \mathbf{E}, \quad (12)$$

where ϵ is the dielectric constant of the immersion fluid, $\mathbf{E}$ is the local electric field in the fluid, and ζ_p is a semi-phenomenological effective surface potential defined by the relation (12). We treat ζ_p as a parameter with a Gaussian distribution of variance σ_{ep}^2 centered on $\bar{\zeta}_p$, yielding a most probable electrophoretic velocity $\bar{\mathbf{u}}_{ep}$ with x - and y -components $\bar{u}_{ep}$ and $\bar{v}_{ep}$, respectively. The PDF of particle displacement with convection and electrophoresis is

$$\begin{aligned} \mathcal{P}\{\delta x, \delta y | \Delta t\} = & \frac{1}{\pi \sqrt{D\Delta t(D\Delta t + \sigma_{ep}^2 \bar{c}_{ep}^2)}} \\ & \times \exp \left[-\frac{1}{\bar{c}_{ep}^2} \left(\frac{(\delta x' \bar{u}_{ep} + \delta y' \bar{v}_{ep})^2}{D\Delta t + \sigma_{ep}^2 \bar{c}_{ep}^2} \right. \right. \\ & \left. \left. + \frac{(\delta x' \bar{v}_{ep} - \delta y' \bar{u}_{ep})^2}{D\Delta t} \right) \right], \quad (13) \end{aligned}$$

where $\delta x' \equiv \delta x - (\bar{u} + \bar{u}_{ep})\Delta t$ and $\delta y' \equiv \delta y - (\bar{v} + \bar{v}_{ep})\Delta t$, and $\bar{c}_{ep}^2 = \bar{u}_{ep}^2 + \bar{v}_{ep}^2$.

Specializing to the case of electroosmotic and pressure-driven flow in microchannels, we note that depth-wise velocity-field gradients dissipate rapidly at low Reynolds numbers. Figure 1 illustrates the flow and resulting velocity PDFs. Only gradients that are actively driven are present to any large extent in these flows. A linear shear may be driven by a ζ -potential difference from the top to the bottom; a parabolic velocity component may be driven by pressure gradients. These gradients may thus be observed throughout low-Reynolds-number microchannel flows. However higher-order gradients can only be locally forced by, e.g., step depth changes. These gradients will only be encountered in regions that are local to the forcing since at low Reynolds number, a disturbance viscously decays by $1/e$ while convecting a distance $\sim a \text{Re}_a$, where a is a characteristic geometrical dimension, e.g., the channel width or height, and $\text{Re}_a = \rho U a / \mu$ is the Reynolds number based upon

that geometrical dimension. If we assume that microchannels are largely planar, we may for the most part ignore higher-order gradients. Hence without excessive loss of generality, we may assume

$$\begin{aligned} \mathbf{u}(x, y | z') &\simeq \mathbf{u}_0(x, y) + z' \mathbf{s}(x, y) + \frac{(z')^2}{2} \mathbf{c}(x, y), \\ \mathbf{s} \cdot \hat{\mathbf{e}}_z &= 0, \quad \mathbf{c} \cdot \hat{\mathbf{e}}_z = 0. \end{aligned} \quad (14)$$

where $\mathbf{u}_0$, $\mathbf{s}$, and $\mathbf{c}$ are the mean velocity, linear shear, and curvature vectors, and z' is the dimensionless (unresolved) depth ranging from $-1/2$ to $1/2$. For flows in which the electric double layer

Figure 1: Depthwise gradients in planar flows and their effect on particle-displacement PDFs.

is thin compared to geometrical dimensions and the ζ -potential of the boundaries is uniform, purely electroosmotic velocity fields are proportional to the local electric field [Cummings et al. 1999]. Furthermore, if the electric field is uniform with depth, but the ζ -potential of the channel boundaries varies linearly with depth, the electroosmotic velocity field contains a depth-wise, linear shear proportional to the electric field and the ζ -potential difference between the top and bottom surfaces. Sufficiently far from a geometrical change in the unresolved (depth-wise) direction, the electric field is nearly uniform with depth. Hence

the electrophoretic velocity fields, linear shear fields, and uniform velocity fields are aligned and proportional to the electric field when (14) is valid, so we may revise (14) to reflect these insights:

$$\begin{aligned}\bar{u}(x, y|z') &\simeq \frac{\epsilon(x, y)}{\mu(x, y)} (\zeta_0(x, y) + z' \Delta \zeta(x, y)) \mathbf{E}(x, y) \\ &\quad + \frac{(z')^2}{2} \mathbf{c}(x, y), \\ \bar{u}_{ep} &\simeq \frac{\epsilon(x, y)}{\mu(x, y)} \bar{\zeta}_p \mathbf{E}(x, y), \\ \mathbf{E} \cdot \hat{\mathbf{e}}_z &= 0, \quad \mathbf{c} \cdot \hat{\mathbf{e}}_z = 0\end{aligned}\tag{15}$$

The probability $\mathcal{P}_z\{\bar{u}|x, y\}$ of encountering a mean convection velocity $\bar{u}$ while observing a point $\{x, y\}$ with no z -resolution for this velocity field is a curve S of δ -functions through the $\{u, v\}$ -plane parameterized by z . The PDF $\mathcal{P}_z$ convolved (over $\{u, v\}$) with $\mathcal{P}_p$ produces $\mathcal{P}_{pz}\{u|x, y\}$, the PDF of particles at a point $\{x, y\}$ integrated over z . This two dimensional convolution is only approximately correct since it neglects the effect of particles diffusing in the z -direction and thereby encountering different velocity groups, c.f., Taylor flow. This approximation is only justified if the particle (diffusional) Peclet number based upon the characteristic transverse dimension and pressure-driven component of the velocity is large. In the low-Peclet-number limit, the particle velocity distribution in flow with depth-wise velocity gradients becomes Gaussian and indistinguishable from that of uniform flow. For intermediate Peclet numbers, we adopt an approximate distribution, replacing the $\mathbf{c}$ -term in (15) by

$$\left[f(\text{Pe}) + (1 - f(\text{Pe})) \frac{(z')^2}{2} \right] \mathbf{c}(x, y), \tag{16}$$

where $f(\text{Pe})$ is a parameter ranging from zero to unity in the infinite- and zero-Peclet number limit, respectively. The computed PDF with this modification is exact in both limits.

For computational efficiency, we construct a finite representation of $\mathcal{P}_z\{\bar{u}|x, y\}$ directly from the curve S parameterized by (14), pad it to twice its size to remove “wrapping” effects, and convolve it with $\mathcal{P}_p$ using fast Fourier transforms to arrive at a discrete representation of $\mathcal{P}_{pz}$. Rather than Fourier transform $\mathcal{P}_p$, we construct $\mathcal{FT}\{\mathcal{P}_p\}$ directly.

4 Processing Software

To implement this analysis scheme, we produced *uPIV*, an interactive and automated GUI-based image processing code written in C++. This code utilizes fast Fourier transforms to perform subimage cross-correlations. The code works with non-interlaced and interlaced images of arbitrary size, in the latter case optionally treating odd and even fields as successive images. An arbitrary number of sequential images can be incorporated into the same analysis, allowing ensemble averaging of the correlation functions for stationary flows.

The user supplies a list of typically 10–1000 image files that contain successive particle-image frames. If the images are interlaced, the even and odd fields are treated as separate frames with the proper spatial shift. This software optionally calculates a mean field and RMS-deviation-from-mean field from all of the frames. This average highlights static elements of the image, e.g., diffraction rings, dust spots, geometrical features, and immobile particles, while smoothing over intensity variations produced by the moving particles. The user may opt to use these fields for background suppression and flat-field normalization as discussed in Section 2.1.

Next, *uPIV* composes a list of frame pairs for cross correlation. The second frame of the correlation is initially a user-supplied number of frames apart from the first. The particle displacement between frame pairs is proportional to the number of skipped frames. The user may opt to skip frames in order to improve the accuracy of measurements in low-speed regions (hence the dynamic range of the technique). The number of frame pairs in the list is equal to the total number of frames minus the number of frames skipped between the first and second subimages.

The analysis of the frame pairs begins by the software generating a regular coarse grid of subimages (e.g., 32×32 pixels or 32×16 pixels for non-interlaced or interlaced images, respectively) of the full images (640×480 pixels, 8 bits, interlaced, in these experiments). The software cross-correlates each subimage of each frame pair in the list and superimposes the correlations into high-fidelity, mean subimage cross-correlations. Optionally, *uPIV* deconvolves each cross-correlation with the subimage-pair or ensemble average autocorrelation and normalizes the combinatorial zero-pixel bias of the correlations as discussed in Section 2.4. A simple correlation-peak-processing routine provides initial estimates of the flow parameters. These estimates may be refined by applying the nonlinear filter, a nonlinear least-

squares fit to theoretical signals discussed in Section 3.1. After this processing *uPIV* checks the inferred parameters for validity, e.g., whether the correlation peak of the inferred velocities falls within the subimage correlation field. Subimages whose inferred parameters fail the validity check are visually flagged. Corrective action may be interactive or automated.

Next *uPIV* refines the subimage grid on the basis of the inferred velocity field. First the locations of the sub-images in each sub-image pair are shifted (nearly) symmetrically to optimize the overlap of the particle images, i.e., to center the correlation peak in the subimage correlation field. This shifting procedure employs an efficient pixel-interpolation scheme to produce effectively continuous pixel shifts of the first subimage. Secondly, the subimage may be subdivided into four equal-sized children subimages, each with the initial parameter set of the parent subimage.

Finally, *uPIV* optionally optimizes the number of frames skipped between each correlation frame pair. In this optimization, the measured speed and subimage size is used to determine how many frames to skip in the next analysis iteration between the first and second image in a correlation pair. The optimum number of skipped frames is that which produces a mean particle displacement between subimages that is approximately equal to the subimage size. This optimization is performed independently for each subimage, hence more frames are skipped between correlation pairs in low-speed regions than in high-speed regions. This optimization increases the dynamic range of the technique while preserving optimal spatial measurement resolution.

The process iterates until the images are the smallest practical size. This procedure has proven to be robust and relatively quick. For example, the complete processing to 4x4 pixel subimages of 50 640x480 images (100 fields) to extract mean velocity measurements takes about 5 minutes on a 500 MHz Alpha 21164 running NT 4.0 with 256 MB RAM. The failure rate of the automated subimage processing depends sensitively upon the geometry of the channel. Most failures occur near channel boundaries. The failure rate is often better than 1 in 10^3 subimages.

Experimental Apparatus

The experiments in this investigation employed nearly neutrally buoyant, highly fluorescent latex nanospheres as flow tracers. Because of the presence of electrical fields in the suspending liquid, particle electrophoresis occurs in addition to convection. To eliminate the ambiguity in the convective

and electrophoretic components of the particle motion, we may simultaneously seed the flow with latex nanospheres with two different electrophoretic mobilities and different fluorescence wavelengths. By measuring the velocity fields of both types of particles independently and simultaneously, we obtain the convective and electric fields in the fluid independently.

The different electrophoretic mobilities between the two types of particles arise from their different surface treatments. One of the particles (a product of Interfacial Dynamics) has only native, highly acidic sulfate groups on the surface, providing a negative surface charge density of ~ 0.02 C/m² at neutral and low pH. These particles have a fluorescent dye coating with an absorption maximum at ~ 490 nm and an emission maximum ~ 520 nm, similar to FITC, applied by Molecular Probes. The other particles are modified to have a high concentration of the more weakly acidic carboxylate group on the surface, providing the particles with a negative charge of ~ 0.3 C/m² at neutral pH. These particles have a Molecular-Probes dye coating that absorbs at 488 nm and fluoresces from 560–600 nm. The presence of the fluorescent dyes apparently does not dramatically affect the surface charge density.

The experimental apparatus is shown schematically in Fig. 2. The imaging system is an argon-ion-laser-pumped, three-color epifluorescence microscope. In this system, the sample is mounted on a two-dimensional translation stage with ~ 1 - μ m resolution. A medium-grade 5 $\times$ or 10 $\times$ microscope objective (*O1*) images the test sample onto the cameras *C1*, *C2*, and *C3*, and flood illuminates the sample with the ~ 20 -mW 488-nm pump laser beam that is reflected into the optical path by dichroic mirror, *M1*.

The fluorescence signals pass through mirror *M1*. Dichroic mirror *M2* reflects fluorescence in the wavelength range below ~ 550 nm into an emission filter *F1* which further limits the signal wavelength range to 500–530 nm. The filtered signal falls on a video-rate monochromatic 1/2-inch CCD camera (Cohu 4910, *C1*). Light that passes through *M2* falls upon a third dichroic mirror, *M3*, which reflects fluorescence at wavelengths shorter than ~ 640 nm into a second arm of the microscope. The reflected fluorescence passes through filter *F2* which passes 560–610 nm light to a second camera (*C2*) of the same type as the first. All filters and dichroic mirrors were obtained from Omega Optical. A third camera *C3* is incorporated into the microscope for a secondary diagnostic. The *uPIV* system resides on a vibration-damped optical table enclosed in a laminar-flow HEPA-filtered clean

hood. The apparatus inside the clean hood has been modified to minimize air currents and vibrations inside the clean hood during sensitive experiments.

Figure 2: Schematic diagram of the μ PIV system. The cameras and optics are mounted on a rigid frame filled with vibration-damping material. Two of the three cameras in this epifluorescence microscope are used for imaging particles that fluoresce in the green and in the red parts of the visible spectrum. The third camera supports a secondary, simultaneous diagnostic.

Test cells were manufactured using microlithographic techniques. The substrate and cover material of the microchannels was soda-lime or silica microscope slide stock in 30-mm $\times$ 30-mm squares with a thickness of ~ 2 mm. Microchannels and microscopic arrays of flow obstacles with minimum feature sizes of $30\text{ }\mu\text{m}$ were patterned onto the substrate using SU-8, a photo-definable, spin-on epoxy. The posts were $\sim 30\text{ }\mu\text{m}$ tall with sides that were effectively normal to the substrate. The assembled test cell was sealed into a fixture that provided fluid reservoirs on opposite sides of the cell and a uniform-electric-field boundary condition at the inlet and exit of the cell. A platinum wire in the bottom of the reservoirs applied the electrical potentials. The height of the fluid in the reservoirs could be adjusted by ~ 4 mm to produce pressure-driven flow in the cell. A regulated high-voltage power supply drove electroosmosis.

5 Results

We have employed the μ PIV system and analysis software described earlier to explore electroosmotic and pressure-driven flow in microfabricated arrays of posts. Of particular interest in these studies are the limit of our resolution and our ability to extract parabolic and mean velocity fields in the unresolved direction. We also seek an understanding of mixed electroosmotic and pressure-driven flows in complicated geometries and in geometries in which boundaries are non-uniform.

The image data was digitized to 8-bit gray scale at 640×480 resolution, interlaced. The effective image size is 640×240 , since even and odd rows must be treated as separate images. These half-images are stretched by a factor of two in the vertical direction so that they retain their proper aspect ratio. Each analysis that is presented employed a 50-frame correlation average (1.7-s video clips) of the particle flow. All the data that are shown were taken on a micropatterned glass/SU-8 test cell consisting of a regular array of marshmallow-shaped posts of SU-8, $\sim 30\text{ }\mu\text{m}$ tall and $\sim 250\text{ }\mu\text{m}$ across. Above the top of the posts is a gap of $\sim 5\text{--}10\text{ }\mu\text{m}$ to the glass cover slide. The fluid in all tests was water at neutral pH containing a suspension of fluorescent nanospheres (100-nm diameter). The ionic strength of the unbuffered water was not measured, but the primary source of ions in the water was the highly-diluted nanosphere suspension liquid. Nevertheless, the electrical double layers were smaller than any resolved dimensions.

Figure 3 shows a particle image of flow between and over two posts. The arrows that are superimposed on the image depict the mean velocity-vector field. The particle flow is nearly completely electrokinetic (consisting of both electroosmosis and electrophoresis) in origin, driven by an applied field of 27 V/mm . Figures 4.a and b are mosaics of independent speed measurements of the flow illustrated in Fig. 3 derived from 8×4 -pixel and 2×2 -pixel subimages, respectively. The mean particle image size is $\sim 2\times 2$ pixels. The mosaics are composed of a tile of the subimage size (stretched vertically by a factor of two) centered on the evaluation point of the subimage correlation. The speed of the flow at a tile is proportional to the tile's gray scale, ranging from black for zero speed to white for the maximum speed in the image, ~ 5 pixels per frame. No smoothing has been applied. The measurements are nominally independent but may be slightly coupled as a result of the grid refinement scheme. The streaks in the speed field follow the streak lines of mobile agglomerated particles

with a significantly different electrophoretic mobility from the mean. Away from the walls of the posts, the RMS scatter in mean particle displacement is ~ 0.2 pixel for the 2×2 -pixel subimages. In the region of the post walls, the illumination intensity is $< 5\%$ of that in the open channel and the scatter is several pixels. We are modifying our illumination scheme and converting to telecentric objectives to eliminate problems near channel edges.

The fields in Figs. 3 and 4 were obtained by convolving the theoretical signal with the autocorrelation of the subimages rather than the deconvolution method described in Section 2. The convolution technique was more robust than the alternative strategy probably because it reduced the stiffness in the fitting routine.

Figure 3: Particle image and superimposed velocity measurements of electrokinetic particle flow past two posts of an array. The electric field is from right to left. The channels and posts are $\sim 250 \mu\text{m}$ across. In the gap of about $10 \mu\text{m}$ between the top of the posts and the cover slip the electrokinetic flow velocity is higher than in the open channel.

We have applied the methodology described in Section 3.1 to dissect the electrokinetic and pressure-driven components of the velocity field in flows produced by both phenomena. The following results were obtained using 8×4 -pixel subimages.

Figure 5 shows measurements of purely pressure driven flow with Reynolds number ~ 0.05 past posts and a bubble. Figure 6 shows the mean-speed mosaic derived from these measurements. While at macroscopic scales, the boundary of a bubble would be considered a free surface that does not support shear, the flow speed near the bubble in Figs. 5 and 6 is much lower than in the center of the channel. Apparently

a. 8×4 -pixel subimages

b. 2×2 -pixel subimages

Figure 4: Mosaics of independent speed measurements vs. spatial resolution. Each measurement is represented by a sub-image-sized tile centered on the measurement location in the particle images. The gray scale of the tile is proportional to the speed: white is the maximum speed and black is zero speed.

surfactant molecules on the bubble surface cause it to resist pressure-driven flow. Flow at the bubble surface convects surfactants downstream, producing respectively a rarefaction and concentration of surfactant at the upstream and downstream stagnation regions. The surfactant gradient produces a surface-tension gradient, evidenced by the variation in curvature of the bubble. The resulting Marangoni forces resist the pressure-driven flow. The obvious defects in the lower left of Fig. 6 are produced by large agglomerations of particles adhering to the surface of

the channel, visible in Fig. 5.

Figure 5: Particle image and superimposed velocity curvature measurements (~ 5000 vectors from 8×4 -pixel subimages) of pressure-driven flow past a bubble and two posts of a post array.

Figure 6: Mean speed-measurement mosaic of the flow in Fig. 5.

At these low Reynolds numbers, the Navier-Stokes equations linearize to the Stokes equation, permitting the superposition of velocity fields. The component of the velocity field in this channel from pressure-driven flow should be independent of other components provided the combined Reynolds number remains low. Using this principle, the PIV methodology is tested by applying a range of voltages across the array, producing different electrokinetic flow components. Figures 7–11 show the results of the dissec-

tion of the electrokinetic and pressure-driven components of the mean flow.

Under the experimental conditions (negligible heating, etc.), the electrokinetic component of the velocity field scales linearly with the applied electric field. At an applied field of 3 V/mm, the electrokinetic component of the particle flow is $\sim 1/3$ that of the pressure driven flow; at a field of 9 V/mm, the two components are approximately equal; and at 27 V/mm the flow is mostly electrokinetic in origin. Figures 7.a–c show the mean (combined) flow fields inferred from the measurements, showing this transition. Figure 7.b shows the formation of a recirculation separatrix in the region above the bubble and near the left post surface. The recirculation near the post occupies an extent that is approximately equal to the region of rotational flow in pressure-driven Hele-Shaw flow. The recirculation above the bubble appears at a lower applied voltage and over a larger extent than that near the post. Figures 10.a–c show a mean-speed mosaic of the flows. The change in mean velocity and speed fields with applied voltage appears deceptively complicated in these images, as the following figures show.

Figures 8 and 9 show the inferred electrokinetic and (assumed constant) pressure-driven components of the mean velocity fields shown in Fig. 7. The velocity vectors are rescaled for different applied voltages to bring out different elements of the flow. However, the vector scales at each applied voltage are consistent across Figures 7, 8, and 9.

Figures 11a–c show mosaics of the inferred magnitude of the electrokinetic component of the velocity. The gray scale of each mosaic is normalized by the applied field. Because of the linear dependence of the electrokinetic flow on the applied electric field, the three mosaics should be identical. While the scatter in the measurements reduces as the relative magnitude of electrokinetic flow increases, the mosaics are duly similar, particularly when compared to the mean-speed mosaics in Fig. 10. The ability to decompose a seemingly complicated flow behavior into simpler independent components represents progress toward interpreting microscale flowfields.

6 Conclusions

We have developed an image processing and optimal nonlinear filtering methodology for PIV and have specialized this methodology to the study of electroosmotic and pressure-driven flows in planar microchannels. The iterative adaptive-gridding processing soft-

ware (*uPIV*) developed to implement this methodology efficiently and robustly measures mean velocity fields with high spatial resolution by adaptive grid refinement and optimization. The optimal nonlinear filter of *uPIV* can extract both the pressure-driven and electrokinetic components of the particle velocity. We have used the system and software to analyze a variety of pressure-driven and electroosmotic flows past an array of obstacles and bubbles. The bubble boundary behaved like a semi-rigid surface to pressure-driven flow and behaved like a surface with a large ζ -potential in electroosmotic flow.

The PIV methodology can be applied equally well to the measurement of turbulent velocity PDFs in unsteady, stationary flow. The optimal nonlinear filter can similarly be employed to extract the variance and moments of the velocity PDF, and other statistical measures of interest to turbulence theorists and modelers.

In addition to improving measurement accuracy, temporal ensemble averaging can be employed in stationary flows to improve spatial resolution. For example, a 1024-frame-pair average correlation from a single-pixel subimage of a stationary flow has similar characteristics to a single-frame-pair correlation from a 32×32 -pixel subimage of a uniform flow. The single-pixel subimage must be padded enough that the correlation array contains the majority of the correlation peak. Practically, with iterative subpixel subimage shifting, this sets a lower limit of the padded subimage of $\sim 4 \times 4$ pixels. Ordinarily, the use of a four-fold padded subimage would seriously slow the image-correlation process. However, single-pixel correlations can be performed directly quite efficiently.

Having independent particle-displacement measurements at each pixel in an image is not the same as having single-pixel velocity measurement resolution, since typical particle displacements are several pixels. In principle, it is possible to convert a displacement field into an Eulerian velocity field with the same measurement resolution. However, the first-order central-differencing scheme generally used to perform this conversion does not preserve the spatial resolution of the displacement measurements.

a. Applied field 3 V/mm.

b. Applied field 9 V/mm.

c. Applied field 27 V/mm.

Figure 7: Particle image and superimposed mean velocity measurements from 8×4 -pixel subimages of electrokinetic and pressure-driven flow. Vector scales differ between a, b, and c.

a. Applied field 3 V/mm.

a. Applied field 3 V/mm.

b. Applied field 9 V/mm.

b. Applied field 9 V/mm.

c. Applied field 27 V/mm.

c. Applied field 27 V/mm.

Figure 8: Inferred electrokinetic component of flow velocity in Fig. 7. Vector scales are consistent with those in Fig. 7.

Figure 9: Pressure-driven component of flow velocity in Fig. 7. Vector scales are consistent with those in Fig. 7.

a. Applied field 3 V/mm.

b. Applied field 9 V/mm.

c. Applied field 27 V/mm.

Figure 10: Mean speed fields of flows in Fig. 7. The speed in each flow is proportional to the gray scale. Black is zero speed; white is the maximum speed.

a. Applied field 3 V/mm.

b. Applied field 9 V/mm.

c. Applied field 27 V/mm.

Figure 11: Electrokinetic velocity magnitude fields of flows in Fig. 7. Each gray scale is normalized by the applied field.

Nomenclature

Coordinates

$\hat{e}_z$	unit vector in z -direction
x -direction	row-wise direction
y -direction	column-wise direction
z -direction	unresolved direction
$\{ , \}$	ordered pair in {col., row} form
$\{\delta x_0, \delta y_0\}$	subimage offset
$\{\Delta x, \Delta y\}$	pixel relative to subimage
$\{x, y\}$	pixel relative to image
Δt	time between image-pair acquisition

Operators

$\mathcal{C}\{\Delta x, \Delta y \delta x_0, \delta y_0\}$	combinatorial bias
$\mathcal{FT}\{\}$	discrete Fourier transform
$\mathcal{P}\{a\}$	probability of a
$\mathcal{P}\{a b\}$	probability of a given b
$\Xi\{\Delta x, \Delta y \delta x_0, \delta y_0\}$	subimage correlation

Parameters

a	characteristic transverse dimension
c	velocity curvature vector
d	diameter of seed particle
$f(\text{Pe})$	empirical function of Pe
k	Boltzmann constant
n	number of columns of the subimage
m	number of rows of the subimage
s	velocity shear vector
u, v	x - and y -components of velocity
$\bar{u}, \bar{v}$	mean components of velocity
$\mathbf{u}$	fluid velocity vector
$\mathbf{u}_0$	uniform velocity vector
ϵ	dielectric constant of the fluid
μ	kinematic viscosity
ρ	fluid density
σ^2	variance of distribution of ζ_p
ζ	effective surface electric potential
A	first image pixels
B	second image pixels
$\mathbf{E}$	electric field
$\tilde{H}$	complex calibration constant
Pe	Peclet number: $\text{Pe} \equiv Ua/D$
Re_a	Reynolds number: $\text{Re} \equiv \rho Ua/\mu$
T	temperature
U	characteristic speed

Subscripts and Superscripts

z	related to the unresolved direction
p	related to particles
m	related to molecules
ep	related to electrophoresis

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