Frame rate free image velocimetry for microfluidic devices

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Here, we introduce Streamline Image Velocimetry, a method to derive fluid velocity fields in fully developed laminar flow from long-exposure images of streamlines. Streamlines confine streamtubes, in which the volumetric flow is constant for incompressible fluid. Using an explicit analytical solution as a boundary condition, velocity fields and emerging properties such as shear force and pressure can be quantified throughout. Numerical and experimental validations show a high correlation between anticipated and measured results, with $R^2 > 0.91$. We report spatial resolution of $2\ \mu\text{m}$ in a flow rate of $0.15\ \text{m/s}$, resolution that can only be achieved with $75\ \text{kHz}$ frame rate in traditional particle tracking velocimetry. © 2013 AIP Publishing LLC.

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Fluid dynamics plays a fundamental role in environmental, biological, and industrial processes ranging from mass transport to separations and heat exchange. Investigation of sand dunes,¹ wind waves,² heat transfer,³ fouling,⁴ and aneurysm failure⁵ are a few examples where an exact solution of the fluid velocity field is required. Over the past decade, microfluidic devices became more common, reducing time, labor, space, and cost of processes.^{6,7} However, current microscale velocimetry methods have considerable difficulty dealing with high flow rates due to current hardware limits.

Existing microscale velocimetry methods include particle-imaging velocimetry, particle tracking velocimetry (PTV), laser doppler velocimetry, and particle streak velocimetry. In particle imaging velocimetry, microscale particles are sequentially imaged. Particle ensembles are recognized in snapshots, and the displacement vector divided by the time delay constitute the velocity field.⁸ PTV applies a similar method, which traces individual particles in a lower seeding density.⁹ Laser Doppler velocimetry calculates fluid velocity based on the frequency of a laser beam scattered from immersed particles,^{10,11} and finally particle streak velocimetry, derives the fluid velocity from the length and direction of single particle pathlines using long exposure photography.¹² Particle streak velocimetry is considered to be significantly less accurate than other velocimetry methods due to long exposure intervals¹³ and is seldom used.

Particle image, particle tracking, and laser doppler velocimetries are commercially available and require significant investment in equipment and computational power, while offering limited spatial resolution.¹⁴ Laser Doppler velocimetry spatial resolution is limited by the fiber diameter, with resolutions of $250\ \mu\text{m}$ (Ref. 15) and $125\ \mu\text{m}$ (Ref. 16) recently reported in literature. The spatial resolution of particle image and particle tracking velocimetries, on the other hand, are limited by the fluid velocity and the

camera frame rate.¹⁷ Recently, a resolution of $129\ \text{nm}$ was reported for a velocity and $0.015\ \text{m/s}$ using single pixel evaluation particle image velocimetry.¹⁸ The problem is compounded when dealing with curved streamlines. Displacement vector in the order of magnitude of the curvature radius will cause significant bias error. The correction of that error is done by estimating the curvature using the second-order shift vector and its gradient.¹⁹

In this work, we present an alternative approach termed Streamline Image Velocimetry (SIV), which allows a rapid quantification of velocity fields in arbitrary geometries for fully developed laminar flows. SIV uses long-exposure images to capture multiple particle pathlines across the entire field. In fully developed laminar flows, pathlines and streaklines coincide with streamlines. Streamlines confine streamtubes, in which the volumetric flow is constant for incompressible fluid, allowing us to derive relative fluid velocities across the field of study. Using an analytically derived velocity field as a boundary condition, we can convert relative to absolute velocities. The method is well suited for microfluidic devices where low Reynolds numbers prevail and the problem is often simplified to two dimensions. A major advantage of SIV over particle image and particle tracking velocimetries is that it is not limited by the camera shutter speed and can therefore be carried out using standard cameras.

A streamline is a curve parallel to the velocity vector.²⁰ In fully developed flow, streamlines coincide with the paths of the fluid particles. A streamtube²¹ is a surface confined between streamlines. The volumetric flow rate in a streamtube is conserved; therefore, the product of the streamtube cross-section A and the average velocity V is constant

$$A_1 \bar{V}_1 = A_2 \bar{V}_2 = A \bar{V}. \quad (1)$$

The application of Eq. (1) results in relative velocity vectors, one for each streamtube, which is converted to an explicit quantitative field, based on explicit analytical solutions. Explicit analytical solutions exist for simple channel geometries, such as circular and rectangular channels.²² The

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velocity profile for Poiseuille flow in a rectangular channel can be expressed as Fourier series as described in Eq. (2)

$$v_x(y, z) = \frac{4h^2\Delta p}{\pi^3\eta L} \sum_{n, \text{odd}} \frac{1}{n^3} \left[1 - \frac{\cosh(n\pi \frac{y}{h})}{\cosh(n\pi \frac{w}{2h})} \right] \sin\left(n\pi \frac{z}{h}\right), \quad (2)$$

where v_x is the velocity, h is the channel height, w is the channel width, L is the channel length, η is the fluid viscosity, z is the vertical location inside the channel, y is horizontal location inside the channel, and Δp is the pressure drop.

For low aspect ratio channels ($h \ll w$), the volumetric flow rate (Q) can be approximated as²²

$$Q \approx \frac{h^3 w \Delta p}{12\eta L} \left[1 - 0.63 \frac{h}{w} \right]. \quad (3)$$

Based on Eqs. (2) and (3), both pressure drop and channel length can be expressed as a function of the volumetric flow rate, and the velocity equation is not dependent on Δp and L . As fluorescent particles are concentrated close to the middle of the channel height due to the wall shear stress, velocity profile can be calculated in the center of the channel height ($z = h/2$). Equation (2) can be simplified to

$$v_x(y) \approx \frac{48Q}{\pi^3(w - 0.63h)} \sum_{n, \text{odd}} \frac{1}{n^3} \left[1 - \frac{\cosh(n\pi \frac{y}{h})}{\cosh(n\pi \frac{w}{2h})} \right] \sin\left(n\pi \frac{1}{2}\right). \quad (4)$$

Equation (4) describes the parabolic velocity profile in a rectangular channel and is used to convert the relative velocity vectors to the quantitative velocity field.

To capture single particle pathlines, we introduced $2 \mu\text{m}$ diameter fluorescence beads at concentrations of 2×10^6 particles/ml to microfabricated channels containing radial distortions (Fig. 1(a)). Particles were suspended in 1% Pluronic F68 solution to minimize non-specific binding to channel walls. Images were taken with camera exposure setting ranging from 0.9 to 1.5 s based on fluid velocity to allow particles to trace complete paths across the entire field of view (Fig. 1(a)). Image processing and velocity profile calculation were coded in a program written in MATLAB. Streamlines were identified by line tracing carried out in

multiple images of the same field of view (Figs. 1(c)–1(f)) fitted to polynomials and superimposed on a single matrix (Fig. 1(g)). Microfluidic devices were fabricated by soft lithography. Briefly, molds were fabricated by photolithography of SU8 on silicon wafers at the Harvey Krueger Center of Nanoscience and Nanotechnology at the Hebrew University of Jerusalem. Channels were replica molded in Polydimethylsiloxane (PDMS) and bonded to glass using oxygen plasma as previously described.^{23,24} Channels were $100 \mu\text{m}$ high with a width of $3000 \mu\text{m}$, and a $200 \mu\text{m}$ radius distortion on one wall. Prior to the experiment, the channel was coated with Pluronic F-68 for 1 h at room temperature to prevent non-specific adhesion of fluorescent particles. Both SIV and particle tracking velocimetry experiments were performed with microbeads diluted in 1% Pluronic F-68 at a concentration of 1 to 2×10^6 particles/ml. Microbeads were perfused using a Fusion 200 syringe pump (Chemxy, Stafford, Texas) and imaged on a Zeiss Axiovert Microscope with a frame rate of 12 frames per second and a shutter time of 1 ms.

Volumetric flow rate between neighboring streamlines is constant for incompressible fluid allowing us to calculate relative velocities within each streamtube. To do that, we calculated a mean polynomial between every two neighboring streamlines. Velocity direction is the derivative of this mean polynomial, while the distance between streamlines is the length of a line perpendicular to the mean polynomial drawn between neighboring streamlines. Neighboring polynomials are approximately parallel to their mean polynomial and the channel cross-section area is given by: $A = h \cdot d \cdot \cos\theta$, where h is the channel height, d is the distance between the polynomials, and θ is the angle of the polynomial tangential. Relative velocity vectors are derived for each streamtube using Eq. (1) and converted to absolute velocity using the explicit analytical solution for rectangular channels given by Eq. (4).

To validate our method, we carried out detailed numerical simulations and critically compared the predictions to SIV and PTV results. Numerical simulations were performed using COMSOL multiphysics simulation platform v4.3 with a direct linear system solver and extra fine mesh (PARADISO). Fluid density was defined as $1 \times 10^3 \text{ kg/m}^3$, with a dynamic viscosity of $1 \times 10^{-3} \text{ Pa}\cdot\text{s}$. PTV measurements were analyzed using MOSAIC 2D Particle Tracker

FIG. 1. Streamline imaging in a micro-channel with a circular cavity. (a) Long exposure fluorescent image of $2 \mu\text{m}$ diameter fluorescent particles streaking across a $400 \mu\text{m}$ circular distortion. Camera exposure time was 1.5 s. (b) Numerical streamline results obtained using COMSOL Mutliphysics®. (c)–(f) Streamline tracing using MATLAB for four different snapshots. Traced lines were highlighted in red. (g) Matrix of superimposed streamline polynomials used for SIV measurements.

Plugin for Image J.²⁵ Particle detection parameters were: particle radius: 3 pixels, particle cut-off: 2, and percentile: 0.55%. We show the results in three cross sections: at the entrance to the channel where only a tangential component of the velocity exists (Figs. 2(a) and 2(d)), in the first quarter of the distortion where a perpendicular velocity component emerges (Figs. 2(b) and 2(e)), and in the middle of the distortion (Figs. 2(c) and 2(f)). Comparisons were carried out at a low flow rate of 10 $\mu\text{l}/\text{min}$ (Figs. 2(a)–2(c)), and a high flow rate of 50 $\mu\text{l}/\text{min}$ (Figs. 2(d)–2(f)). The results show that at 10 $\mu\text{l}/\text{min}$, both SIV and particle tracking velocimetry showed an excellent correlation of $R^2 > 0.97$ to the numerical results. However, when flow rate increased to 50 $\mu\text{l}/\text{min}$ SIV still showed correlation of $R^2 > 0.97$ to the numerical simulation, but particle tracking velocimetry correlation decreased to $R^2 < 0.51$ due to frame rate limitations. Figure 2(g) shows a good correlation of SIV for both the perpendicular and tangential velocities in the cavity, with R^2 of 0.93 and 0.95, respectively. To further validate SIV in high flow rates, we compared it to numerical predictions at flow rates of 2 ml/min (0.15 m/s) reaching R^2 of 0.91 (data not shown).

Shear stress is an important physical phenomenon that governs hemodynamics, fouling, and other effects.^{1,4,5} Figure 2(h) shows shear stress 10 μm from the wall of the cavity. The data show an excellent agreement between the numerical model and SIV results, with R^2 of 0.96. The velocity field output can also be shown as an arrow field, providing a macro overview of our velocity field description (Fig. 2(i)).

One advantage of experimentally measuring fluid velocities is the ability to quantify flow in structures with unknown boundaries, such as porous membranes. Recently, Fachin and colleagues fabricated a microfluidic device integrated with nanoporous 500 μm diameter columns composed of vertically aligned carbon nanotubes for chemical and mechanical isolation of cells (Fig. 3(a)).²⁶ Numerical simulation of flow in such devices will require exact knowledge of the porous properties of the boundary. However, SIV is able to derive the velocity field based solely on the visualized streamlines (Fig. 3(b)). The velocity arrow field around the porous post is shown in Figure 3(c). The reliance of SIV on polynomial functions also allows us to derive smooth wall shear stress functions for this problem as a function of radial position around the column (Fig. 3(d)).

SIV is derived from the continuity equation which is valid for both laminar and turbulent flow.²⁷ However, streamlines can only be reliably imaged as path-lines in fully developed, laminar flow, up to Reynolds numbers of a few hundreds. When Reynolds numbers are greater than 2000, path-lines start crossing each other²⁸ and the error related to the streamtubes dimension significantly increases. However, even in turbulent flow, local streamtubes visualization can be carried out within the laminar boundary layer. We note that SIV is particularly appealing in microfluidic applications where Reynolds numbers are low.

The demand for fully developed streamlines limits the ability to utilize SIV in visco-elastic fluids. Particularly, streamlines in visco-elastic fluids tend to destabilize in

FIG. 2. Comparison of SIV results (clear circles) to PTV results (filled circles) and numerical predictions (solid lines) at multiple cross-sections. Flow rate for (a), (b), and (c) was 10 $\mu\text{l}/\text{min}$. (a) Absolute velocity field cross-section at the beginning of the cavity, where only a tangential velocity component is present. Both SIV and PTV show a high correlation, with $R^2 = 0.97$ and 0.99 , respectively. (b) Absolute velocity field cross-section at the quarter of the cavity where both perpendicular velocity and tangential velocity components are present. Both SIV and PTV show a high correlation, with $R^2 = 0.97$ and 0.98 , respectively. (c) Absolute velocity field cross-section at the middle of the cavity where only a tangential velocity component is present. Both SIV and PTV show a high correlation, with $R^2 = 0.98$ and 0.93 , respectively. Flow rate for (d), (e), and (f) was 50 $\mu\text{l}/\text{min}$. (d) Absolute velocity field cross-section at the beginning of the cavity. While SIV correlation remained high, with $R^2 = 0.96$, PTV correlation to the numerical model fell to 0.41 . (e) Absolute velocity field cross-section at the quarter of the cavity. While SIV correlation remained high, with $R^2 = 0.98$, PTV correlation to the numerical model fell to 0.51 . (f) Absolute velocity field cross-section at the middle of the cavity. While SIV correlation remained high, with $R^2 = 0.93$, PTV correlation to the numerical model fell to 0.49 . (g) SIV derivation of tangential and perpendicular velocity components in a cross-section at the quarter of the cavity at 50 $\mu\text{l}/\text{min}$. Both perpendicular and tangential velocities show good correlation with, with $R^2 = 0.93$ and 0.95 , respectively. (h) SIV derivation of shear stress 10 μm from the wall, across the 400 μm diameter cavity compared to numerical prediction. (i) Overview of the velocity field at 50 $\mu\text{l}/\text{min}$ presented as an arrow field.

curved channels. Therefore, Pakdel and McKinley's dimensionless criteria²⁹ for flow stability should be considered.

Measurement errors that affect image velocimetry methods are random error and bias error.¹⁸ The most significant random error in image velocimetry is Brownian motion. The error in tracer location due to Brownian motion is given by¹⁷

$$\varepsilon_B = \frac{1}{u} \sqrt{\frac{2D}{\Delta t}}, \quad (5)$$

where Δt is the time shift, u is the fluid velocity, and D is the diffusion coefficient of the particle. This random error establishes the lower limit of the discrete time interval for particle image and particle tracking velocimetries. In our measurements, the diffusion coefficient of the tracers was $4.39 \times 10^{-13} \text{ m}^2/\text{s}$, with linear velocity ranging between 0.8 mm/s and 4 mm/s, and Δt of 1/12 s, producing random error of 0.4% and 0.08% for flow rates of 10 $\mu\text{l}/\text{min}$ and 50 $\mu\text{l}/\text{min}$, respectively. High resolution particle tracking velocimetry, achieved at 1 kHz frame rates will increase random error to 4% and 0.7% of the measurement, respectively. In SIV, pathlines are imaged in long exposure of 0.9 to 1.5 s, which is significantly longer than the characteristic diffusion time. For this reason, random error is averaged when the pathline is fitted to a polynomial and can be ignored.

Bias error becomes significant in curved channels where displacement vectors replace the curved motion (Fig. 4(b)).^{19,30} Displacement vector in the order of magnitude of the curvature radius will cause significant bias error, while smaller displacement vectors will lead to a finer division and a more accurate display of the curve (Fig. 4(a)). Smaller displacement vectors, and smaller bias error, can be achieved with higher frame rates or lower fluid velocities. Several approaches were taken to correct this bias. For

example, Scharnowski *et al.* estimated the curvature using the second-order shift vector and its gradient.¹⁹

One way to compare between velocimetry methods is to assess the ability of particle tracking velocimetry to match SIV spatial resolution. SIV can be used to calculate the velocity of every point on the mean polynomial. In our hands, a point spread function of 2 μm and velocity up to 0.15 m/s results in an effective time shift of 13.3 μs or an equivalent particle tracking velocimetry frame rate of 75 kHz. In common practice, high-resolution particle tracking velocimetry methods reach frame rates of only a few kHz.^{31,32} Therefore, SIV exhibits significantly higher resolution than common particle tracing velocimetry methods for fully developed laminar flow.

One uncertainty that does not occur in other particle tracing methods is SIV requirement for a boundary condition to convert relative to absolute velocities. In most microfluidic applications, exact knowledge of volumetric flow rate and channel geometry can be calculated to within a minor 1% error.

Finally, we note that all particle tracing methods, particle image and particle tracking velocimetries, and SIV are limited by particle behavior in fluid defined by the Stokes number (Stk) given by

$$Stk = \frac{\rho d^2 u}{18 \mu D}, \quad (6)$$

where ρ is particle density, d is particle diameter, u is fluid velocity, μ is fluid viscosity, and D is the obstacle length scale. For our system, Stokes ranges from 2×10^{-5} to 3×10^{-3} and particles follow streamlines closely. At Stokes numbers above 0.1 corresponding to velocities greater than

FIG. 3. Streamlines imaging in a microchannel with a porous element.²⁶ (a) Long exposure fluorescent image of 10 μm diameter fluorescent particles streaking across a 500 μm porous column. (b) Streamlines tracing using MATLAB for the published snapshot. Traced lines are highlighted in red. (c) Overview of the velocity field at 10 $\mu\text{l}/\text{min}$ presented as an arrow field. (d) Wall shear stress function derived by SIV as a function of angle around the porous column.

FIG. 4. Schematic representing bias error in particle tracing methods. (a) PTV accuracy increases as displacement vector shortens due to faster frame rates or slower fluid velocities, where n is the number of displacement vectors. (b) Real route and PTV derived route ratio as function of fluid velocity for a constant frame rate.

5 m/s, a 1% bias error occurs due to decrease in particle fidelity in all methods.³³ Stokes numbers greater than 1 are only relevant to particle image and particle tracking velocimetry, as flow is seldom laminar, and particles detach from flow especially in regions of sudden deceleration.

To conclude, as opposed to current velocimetry methods like particle tracking velocimetry, SIV resolution is not limited by frame rate and exposure time. Numerical and experimental validations show a high correlation between anticipated and measured results, with $R^2 > 0.91$. We report spatial resolution of $2\ \mu\text{m}$ in a flow rate of 0.15 m/s, resolution that can only be achieved with 75 kHz frame rate in traditional PTV. SIV is simple, quick, and can be used for a range of applications ranging from *in vivo* studies of hemodynamics to the design of complex microfluidic circuits.

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