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3D Imaging of Flow Patterns in an Internally-Pumped Microfluidic Device: Redox Magnetohydrodynamics and Electrochemically-Generated Density Gradients

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Abstract

Redox magnetohydrodynamics (MHD) is a promising technique for developing new electrochemical-based microfluidic flow devices with unique capabilities, such as easily switching flow direction, adjusting flow speeds and flow patterns as well as avoiding bubble formation. However, a detailed description of all the forces involved and predicting flow patterns in confined geometries is lacking. In addition to redox-MHD, density gradients caused by the redox reactions also play important roles. Flow in these devices with small fluid volumes has mainly been characterized by following microbead motion by optical microscopy either by particle tracking velocimetry (PTV) or by processing the microbead images by particle image velocimetry (PIV) software. This approach has limitations in spatial resolution and dimensionality. Here we use fluorescence correlation spectroscopy (FCS) to quantitatively and accurately measure flow speeds and patterns in the $\sim 5\text{--}50\text{ }\mu\text{m/s}$ range in redox-MHD-based microfluidic devices, from which 3D flow maps are obtained with a spatial resolution down to $2\text{ }\mu\text{m}$. The $2\text{ }\mu\text{m}$ spatial resolution flow speeds map revealed detailed flow profiles during redox-MHD in which the velocity increases linearly from above the electrode, and reaches a plateau across the center of the channel. By combining FCS and video-microscopy (with PTV and PIV processing approaches), we are able to quantify a vertical flow of $\sim 10\text{ }\mu\text{m/s}$ above the electrodes as a result of density gradients caused by the redox reactions and follow convection flow patterns. Overall, combining FCS, PIV and PTV analysis of redox-MHD is a powerful combination to more thoroughly characterize the underlying forces in these promising microfluidic devices.

Keywords

Microfluidics; Fluorescence Correlation Spectroscopy; Particle Image Velocimetry; Single Particle Tracking Velocimetry; Ionic Current Density; Microelectrode Array; Convection; redox-magnetohydrodynamics

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SUPPORTING INFORMATION AVAILABLE Experimental details, effects of time on measured current under constant voltage, separation between active electrodes on flow velocity, and electrode polarity of flow profiles, typical FCS curves, characterization of parameter error dependency on measurement time, PTV data showing negligible bead movement due to electrophoresis, errors in flow maps from FCS fitting, temporal fluctuations in flow velocities, and effect of electrode polarity on convection. Also, video clips of microbead movement during redox-MHD flow and convection caused by density gradients. This material is available free of charge via the internet at <http://pubs.acs.org>.

INTRODUCTION

Microfluidic devices are of interest for a range of analytical applications in chemistry, physics, and the life sciences and involve handling small volumes of fluids in confined spaces. While mechanical pumps are usually used for controlling the fluid flow, they can be quite expensive, difficult to miniaturize and, due to the required pressure, the coupling of the pump to the channel can be the least controllable aspect of the device.¹ These drawbacks of mechanical pumps generally complicate the engineering of microfluidic devices. The electroosmotic approach for directing fluid flow is growing in popularity and does not share these drawbacks, but the fluid velocity is sensitive to the physicochemical properties of the walls of the flow path and is most suitable for narrow cross sections (up to ~100 μm).^{2, 3} One novel way of achieving localized control of solution flow without moving parts, with fluid velocities similar to electroosmotic pumping (a few $\mu\text{m/s}$ to a few mm/s) but over larger volumes, and without the requirement for sidewalls is by magnetohydrodynamics (MHD).^{4, 5} MHD-based fluid flow is the result of the (body) force, $\mathbf{F}_B$, induced by the cross product of an ionic current density, $\mathbf{j}$, and a magnetic flux density, $\mathbf{B}$, and obeys the right-hand rule,⁶⁻⁸

$$\mathbf{F}_B = \mathbf{j} \times \mathbf{B}. \quad (1)$$

(See footnotes in ref ⁹ for choice of notation.)

Redox-MHD offers unique capabilities: It avoids bubble formation and electrode corrosion because the ionic current, $\mathbf{j}$, results from electroactive redox species added to the system that oxidize and reduce at the electrodes.¹⁰⁻¹² A variety of fluid compositions are compatible with electrochemistry, and thus pumping both aqueous and non-aqueous solutions is possible. Redox-MHD has the flexibility of creating flow patterns simply by activating different electrodes on the device in a programmable fashion.⁹ Thus, it can switch flow direction easily and has the capability to mix fluids under laminar flow conditions. Also, by controlling the current, it can finely tune fluid velocities to a few $\mu\text{m/s}$.¹³

However, quantitatively predicting flow velocities from equation 1 in a microfluidic system caused by redox-MHD is not an easy task. First, calculating the ionic current density in a particular region of space at a particular time, $\mathbf{j}(x,y,z,t)$, in an asymmetric electrochemical cell, based on the net current passing through the electrodes, is not trivial. Second, the geometry of the system, including wall placement relative to the location of active electrodes, greatly affects the flow pattern and velocity. Quantifying velocity in small volumes from picoliters to microliters with micrometer spatial resolution is needed to study the different forces that affect solution flow and mixing and to guide the design of new redox-magnetoconvective-based microfluidic devices.

An attractive method to evaluate fluid flow in such small systems can be accomplished by directly visualizing particles in the flow path using particle image velocimetry (PIV) or single particle tracking velocimetry (PTV) analysis. Guidelines for and the use of particle image velocimetry (PIV) to follow fluid flow have been reviewed.^{14, 15} The emphasis in PIV has been on two-dimensional visualization. Demonstration of PIV in two dimensions for very high spatial resolution (1 μm) in a microfluidic device was reported by Meinhart et al.,¹⁶ using only one observation window and 200 nm diameter particles, but more typical PIV measurements are limited to 1 μm particles and therefore poorer resolution (see ref ¹⁷ and references therein). There remains a strong need for three-dimensional imaging, especially for more complex microfluidic systems having asymmetry in terms of geometry or applied forces, such as in the redox-MHD device described herein. The advantages and

disadvantages for particle imaging methods for microfluidics in three dimensions have been recently reviewed by Cierpka and Kahler.¹⁷

In systems involving magnetoconvective effects in small volumes containing redox species, there are only a few reports of visualizing fluid flow using tracer particles or microbeads.^{9, 18-21} A promising approach is astigmatism PTV, which requires only one observation window, and a single camera.²² Visualization at a 30 μm spatial resolution was demonstrated, using 5 μm fluorescent beads in a 500 μm wide channel and conventional pumping. This method has been used to image magnetoconvective fluid flow in three dimensions, but in a cell of much larger dimensions, $10 \times 10 \times 20 \text{ mm}^3$, than is typical for a microfluidic device.²⁰ Higher resolution imaging in devices having smaller dimensions remains challenging. Koenig et al.¹⁹ describe a laser Doppler profile sensor that promises sub-micrometer spatial resolution with a long-working distance to assess microenvironments in larger volumes. However, velocity profiles in a magnetoconvective system were only demonstrated at 15 μm spatial resolution with 2 μm fluorescent particles, also in a relatively large volume of a 20 mm cuboid cell. Very low concentrations of particles were necessary so that only a single tracer particle was present in the measurement volume at a time. With this low data rate, time-resolved velocities (2.5 s) were possible only at a fixed position ($200 \mu\text{m} \pm 25 \mu\text{m}$ from the electrode) and required large numbers of replicates ($25 \times$). In that same paper (Koenig), PIV was used to validate the Doppler approach. Although in theory, these other approaches are capable of measuring fluid flow in smaller cells, the smallest cells involving magnetoconvection that have been studied thus far (350 μm deep cell⁹) have used the simple method of tracking individual 10 μm microbeads with transmission video microscopy with an estimated depth of correlation of about 20 μm and a lateral resolution of about 10 μm . Although such particle tracking is highly accurate, it is very time consuming, especially when overall fluid flow patterns in a single image need to be visualized, and it only provides two-dimensional, two-component velocity information (2D2C), and three-dimensional, two-component velocities (3D2C) through acquiring data by focusing at different heights. We have recently demonstrated that the videos can be processed with software used for PIV to visualize flow patterns in 2D more easily, although the spatial resolution decreases because of the need to follow groups of beads and the effect of following blurred bead movement above and below the focal plane.²³ (Note that from here on, we will use the term “PIV” to refer to the *processing* of optical microscopy videos of microbeads.)

Fluorescence correlation spectroscopy (FCS) offers both high sensitivity and spatial resolution,²⁴⁻²⁶ because it uses a diffraction-limited focal volume in all three dimensions and fluorescence detection. It has been shown that FCS can be used to obtain quantitative and accurate flow velocity maps in 3D in microfluidic devices.²⁷⁻³⁵ In most literature reports, fluid motion has been imaged within devices employing mechanical pumps with relatively high flow speeds of 50 $\mu\text{m/s}$ – 10 cm/s. Additionally, flow direction information can be obtained using dual-focus³⁰ FCS and fast imaging times can be realized using a spinning disk confocal microscopy setup,³⁵ although these instruments require more technical expertise or are more expensive than conventional FCS, which can be performed on any scanning confocal fluorescence microscope (SCFM). The optimization and limitations of FCS for measuring flow has been systematically studied for microfluidic devices with pumps using compressed N_2 or gravity for velocities between hundreds of $\mu\text{m/s}$ and hundreds of mm/s,³⁶ but such optimization has not yet been done for the slower flow velocities that are accessible by redox-MHD.

Here we used a SCFM and conventional FCS to image 3D flow patterns of a microelectrode array-based, redox-MHD microfluidic device, and obtained accurate flow velocities from 5-50 $\mu\text{m/s}$ and at 2 μm spatial resolution. We combined FCS and video optical microscopy/

PIV-processing approaches to both validate our data, and to obtain directional information. We verify that, when using bead sizes that are compatible with both FCS and PIV (1 μm), the same velocities are obtained, but that FCS also provides 3D information at higher spatial resolution and can extract the vertical component of velocity. The 2 μm spatial resolution flow map obtained by FCS revealed a flow profile between the active electrodes that increases rapidly and reaches a plateau across the center of the redox-MHD flow channel. Moreover, because the third, vertical component of the velocity can be extracted from the combination of FCS and PIV, we are also able to quantify flow due to convection caused by the electrochemically-generated density gradients in small sample volumes ($\sim 30 \mu\text{l}$) directly over the electrodes. Overall, this unique combination of electrochemical pumping and optical analytical techniques allows us to obtain low fluid flow velocities (down to a few $\mu\text{m/s}$) with novel flow profiles and to accurately quantify both flow speed and direction with high spatial resolution in 3D, all with relatively simple experimental apparatuses.

EXPERIMENTAL

Complete experimental details are provided in the supporting information. Briefly, FCS is acquired using a Microtime 200 microscope (Picoquant GmbH, Berlin, Germany). This system consists of a 3D piezo scanner (PI, Berlin, Germany) with sub-nm spatial resolution, 485 nm and 640 nm DPSS lasers (Picoquant), a UplanApo 60 $\times$ /1.3W objective (Olympus, Japan), a 100 μm pinhole and single photon avalanche diode (SPAD) detectors (MPI, Microphotonic devices, Bolzano, Italy).³⁷ 3D imaging is performed by taking fluorescence intensity traces as a function of time at multiple points in the X-Y plane at a given Z position, adjusting the Z position and repeating the trace collection until all the required X-Y-Z coordinates have been sampled. The schematic of the microfluidic setup as monitored by FCS is given in Figure S1. A user macro was written in SymPhoTime (Picoquant) to calculate and extract the FCS curves from each trace. Home-made software was written in Igor Pro (WaveMetrics, Inc, Oregon) to import the extracted FCS traces and fit each with a combined diffusion and flow model,³⁴ as follows

$$G(\tau) = G(0) \cdot \frac{1}{(1 + \tau/\tau_D) \left((1 + a^{-2} \frac{\tau}{\tau_D})^{1/2} \right)} \cdot \exp \left\{ - \left(\frac{(v_x^2 + v_y^2) \tau^2}{r_0^2} \right) \cdot \frac{1}{1 + \frac{\tau}{\tau_D}} \right\} \cdot \exp \left\{ - \left(\frac{v_z^2 \tau^2}{z_0^2} \right) \cdot \frac{1}{1 + a^{-2} \frac{\tau}{\tau_D}} \right\} + G(\infty) \quad (2)$$

where τ represents the correlation lag time, τ_D represents the bead diffusion time in the confocal laser focus, and v_x , v_y and v_z represent the flow speeds through the laser focus in the X, Y and Z axis. $a = z_0/r_0$ is the elongation of the detection volume in the Z axis relative to the X-Y axes, and is used to describe 3D diffusion. r_0 is the Gaussian laser focus waist in the X or Y axis, and z_0 is the Gaussian laser focus waist in the Z axis. $G(0)$ is the amplitude of the autocorrelation function at $\tau = 0$, which is proportional to $1/\langle N \rangle$ ($\langle N \rangle$ is the average number of beads in the laser focus), and $G(\infty)$ is the amplitude of the correlation function for the largest τ (chosen so that $G(\infty)$ is usually 0). In our experiments, redox-MHD or density gradient-generated flow is limited to the X-Y plane ($v_z = 0$) or along the Z axis ($v_x = 0$, $v_y = 0$), respectively. This simplifies fitting the data to equation (2), and is further discussed below and in the supporting information.

For PIV/PTV analysis, bead movement was observed with a Nikon Eclipse ME600P microscope equipped with a CF plan 10 $\times$ /0.30 objective, and videos were recorded using a Sony Handycam digital camera (model no. HDR-XR500V, 30 frames/s with 720 $\times$ 480 pixels/frame). The microscope was focused halfway between the microelectrode chip and coverslip and the viewing region was centered halfway between the active electrodes. DynamicStudio v.3.00 software (Dantec Dynamics, Copenhagen, Denmark) was used to obtain flow velocities. These were depicted graphically using PrestoVectors, a software

developed previously by us,²³ so that vector length and color are coded according to their speed.

RESULTS AND DISCUSSION

Properties of the Microfluidic Device

In this study, we use the term microfluidic device in its most general sense of having at least one dimension on the micron length scale (in this case, z). The height of the microfluidic cell used here is $\sim 200\ \mu\text{m}$, which is smaller than has been reported for redox-MHD devices. This allows us to take advantage of the high resolution 3D imaging capabilities to evaluate redox-MHD fluid flow in strongly-confined geometries, which holds promise for highly efficient, low-volume lab-on-a-chip applications, and yet is relatively unexplored. Figure 1 shows images of the microelectrode array chip used at various magnifications. Figure 1(a) shows the whole microelectrode array chip, where the individually-addressable contact pads are connected via leads to individual electrodes in the microelectrode array. Figure 1(b) is an optical image of the microelectrode array. Figures 1(c) and (d) were acquired at higher magnification using the SCFM by monitoring the reflection of a 640 nm laser (PDL 640, Picoquant). The 640 nm light is not absorbed by the fluorescent beads in the solution but is reflected strongly by the gold electrodes, and less so from the silica substrate, providing *in situ* (i.e. with the fluorescent beads already loaded) high resolution imaging of the electrodes. Based on these images, it is verified that the electrodes have widths of $\sim 40\ \mu\text{m}$ and center-to-center distances of $\sim 100\ \mu\text{m}$ between two adjacent electrodes, consistent with the design parameters for the microelectrode array chip. In our FCS setup, the electrodes run parallel to the Y axis and, thus, once the microelectrode array is imaged, we identified and numbered electrodes from left to right in the X axis by manually moving the microscope stage in $100\ \mu\text{m}$ intervals to setup the imaging coordinates.

A reinforcing flow configuration, where parallel microband electrodes are oppositely polarized was selected, as it has a flow profile that has been recently characterized.⁹ It also results in unidirectional flow to simplify the assignment of flow vectors. Furthermore, a two-electrode configuration was used, rather than using more distant large counter and reference electrodes on the chip,³⁸ to confine the electric field to a narrow, well-defined region in the microfluidic device. Finally, the current (at a constant applied voltage) as a function of time was determined (Figure S2). The current decreases rapidly within the first 20 s after applying the potential, and then reaches a relatively stable value; thus velocities were only determined after this initial 20 s settling time. It was also found that activating electrodes 1 and 4, which have a separation of $\sim 260\ \mu\text{m}$ provided the maximum flow velocity for this device, as determined by video microscopy and PIV analysis (Figure S3). A trend of increased speed with electrode separation, followed by a subsequent decrease with increased spacing was observed and is consistent with previous studies,^{9, 38} although the exact maximum of $260\ \mu\text{m}$ separation observed is less than previous studies, a consequence of the smaller device dimensions used here. All our subsequent experiments are carried out using electrodes 1 and 4 as the active electrodes.

Optimization of FCS Parameters

The major goals of this research are to (1) demonstrate that a combined FCS and PIV analysis can be used to simply and accurately quantify flow patterns in a novel electrochemically-pumped (redox-MHD) microfluidic device, (2) evaluate the limits of velocity sensitivity for such analyses, and (3) extend the quantification to three dimensional convective forces resulting from electrochemically-generated density gradients above the electrodes. In order to accomplish these goals, it is necessary to optimize the conditions to ensure comparisons between the analytical methods are valid, and that the effects of

experimental variables are thoroughly understood. While the optimization of FCS parameters have been previously reported for diffusing species³⁹ and pressure-pumped microfluidic devices with relatively fast flow velocities,³⁶ it has not been studied for redox-MHD-based microfluidic devices, and particularly for flow velocities below $\sim 100 \mu\text{m/s}$.

FCS analysis requires knowledge of the effective illumination width, r_0 , and height, z_0 , for each fluorescent species. To determine these parameters, we immobilized two different sized beads ($0.1 \mu\text{m}$ and $1 \mu\text{m}$) on a glass coverslip at high dilution and imaged them (Figure 2). As expected, the $0.1 \mu\text{m}$ beads appeared as diffraction-limited spots with $r_0 = 0.32 \mu\text{m}$ – the width of the beam profile at $1/e^2$ of the maximum intensity. For the $1 \mu\text{m}$ beads, r_0 is $0.90 \mu\text{m}$. That is to say that, although the diffraction-limited confocal laser focus is only $\sim 0.3 \mu\text{m}$ wide, the $1 \mu\text{m}$ bead will be illuminated for the whole $0.9 \mu\text{m}$ distance that it is excited by the laser. This is an important parameter in order to extract the flow velocity from equation 2. In order to calculate 3D diffusion, and to perform vertical flow analysis, we also need to know the waist of the beam in the Z axis. An X-Z scan of the immobilized beads was acquired and a value of $z_0 = 1.2 \mu\text{m}$ for $0.1 \mu\text{m}$ beads and $2.9 \mu\text{m}$ for $1 \mu\text{m}$ beads was found; the larger z_0 for the $1 \mu\text{m}$ beads is expected due to the convolution of the bead with the similarly-sized Gaussian illumination beam in the Z axis.

For $0.1 \mu\text{m}$ beads in the absence of an applied voltage, the obtained FCS curves can be fit to a diffusion-only model with x , y and $z=0$ (Figure S5, red curve). With $0.1 \mu\text{m}$ beads in the presence of an applied voltage and magnet, the beads can both diffuse and flow by redox-MHD (Figure S5, blue curve). $1 \mu\text{m}$ beads are too large to show Brownian diffusion on these timescales and thus show no autocorrelation function in the absence of an applied voltage. In the presence of an applied voltage, they show only flow (i.e. $D \approx$ in equation 2) (Figure S5, green curve). The fact that the FCS curves show excellent fits to this equation supports our expectation that redox-MHD based solution flow is laminar along the microelectrode direction perpendicular to the optical axis, and will be further supported with particle tracking methods below.

It is well known that collection statistics for FCS influence the signal:noise ratio; the noise in the FCS curves (Figure S5) decreases as the square root of the collection time, but also depends on other experimental parameters such as binning time and fluorophore concentration and brightness.^{36, 39} The duration of each FCS point collection time multiplied by how many points are collected determines both the total collection time and the error in the recovered diffusion and flow parameters. It is important to balance the experimental time with obtaining enough statistics for accurate recovery of the flow and/or diffusion, and it is not immediately obvious what this balance should be. There are two origins of error that need to be quantified; relative standard deviation between multiple measurements, and the error from the least-squares fitting of the collected FCS curve to equation 2. Figures S6 and S7 characterize the relative standard deviation (RSD) and the fitting errors associated with taking multiple measurements as a function of measurement time, when both flow speeds and diffusion coefficients (D) of $0.1 \mu\text{m}$ beads are considered as variables and allow to be fitted during the FCS curve fitting. For any given measurement time, the RSD is higher than the fitting error, although both increase sharply as measurement time decreases below 30 s. RSDs do not drop much below 10-20% RSD even for long (>100 s) measurement times, while the fitting error is already $<3\%$ at 30 s, and continues to drop as measurement time increases. Based on the results of Figures S6 and S7, we choose a 30 s collection time per point and took $30 \mu\text{m}$ steps for a wide field of view and $2 \mu\text{m}$ steps for higher spatial resolution imaging with a smaller field of view. In principle, one can collect wide fields of view with high spatial resolution and longer trace length, but the experimental time would increase accordingly. The FCS imaging process can be automated to achieve this goal, if required.

In principle, one can either fix the diffusion coefficient, D , and fit only for the flow speed, or simultaneously fit for both parameters in equation (2). We performed both types of analysis and found that the recovered velocity converges less and the fitting error is larger when D is fixed rather than fitted (Figure S8). The reason for this is that there is an inherent error in the recovered D values, due to the limited statistics (Figure S9), which is compounded to the fitting error in the velocity when it is fixed to an average value. We also confirmed that bead movement between the active electrodes 1 and 4 (in the x -direction) due to electrophoresis is negligible (Figure S10), and therefore the recovered beads flow speeds from FCS and PIV represents flow resulting from redox-MHD.

3D Imaging of Redox-MHD Flow

Once the microfluidic device, microscope and experimental parameters were characterized, we obtained 3D flow maps of the redox-MHD flow in our highly confined geometries. We plot the 3D flow velocity and diffusion coefficient maps from the $0.1\ \mu\text{m}$ beads in solution in Figure 3. The flow velocity varied in all three dimensions, but was not parabolic. The highest velocity was recorded closer to the electrode surface than the cover slip surface (the $60\ \mu\text{m}$ plane of a $180\ \mu\text{m}$ thick channel) and was $\sim 50\text{--}60\ \mu\text{m/s}$, decreasing to $0\text{--}10\ \mu\text{m/s}$ at the surfaces and above the active electrodes (labeled as 1 and 4 above the maps). In the central Z plane ($90\ \mu\text{m}$), the flow profile was somewhat flatter, which was supported with higher spatial resolution imaging (as will be shown below). Extracting this flow profile asymmetry in the Z axis highlights the importance of performing 3D imaging of microfluidic cells, which is easily realized using FCS. The diffusion coefficient was $2\text{--}5\ \mu\text{m}^2/\text{s}$ and showed minimal variation across the device, as would be expected, and is in agreement with that expected from the Stokes Einstein relationship for $0.1\ \mu\text{m}$ spherical particles ($\sim 3\ \mu\text{m}^2/\text{s}$). In the supporting information, we also provide the fitting errors in both velocity and diffusion coefficient associated with each pixel (Figure S11). The error in the diffusion coefficient is relatively homogeneous across the channel, remaining at $10\text{--}20\%$. The error in the fitted velocity remains at $10\text{--}20\%$ until the velocity drops below $\sim 5\ \mu\text{m/s}$.

$0.1\ \mu\text{m}$ beads are not visible in transmission mode for PIV or PTV analysis. Therefore, in order to compare the FCS results with these more common techniques, we obtained a 3D flow velocity map using $1\ \mu\text{m}$ beads. Since $1\ \mu\text{m}$ beads show negligible diffusion (based on the Stokes-Einstein equation, we estimated $1\ \mu\text{m}$ beads to have $D \sim 0.3\ \mu\text{m}^2/\text{s}$), it also allows us to probe the dependency between fitted diffusion coefficients and flow velocities. The flow velocity and error maps are plotted in Figure S12, showing a very similar flow map as for $0.1\ \mu\text{m}$ beads (Figure 3), including the asymmetry in the Z axis, but highlighting negligible dependency on the fitted parameters.

For both flow maps, there are a few discontinuous pixels. A number of sources may account for this, including bead heterogeneity (both in size and brightness), data collection statistical fluctuations caused by the limited FCS trace collection time and the low bead concentration (this effect is much more obvious for $1\ \mu\text{m}$ beads since they have lower concentration and stronger scattering), as well as possible fluctuations in flow velocities resulting from variations in the rates of oxidation and reduction of the electrolyte species. This latter effect has been quantified in the supporting information (Figure S13). From Figure S13, the histograms for fitted flow velocities over time are not symmetric around the average value, showing skew towards lower velocities. This asymmetry may be indicative of fluctuations in the flow caused by temporal reduction in the redox reaction rates, but requires further study to quantify in more detail. However, this result does suggest that it may be one source of the discontinuity in the pixels obtained in the 3D flow maps.

High Spatial Resolution Imaging of Redox-MHD Flow by FCS

To highlight the information that can be gleaned from a high spatial resolution imaging approach, as can be obtained from FCS, a $2\text{ }\mu\text{m}$ spatial resolution map of a $20\text{ }\mu\text{m} \times 160\text{ }\mu\text{m}$ region is imaged in 2D (Figure 4). Again, the diffusion coefficient of beads in each pixel is provided in the supporting information (Figure S14). This $2\text{ }\mu\text{m}$ resolution map shows a more detailed flow profile from the central z plane ($90\text{ }\mu\text{m}$), which increases in a linear fashion from $0\text{--}100\text{ }\mu\text{m}$ (0 being above the active electrode) and then reaches a plateau from $100\text{--}160\text{ }\mu\text{m}$ (the left half of the channel). The level of detail in the flow profile over the X axis is not as clearly observable in the lower resolution flow maps (with spatial resolution of $\sim 20\text{--}30\text{ }\mu\text{m}$), and highlights the importance of high resolution imaging in revealing the detailed flow patterns in redox-MHD based microfluidic flow cell. To the best of our knowledge, this is the first time that such high resolution imaging in such a strongly confined redox-MHD microfluidic cell has been obtained, which has been realized by the application of FCS. It is interesting that a relatively flat flow profile in the center of the channel can be obtained in such confined geometries, and may be promising for a range of applications in the bioanalytical and separation sciences.

Comparison of Redox-MHD Fluid Flow Obtained by FCS and PTV/PIV

A major reason to image the $1\text{ }\mu\text{m}$ beads by FCS is that, unlike $0.1\text{ }\mu\text{m}$ beads, this size is also visible in transmission mode by wide-field video microscopy and can be tracked. Bead tracking with video microscopy is usually performed with larger beads in the range of $5\text{--}10\text{ }\mu\text{m}$ for easier visualization,^{9, 38} but $1\text{ }\mu\text{m}$ beads can be analyzed in this way. To compare, we imaged both $1\text{ }\mu\text{m}$ and $6\text{ }\mu\text{m}$ beads using video microscopy, and performed two types of particle tracking analysis (PIV and PTV). Figure 5 shows the PTV and PIV images and analysis from the redox-MHD flow using $6\text{ }\mu\text{m}$ and $1\text{ }\mu\text{m}$ beads. As shown in Figure 5, PIV provides a 2D flow velocity map of a large $\sim 1\text{ mm}^2$ area in a single X-Y plane, and with a spatial resolution of $\sim 60\text{ }\mu\text{m}$. The flow velocity maps show that the flow velocities in the center of the channel for both $6\text{ }\mu\text{m}$ and $1\text{ }\mu\text{m}$ beads are $\sim 50\text{--}60\text{ }\mu\text{m/s}$, consistent with the velocities in the center of the channel obtained in the FCS flow maps (Figure 3). We also observe a slight dependence on bead size, just as we did between the $0.1\text{ }\mu\text{m}$ and $1\text{ }\mu\text{m}$ beads in FCS. The flow velocity decreases across the X axis from a maximum in the center of the channel towards electrodes 1 and 4 (the active electrodes). Particle tracking velocimetry (PTV) verifies that the velocity of the $6\text{ }\mu\text{m}$ beads gives consistent velocities as those obtained by PIV (Figure 5(b)) when the same sized bead is used.

In order to quantitatively compare the data from each method, we used the $60\text{ }\mu\text{m}$ and $90\text{ }\mu\text{m}$ Z axis layers from the 3D flow maps obtained from FCS because they correspond to the same imaging region in which the PIV and PTV data were collected. As can be seen from Table 1, the comparison of flow velocities for the $1\text{ }\mu\text{m}$ beads analyzed by FCS and PIV shows extremely highly consistent results. However, a consistent size dependence on the recovered flow velocities is present for all methods. Surprisingly, the larger beads seem to consistently recover faster velocities, contrary to intuition. It is not yet clear to us why we should see this effect, and plan to investigate it further. One possibility is that the $6\text{ }\mu\text{m}$ beads are sulfonated while the $1\text{ }\mu\text{m}$ and $0.1\text{ }\mu\text{m}$ beads are carboxylated, but the fact that the size trend is also present between $1\text{ }\mu\text{m}$ and $0.1\text{ }\mu\text{m}$ beads suggest other factors are involved. Another possibility is that the negatively charged groups of the beads (sulfonate or carboxylate) interacts with the flowing electrolytes, leading to reduced friction between the flowing K^+ ions and the negatively charged beads.

Overall, particle tracking analyses using PIV and PTV have the advantage of visualizing the solution flow in a larger area ($1\text{ mm} \times 1\text{ mm}$ scale) in real time as well as providing flow direction information, but only offers spatial resolution of $\sim 50\text{--}150\text{ }\mu\text{m}$ and in only two

dimensions. FCS has the advantage of high spatial resolution in all three dimensions, although it does not provide directional information without using a more complex setup, such as dual focus.³⁰ Combining these approaches is a powerful combination to provide a more complete characterization of flow patterns in such microfluidic devices.

Electrochemically Generated Density Gradients Measured with FCS

There is another force involved in redox-MHD flow, which is the convective force caused by density gradients. The density gradients are electrochemically generated during the redox reaction of Fe(CN)_6^{3-} to Fe(CN)_6^{4-} at the cathode and Fe(CN)_6^{4-} to Fe(CN)_6^{3-} at the anode. Due to the slight density difference between Fe(CN)_6^{3-} and Fe(CN)_6^{4-} , and the movement of the additional K^+ counter ions needed to balance the accumulated, reduced Fe(CN)_6^{4-} at the cathode or the movement away of the extra K^+ from the oxidized Fe(CN)_6^{3-} at the anode will cause convection in the microfluidic flow cell. In order to follow this process, we performed the experiments under an applied voltage, but without the magnet, and thus redox-MHD will not occur. PIV and/or PTV analysis can be used to follow the horizontal flow (two-dimensional and two-component velocity), while FCS can be used to quantify both horizontal and vertical flow (three-dimensional and three-component velocity), based on equation (2). Figure 6 shows the PIV imaging data for the horizontal flow caused by the density gradients. A horizontal flow velocity of $\sim 10 \mu\text{m/s}$ is observed close to the electrodes, both near the chip and coverslip surfaces. The flow rapidly decreases as a function of distance from the active electrode. The inverse flow direction near the top and bottom surfaces is strongly indicative of convective flow in the microfluidic flow cell. Using PIV, we are not able to see vertical flow, since the beads move out of focus and become difficult to track. Here, we take advantage of the unique ability of FCS to extract the vertical component of fluid velocity. Figures 7 and S15 shows the corresponding flow measurements caused by the density gradients in the FCS microscope with different electrode polarity configurations. The flow directions in Figures 7 and S15 are inferred from the PIV measurements in Figure 6 (and the right hand rule). The recovered horizontal flow speeds near the surfaces are $\sim 5\text{--}10 \mu\text{m/s}$, which is in good agreement with the PIV experimental results. In the case of vertical flow, flow velocities of $\sim 10 \mu\text{m/s}$ are obtained by FCS curve fitting. We verified that there is no horizontal flow component for electrochemically-generated density gradients directly above the active electrodes (Figure S16). However, with such small velocities, the RSDs are comparable to the average value, and quantifying convective forces requires more in depth study, which we are currently undertaking.

The recovered flow speeds for the center of the flow cell by FCS is $\sim 0 \mu\text{m/s}$, indicating that the solution is not moving (or is moving slower than $\sim 5 \mu\text{m/s}$) at this point. This experimental result, together with the horizontal flow speeds measured near the surfaces (by both PIV and FCS) strongly suggests convection in the microfluidic cell caused by density gradients, even in such confined geometries and small electrode sizes as used here. Because the density gradient flow depends on current, it should exist during redox-MHD flow and may affect the flow velocity close to the surface and above the active electrodes. As shown in Figures 7 and S15, the flow velocities caused by density gradients are relatively small compared to the redox-MHD flow, although it certainly is not negligible over the active electrodes. However, it appears to not perturb flow in the center of the cell. Convective forces close to the electrode and coverslip may be one source of the asymmetric flow profile in the Z axis (Figure 3), but more work is needed to evaluate this possibility, and we have shown that FCS is a powerful tool for such a study.

SUMMARY AND CONCLUSIONS

Redox-MHD microfluidics can presumably be easily programmed toward different flow geometries using electronics rather than pre-specified microfabrication parameters. Consequently, it is anticipated that devices based on this phenomenon should be much easier to fabricate and miniaturize for lab-on-a-chip applications. For their maximum potential to be realized, the various forces involved must be thoroughly characterized and understood in all three dimensions. We have shown that FCS can be used to obtain quantitative and accurate 3D flow maps with 5-50 $\mu\text{m/s}$ velocities and with up to 2 μm spatial resolution to accurately quantify flow profiles in strongly confined geometries. The combination of FCS and video microscopy with PIV/PTV analysis offers us the combined advantages of three-dimensional and three-component velocity imaging, high spatial resolution, and wide-field imaging in real time. We have uncovered interesting 3D flow profiles underlying both redox-MHD and convection caused by electrochemically-generated density gradients in confined geometries. We are confident that this type of analysis will lead to improvements in designing and optimizing redox-MHD devices for a wide range of analytical and biological applications, and include manipulating fluids for multi-step assays, controlling solution mixing, and playing a role in biological, pharmaceutical and chemical separations, all with small sample volumes and low waste.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Image of complete microelectrode chip. (b) 10 $\times$ magnification of microelectrode array region of chip. (c) and (d) 60 $\times$ magnification of a single electrode and the gap between 2 electrodes obtained using reflected 640 nm laser light with a total imaging area of 80 $\mu\text{m} \times 80 \mu\text{m}$. The edge-edge distance for the two neighbor electrodes are $\sim 60 \mu\text{m}$ and the electrode width is $\sim 40 \mu\text{m}$. The cathode and anode are labeled with – and +, respectively. In order to minimize the depletion of redox species at the electrodes, current switching is used during 3D FCS flow speed imaging, which changes the polarity of the electrodes alternatively after each z slice (Figure S4). The direction of the magnetic field is also given.

Figure 2.

Fluorescence intensity images of (a, b) 0.1 μm and (c, d) 1 μm immobilized beads. (a) and (c) are x-y scans, and (b) and (d) are x-z scans showing the 3D point spread functions (PSF). The r_0 values to be used in equation 2 are 0.32 μm and 0.9 μm for 0.1 μm beads and 1 μm beads respectively. The z_0 values are 1.2 μm and 2.9 μm for 0.1 μm beads and 1 μm beads respectively.

Figure 3.

3D maps for flow speeds (a) and diffusion coefficient (b) obtained from 0.1 μm beads in redox-MHD microfluidic flow cell. The spatial resolution in the x-y plane is 20 μm and is 30 μm in z. The relative position of the microelectrodes is also shown above the high resolution maps. Black colored electrodes are the active electrodes, and gray are the non-active electrodes. The thickness of the microfluidic cell is $\sim 180 \mu\text{m}$.

Figure 4.

(a) High-resolution ($2\ \mu\text{m}$ in x and $5\ \mu\text{m}$ in y) imaging of redox-MHD flow for a $160\ \mu\text{m} \times 20\ \mu\text{m}$ area in the x - y , acquired at the center plane of the channel in z . The position of the active electrode 1 and non-active electrode 2 are also shown for comparison to the lower resolution, wider field images in Figure 3. (b) Flow speeds fitting error map from (a). (c) Flow profile as a function of horizontal distance from the active electrode 1. The dependence linearly increases between 0-100 μm , and reaches a plateau between 100-160 μm .

Figure 5.

Video microscopy images showing 2D redox-MHD flow imaged using (a) PTV with 6 μm beads and PIV with (b) 6 μm beads and (c) 1 μm beads. PTV shows the trajectory of 4 individual beads, while PIV shows the average velocity of beads within a 50 μm region, plotted as color coded vectors. The cathode and anode are labeled with – and +, respectively. The other electrodes are inactive.

Figure 6.
PIV images showing 2D density gradient flow acquired with current but without magnet.
The color bar shows the bead speed in $\mu\text{m/s}$.

Figure 7.

FCS curves showing 3D density gradient flow between the active electrodes 1 and 4 acquired with current but no magnet. The arrangements of the five FCS curves represent where the FCS curves were collected. The flow direction is inferred from the right hand rule and measured using PIV as shown in Figure 6.

Table 1

Summary of average flow speeds in center of channel determined using different methods.

Method	Bead size / flow speed		
	6 μm	1 μm	0.1 μm
Bead Tracking	62 $\pm$ 2 $\mu\text{m/s}$	N/A	N/A
PIV	58 $\pm$ 4 $\mu\text{m/s}$	52 $\pm$ 1 $\mu\text{m/s}$	N/A
FCS	N/A	52 $\pm$ 3 $\mu\text{m/s}$	44 $\pm$ 6 $\mu\text{m/s}$