Published in final edited form as:

Lab Chip. 2010 October 7; 10(19): 2614–2617. doi:10.1039/c004766j.

Visualization and Measurement of Flow in Two-Dimensional Paper Networks

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Abstract

The two-dimensional paper network (2DPN) is a versatile new microfluidic format for performing complex chemical processes. For chemical detection, for example, 2DPNs have the potential to exceed the capabilities and performance of existing paper-based lateral flow devices at a comparable cost and ease of use. To design such 2DPNs, it is necessary to predict 2D flow patterns and velocities within them, but because of the scattering of the paper matrix, conventional particle imaging velocimetry is not practical. In this note, we demonstrate two methods for visualization of flow in 2DPNs that are inexpensive, easy to implement, and quantifiable.

Introduction

Lately there has been an upsurge in interest in the use of capillary flow-based devices for performing biomedical diagnostic tests. Such devices have the potential to perform many of the sophisticated processes that have been demonstrated for microfluidic systems, but with far less complexity or cost. Some of these new devices have been based on simple two-dimensional structures^{1,6}, and some on more complex three-dimensional structures^{7,8}. Our laboratory is focusing on minimizing the cost of medical diagnostic tools, so simplifying manufacturing is of supreme importance. We are, therefore, developing the simplest possible two-dimensional systems, or two-dimensional paper[‡] networks (2DPNs); one potential use for them is to produce immunoassay devices that transcend the performance of traditional lateral flow strips⁹⁻¹¹. Specifically, we have been demonstrating the potential of 2DPNs with multiple inlets to perform sequential delivery of multiple reagents to a common detection zone, and thus perform sophisticated processes that are not possible in either conventional lateral flow strips or the paper structures presented in previous work by other groups.

To reliably design such multi-inlet paper networks, there must be validated methods of predicting flow velocities and patterns in 2DPNs. This is especially important where multi-step processes require accurate and precise control of multiple reagent delivery. Numerical modeling of transport in 2DPNs¹¹ is critical, but models must be validated, so we have developed methods for visualizing flow in 2DPNs to allow such validation. Conventional particle-imaging velocimetry (PIV) is not useful in 2DPNs because: 1) the paper matrix

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‡Note that we use a broad definition of the term “paper” that includes related porous materials.

interferes with movement of particles, and 2) the scattering of the matrix makes the visualization of most simple particles impossible. In this article, we demonstrate two complementary optical methods for flow visualization that are easy to implement with very low-cost equipment, and could provide information equivalent to that provided by PIV. The methods involve changing the optical characteristics of a small volume of fluid within the 2DPN, allowing that “marked” volume to be imaged as it moves through the device. These methods can be useful in all stages of the development of paper-based devices (whether 1D, 2D or 3D), including materials characterization, device design, and device characterization.

Experimental

Schematics of the two marking methods are shown in Figure 1. The paper devices used were composed of backed nitrocellulose (Millipore, Billerica, MA) cut by a CO₂ laser (Universal Laser Systems, Scottsdale, AZ)¹². Paper devices were taped down to glass or PMMA surfaces using double-sided tape (3M, St. Paul, MN). Absorbent material (Millipore, Billerica, MA, Whatman, Maidstone, Kent) was used for the source and wicking pads. For each of the methods, marking solution was introduced to the source pad of a device using a pipette. Sequences of images of the devices were recorded using a simple webcam (Logitech Webcam Pro 9000). A time-lapse recording program (HandyAvi, Azcendant Software, Tempe, AZ) was used to record webcam images. Velocity measurements were made from the displacement of the tracer species *versus* time using individual frames from the time-lapse data.

For the fluorescence-based marking method, a caged fluorophore solution, 3 mM CMNB caged fluorescein (F-7103, Invitrogen, Carlsbad, CA) in 0.1M Na₂HBO₄ (pH 9.1) was used. Caged fluorophores are compounds that greatly increase in fluorescence quantum efficiency after a photolysis reaction. A 25 watt-second commercial photo flash unit was used as a pulsed (~1 msec) 360 nm light source for the photolysis. The photo flash unit was masked with aluminum shim stock to define the region of illumination, thereby producing a narrow band, 0.3 mm wide, of uncaged fluorescein for each pulse. The masked photo-flash unit was held close (<0.5mm) to the paper and manually flashed with a switch on the unit. For the fluorescence measurements, the webcam was fitted with a long-pass filter (FEL0550, Thorlabs, Newton, NJ), and the paper was illuminated with blue LEDs (889-B42182, Mouser Electronics Mansfield, TX) to excite the fluorescein produced by the photolysis reaction. The LED can be filtered with an optional shortpass filter (FES0500 from Thorlabs) for improved contrast. After marking, the paper network was placed under the excitation LEDs, additional caged fluorophore solution was added to the source pad, and flow of the uncaged fluorophore band was recorded with the camera.

For the electrochemical marking method, a pH sensitive dye solution of 1 mg/ml phenol red in pH 7.4 phosphate buffered saline (PBS) was used. The wire electrodes were made from single-stranded #30 silver-plated wire with Kynar™ insulation stripped back to allow contact with the wetted paper. The wire electrodes were placed on the paper strip next to the source pad and were held in contact with the paper using tape or with a transparent (PMMA) support that was laid on the source pad. The positive electrode was sandwiched between the paper and the source pad. Note that the pressure of the wires on the paper must be sufficient

to make electrical contact, but not so strong as to crush the matrix. The electrodes were driven either with a computer-controlled 5 volt power supply to control pulse width and electrode polarity, or with manual switches connected to a 9V battery or supply. The marking electrode in the case of phenol red dye was the negative electrode, resulting in a local increase of pH ($2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^-$) and a change of dye color from yellow to dark red. The pKa of phenol red is 8.0, so the color change occurs over a range of 6.8 to 8.2. The pH of the marked band was measured at between 10 to 11 using pH sensitive paper (Micro Essential Laboratory, Brooklyn, NY). To the marked zones, shorter reverse polarity (5 volt positive) pulses were placed on either side of a longer marking (negative) pulse, thus 'bracketing' the high pH region with lower pH zones. The paper networks were illuminated with a white LED array (AASL4805QR424S/5-N2, Kingbright, City of Industry, CA), and the camera was unfiltered. The program ImageJ¹³ was used to make measurements of the position of the bands as a function of time. Position measurements were made at the front of the bands.

Results and Discussion

A demonstration of the fluorescence-based marking method is shown in Figure 2. The uncaged fluorophore can be seen as a band moving upward in the sequential images. The plot of Figure 2 shows that the fluorophore moves at a constant velocity in a fully wetted constant-width strip. For the lengthscales investigated, a series of 3 flashes produced a well-defined band for flow velocity measurement. This fluorescence-based method is minimally invasive (i.e. there is no physical contact with the paper strip) and the location(s) and size(s) of the marking(s) can be varied by fabrication of an appropriately-patterned mask. Alternatively, spot marking (~1 mm in diameter) can be accomplished by using a 265 nm UV LED (data not shown). Alternate tracer species are available, with some that uncage at longer wavelengths. Though this method requires specialized lighting/filtering to visualize the fluorescent bands, the cost of a detection system can be low; the home-built fluorescence set-up demonstrated here cost approximately \$200.

A demonstration of the electrochemical marking method is shown in Figure 3. In the image, 10 sec negative pulses to the "marking" electrode were spaced two minutes apart. The marking pulse was bracketed by positive pulses 1 sec in duration. Timing accuracy is dependent on computer clock precision, and is usually better than 10 ppm, independent of the pulse width. For the strip of varying width on the right in Figure 3, note the expected reduction in the spacing of the bands that indicate the slowing linear velocity as the strip increases in width¹¹. Comparison between the velocities in the narrow segments of the two different strips indicates that the speed is greater in the strip on the right compared to on the left, and is consistent with an analysis of flow in these simple geometries¹¹. Also note the apparent reduction of the flow rate at the edges of the paper, even in the narrow segment. This phenomenon is often observed, and it may be attributable to localized effects of laser cutting (data not shown). The plot of Figure 3 shows distance *versus* time for a series of 3 consecutive pulses in the constant-width strip. The gradual slowing of sequential pulses is likely due to the depletion of fluid in the source and filling of the wicking pad. Thus, a marking method is an ideal tool to troubleshoot fabrication and system set-up, as well as to investigate transport in different geometry 2DPNs.

Figure 4 shows a series of images of flow in a simple 3-inlet 2DPN. In a previous report¹⁰, the sequential delivery of reagents was demonstrated in a similar device. Current pulses approximately 3 seconds in duration were generated simultaneously in the inlet legs. Note that, because of differential resistances between the three source pads and the single wicking pad, transport of the tracer species differs substantially between the 3 inlets. Thus, a marking method is an ideal tool to investigate the timescale and patterns of flow for device optimization.

For the electrochemical marking method, the cost of the detection system is approximately \$100, with the majority of the cost in the webcam. Spot marking is also possible using this method by restricting the area of marking electrode (e.g., we have generated small spots by contacting only the tip of the wire to the paper). Alternate tracer species include other pH indicator dyes as well as molecules capable of redox reactions. We tested dimethyl benzidine (o-tolidin) and found that it worked well, but required a very low pH (<1), and faded more quickly than the pH dye method demonstrated above (data not shown). A potential complication would occur if the tracer species had a nonzero affinity for the stationary phase (i.e. there is chromatography occurring with an R_f value less than 1.0). That is not the case here; phenol red (at both low and high pH) and the uncaged fluorophore move with the wettable fronts (data not shown).

The two methods shown here have their strengths and weaknesses. Any marking band formed in this way will broaden over time by diffusion of the observed chemical species, although this could be lessened by using a marking dye with a low diffusion coefficient. Observation of a dark spot on a light background (or vice versa) using a camera and white light is clearly simpler than using fluorescent markers. However, localized pH changes are transient, and will eventually revert to something close to the initial buffer pH through buffering by the paper or by in-diffusion from the fluid in front of or behind it the marker band—the smaller the band, the faster this will happen. While the uncaged fluorophores are inherently stable (except against photobleaching), the bands formed by them will still broaden over time by diffusion. The equipment needed to uncage them is somewhat more complex, and the dyes used are also more expensive.

These methods can also be compared to that used by Mendez et. al.⁵, in which membranes were alternately dipped into dyed fluid and water by hand to visualize flow patterns and measure velocity. The two methods described here provide several advantages over their manual dye dipping method. First, the current methods require no handling of the membrane during marking. Second, variation of the optical mask or the electrode geometry allows for the creation of marks in alternate shapes, such as dots or streaks, in a two dimensional pattern that can aid the tracking of flow in complex 2DPNs. And third, in the case of the electrochemical marking method, the automation of voltage pulse timing results in the creation of accurately-timed marks with highly precise widths.

Conclusion

The two methods enable the visualization and measurement of flow in paper matrices—a capability that is useful for all stages of development of paper-based devices. The methods

can be used to investigate transport during wet-out, as well as during fully-wetted flow in both simple paper strips and in 2DPNs, thus enhancing our understanding of transport in these devices. The application to 2DPNs enables a host of engineering processes ranging from design optimization to quality control during manufacturing. In addition, the methods have great utility for the characterization of the materials (e.g. different papers and absorbent pads) relevant to paper device fabrication. Both methods are inexpensive and easy to implement, and so should be accessible to researchers, device developers, and educators. The pH marking method has the simplest implementation, but does involve a pH change of the fluid, which could be incompatible with some running solutions. The caged fluorophore technique, while more complex, is compatible with a wide range of solutions that might contain pH-sensitive molecules. Future work from this group will 1) optimize the methods with respect to choice of buffers to enhance the imaging methods, and 2) utilize the methods for detailed quantitative mapping of flow across devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We gratefully acknowledge the support of NIH ARRA Challenge Grant No. 1RC1EB010593 and funds from the University of Washington.

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Figure 1. Schematics of the two methods for visualization and measurement of flow. Fluorescence-based marking method (top) uses as a tracer species, an initially caged fluorophore species that is uncaged and fluoresces when exposed to 355 nm light. Set-up for the electrochemical marking method (bottom) in which a high pH zone is electrochemically generated in a lower pH background such that a pH indicator dye acts as a tracer species.

Figure 2.

Demonstration of flow visualization and measurement using the fluorescence-based marking method. A series of images of the transport of a caged fluorophore is shown in the top panel. The images correspond to the start of data acquisition, 10 s, 30 s, and 60 s, from left to right, respectively. A plot of the distance traveled by the uncaged fluorophore *versus* time shows the constant velocity of the fluorophore, 0.17 mm/s (with a standard error of 0.002 mm/s).

Figure 3.

Demonstration of flow visualization and measurement using the electrochemical marking method. In the image, the time between pulses is two minutes, and the pulse width is 10 seconds bracketed on either side with 1 second reverse polarity pulses. The plot shows distance *versus* time, for the three sequential pulses in the constant width strip in the image.

Figure 4.

Visualization of the flow patterns in a simple 3 inlet 2DPN. In this case, pulses were generated manually, approximately 1 sec in duration. Images were acquired at 6, 12, 24, 44, and 116 s after t_1 for the images taken at t_2 through t_6 respectively. Note the very different timescales of transport of the tracer species in the 3 inlets of the device.