Volumetric measurements by image segmentation on centrifugal microfluidic platforms in motion†

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An image segmentation based method was developed to perform volumetric measurements of liquid aliquots in centrifugal microfluidic platforms in motion. The method was designed to be as automated as possible to allow its applicability to the large variety of available design features that tend to be included on such platforms. Experiments have indicated a relative standard deviation (RSD) of 0.3% for replicate measurements and 1% for same volume aliquots injected into different sized chambers. The versatility of the method in regards to chamber shape and size, liquid colour and platform rotational frequency was demonstrated. This flexibility should allow it to be used for a variety of applications including real time metering of volumes in platforms, quantitative monitoring of a design’s performance in real time and could result in the elimination of metering chambers for some applications.

Introduction

Centrifugal microfluidic (CM) platforms have been developed to perform a variety of analytical operations through the use of centrifugal force to direct liquid flow. A wide range of applications have been rendered possible on such platforms thanks to the native advantages granted by the centrifugal force. These advantages include the capability to pump liquids irrespective of their physicochemical properties, a high degree of parallelism given by the uniformity of centrifugal force at every radial position and the potential for portability resulting from the elimination of external connections.1–5

Despite the flexibility offered by CM platforms, they face the important constraint of radially-limited usable space (sometimes called “real estate”) due to the unidirectional nature of centrifugal force. While this limitation can be partially overcome through pumping features designed to return the liquid to the center of a disk,7–9 it is valuable to engineer features that utilize the least real estate while providing the desired functionality.

Volume metering chambers are commonly used in CM systems to dispense a fixed amount of liquid as required by many analytical methods. In this chamber type, the metered volume is determined by the physical dimensions of the chamber with all additional liquid being directed into a waste chamber through an overflow channel.10 During this process, the metered aliquot is retained through a passive13 or active valve12 or siphon13 and is released afterwards through the corresponding mechanism. Despite their widespread use, volume metering chambers have two disadvantages: they consume valuable real estate on the disk and are inflexible as they are specific to a single volume per chamber.

The ability to measure volumes directly on CM platforms in motion could not only allow experimenters to avoid the use of metering chambers but also to quantify liquid flow in real time. This allows evaluation of the performance of CM platforms while in motion. Measurement while in motion is of importance since CM platforms are often designed to be subjected to continuous centrifugal force during operation. Stopping a CM platform may result in unintended capillary induced flow in channels that could cause both a loss of sample and a disruption of the intended operation of the platform.7

While application-specific volume measurements on microfluidic platforms have been successfully accomplished through methods such as the use of impedance measurements on an electrowetting on dielectric (EWOD) platform,14 total internal reflection of a laser beam on a single channel in a CM platform,15 or microscopy images of water-in-oil droplets,16 there is currently a lack of a robust, multipurpose volume measurement technique for CM platforms in motion. This lack can be remedied through dimensional measurements obtained from the segmentation of real time platform images, which are often acquired to conduct a visual, qualitative evaluation of a CM platform’s performance.17

Image segmentation18 is a potent computational tool that has been used in a variety of domains to perform dimensional measurements, including the areas, volumes and deformability of erythrocytes injected into a microfluidic device,19,20 the
haematocrit of blood on a diagnostics platform\textsuperscript{21} and the volume of generated droplets on an EWOD device.\textsuperscript{22} To extend this technique to volume measurements on CM platforms in motion, we present a method based on the automatic edge-based segmentation of platform images. The figures of merit of the technique were evaluated and studies were conducted to demonstrate the versatility of the technique in regards to the colour and surface tension of the target liquid, the size and aspect ratio of the platform chambers and the rotational frequency of the platform during operation.

Volume measurement technique

Basic principles

Images of centrifugal microfluidic platforms provide the opportunity for precise analysis in two dimensions \((x,y)\). As volume measurement involves a third dimension, approximations regarding the platforms’ height component \((z)\) must be made for volumetric data to be obtained from CM platform images. Due to the fabrication method utilized to construct test platforms, the height of all chambers located on the same platform may often be assumed to be equal (Fig. 1A). In our experiments, the camera was placed at a 90 degree angle from the platform’s surface, which completely eliminates the height component on images (Fig. 1B).

To obtain an accurate calibration without the need for \(z\) knowledge, a pre-metered aliquot injected into a calibration chamber is employed as a reference. Using this technique, the volume of liquid aliquots located inside any of the platform’s chambers can be measured by considering it as proportional to its area on images taken during platform rotation. Using automatic image segmentation (Fig. 1C), each liquid aliquot forms a closed outline when imaged inside a platform. This step is enhanced through a black coating applied to the middle platform layer that provides sharp edges along chamber outlines. Subsequently, the segmented outlines are filled to directly measure each aliquot area (Fig. 1D). The ratio of these areas with that of a pre-metered aliquot located on the same image then allows the direct evaluation of aliquot volumes. While the segmentation process excludes the volume within the meniscus of the aliquot, this volume can be considered negligible for most applications\textsuperscript{11} and can easily be determined through calibration when required.

The technique was implemented using a graphical user interface (GUI) in MATLAB R2013B (The Mathworks Inc., Natick, MA, USA) and its associated image processing toolbox. The MATLAB code of the GUI along with an example of its use are available in the ESL\textsuperscript{†} The different steps of the technique are demonstrated on an ideal and real platform (Fig. 2) and detailed in the following section.

Image acquisition and grayscale conversion

Experimental 24 bit colour images were first acquired from a research-grade digital camera setup previously described by Duford.\textsuperscript{17} To keep the method applicable to any kind of liquid, independent of colour, the images were first converted from an RGB colour space to grayscale that retains only the luminance or brightness of the image. Since the data of each colour is encoded in 8 bits, the resulting grayscale level is also composed of 8 bits. This imposes a potential limitation on segmentation with 256 available intensity levels.

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Fig. 1 Basic principles of the volume measurement technique. A) 3-D model of liquid aliquot inside a contrast-enhanced CM platform in motion. The top and bottom platform layers are abstracted due to their transparent properties. Centrifugal force is shown flattening the meniscus. The height \((z)\) is uniform across the platform. B) Model of ideal image of a platform containing three liquid aliquots taken at 90 degrees. Black coating of the middle layer provides a sharp edge at the platform-liquid interface while the liquid meniscus provides an edge at the liquid-air interface due to change in refractive index. C) Segmentation of image B results in automatic detection of the aliquot outlines. D) Filling of the aliquot outlines in C allows direct computation of aliquot areas. Volumes of the measured aliquots are obtained through the ratio of their areas over that of the calibration aliquot.
Image segmentation

Edge-based image segmentation was performed to automatically partition the area occupied by the liquid of interest. Image segmentation involves the partitioning of an image into distinct regions sharing common characteristics.\(^\text{23}\) Edge-based segmentation\(^\text{18}\) utilizes an intensity gradient present on an image to segment features, where edges are defined as a rapid and significant variation in intensity. This type of segmentation was selected due to the large changes in intensity expected at the platform–liquid interface thanks to a contrast-enhanced platform (black middle layer) and at the liquid–air interface due to a variation in refractive index.

Amongst a variety of available edge detection operators,\(^\text{24-29}\) the Laplacian of a Gaussian (LoG) method established by Hildreth and Marr\(^\text{30,31}\) was found to be the most suited to the task due to its acute sensitivity in edge selection. The LoG operator is the convolution of a discrete approximation to a Laplacian filter with a Gaussian filter. Its application first results in a slight blurring of the image to reduce imaging noise and the possibility of flagging false edges. This blurring is then followed by the computation of the second derivative (gradient magnitude) of the intensity at each pixel.

Since an edge is defined as a sharp transition in intensity, its first derivative will be shaped as a peak and its second derivative will have a zero-crossing found at its location (Fig. 3).\(^\text{10}\) However, given the discrete nature of pixels in an image, a real zero will often not be found for all edges. In order to obtain a consistent segmentation, the edge was traced along the negative side of the gradient magnitude at zero-crossings in cases where a real zero could not be found, producing a binary image where edge points were flagged as 1s.

Area measurement and volume evaluation

Following segmentation, a closed shape was obtained that outlined the liquid volume of interest. A pixel was then

![Fig. 2](image-url) Overview of the steps of the volumetric measurement technique demonstrated on images of an ideal (A–D) and a real (E–H) platform. A) Raw image of an ideal platform. B) Conversion to grayscale to retain only the intensity information. C) Segmentation using the Laplacian-of-Gaussian operator. D) Area measurement of user selected chambers after filling by morphological reconstruction. E–H) Steps A–D performed on a real CM platform in motion (1200 RPM) subsequent to injection of a 10 μL aliquot of distilled deionized water containing 0.03% of Triton-X in each chamber.

![Fig. 3](image-url) Illustration of derivative-based segmentation techniques on a one dimensional edge. \(f(x)\) represents the intensity of the image as a spatially distributed signal over pixels in one dimension \(x\). \(df/dx\) is the first derivative of the image, while \(d^2f/dx^2\) is its second derivative. The center of the edge presents a peak in \(df/dx\) that can be hard to localized on real images. When \(d^2f/dx^2\) is used instead, the zero crossing found at the edge position renders the edge localization more reliable and reproducible.
selected within this outline. The four closest connected objects (1’s) to this pixel were found by searching in the four axial directions and retained on a separate image. This process was followed by a bridging algorithm that connects 1’s that have a single 0 lying in between to prevent single pixel discontinuities. This resulted in a closing of the liquid’s outline if that was not already the case.

The liquid outline was then filled by morphological reconstruction\(^3\)\(^3\) to obtain its area. Morphological reconstruction requires a ‘mask’ image on which an elementary morphological operation such as dilation or erosion is performed iteratively while constrained to another image called the ‘marker’. While both dilation and erosion based reconstruction methods are available, dilation was chosen for its simplicity. Morphological dilation can be defined as an operation where a structuring element is slid over every ‘1’ in the image, converting neighbouring pixels to ‘1’s’ where they overlap with the structuring element. The dilations performed during reconstruction are additionally constrained by the marker, where the conversion of a pixel to a ‘1’ only occurs if it is also a ‘1’ on the marker image. To perform reconstruction, these dilations are performed iteratively using a structuring element consisting of a three by three square until stability is achieved. The complement of the binary image containing the outline was chosen as the marker. The mask was chosen to be an image of the same size as the outline image with all pixel values set to 0’s with the exception of the image border where the pixel values are set to be the complement of the original outline image. The complement of the reconstructed image results in the original outline image being completely filled.

Following reconstruction, the pixels of the filled area were counted to obtain the area of the liquid (Fig. 2D, H). The same process was simultaneously accomplished on a pre-metered aliquot of liquid injected into a calibration chamber located on the same image. The volume of each aliquot \((V_{\text{meas}})\) could then be modelled according to eqn (1):

\[
V_{\text{meas}} = V_{\text{cal}} \times \frac{A_{\text{meas}}}{A_{\text{cal}}}
\]  

(1)

Where \(A_{\text{meas}}\) is the segmented area of the aliquot on the image, \(A_{\text{cal}}\) is the segmented area of the calibration aliquot on the image, \(V_{\text{cal}}\) is the pre-metered volume of the calibration aliquot.

**Experimental**

**Design and fabrication of test platforms**

Centrifugal microfluidic platforms were constructed in five layers (Fig. 4) using a subtractive rapid prototyping technique.\(^3\)\(^3\) Transparent polycarbonate (PC) digital video disks (DVDs) (U-Tech Media Corporation, Taiwan) were used as a base substrate for the main platform layers (Fig. 4A, C, E) while double-sided adhesive (FLEXmount DFM-200-Clear V-95 150 poly V-95 400, FLEXCon, Spencer, MA, USA) was selected for the bonding layers (Fig. 4B, D). The individual layers were

Fig. 4 CM platform layers. A) Top DVD layer containing drilled injection and vent holes. B) Double sided adhesive layer bonding layers A and C. C) Middle DVD layer containing chambers and channels. This DVD is spray-coated with black paint before machining to enhance contrast during imaging. D) Double sided adhesive layer bonding layers C and E. E) Bottom DVD layer.
while the 4 and 5 mm chambers were designated as measurement chambers.

In order to explore the effect of feature size and shape, three additional platforms were designed containing rectangular chambers of varying aspect ratios (1:1, 1:1.8, 1:2.3) that retained the areas of the circular chambers of disk A. Disk B (Fig. 5B) was comprised of sixteen identical wedges containing square chambers (1:1 ratio) of varying sizes. This disk was used as a baseline for comparison with disk C and disk D (Fig. 5C and D), which contained rectangular shapes of differing aspect ratios (1:1.8 and 1:2.3).

**Experimental setup**

The setup (Fig. 6) used to test the volumetric measurement technique consisted of a previously described experimental apparatus updated with a colour digital camera.17 This configuration consisted of a servomotor (Parker MPJ0922D3E-NPSN, Cadence Automatisation, Sainte-Thérèse, QC, Canada) and a servo drive (Parker AR-08AE, Cadence Automatisation) synchronized with a high-speed 24 bit colour digital camera (GRAS-14S5C-C, Point Grey Research Inc., Richmond, BC, Canada) and xenon arc strobe light (Shimpo DT-311A, Primo Instruments, Montreal, QC, Canada) via a custom LabVIEW program (LabVIEW 8.6, Developer Version, National Instruments, Vaudreuil-Dorion, QC, Canada). This configuration allowed the acquisition of ‘still appearing’ images during platform rotation. The camera was placed at 90 degrees from the platform while the strobe light was positioned at an angle to illuminate the platform from above without causing specular reflection. A white sheet of paper was added at the base of the motor spindle to produce a white background. The magnification of the imaging system was set to be fully zoomed in on one wedge of a platform at a time to give the best resolution possible. All images were taken at a platform rotational frequency of 1200 rotations per minute (RPM).

**Reagents**

Initial experiments evaluating the figures of merit of the technique were conducted using an aqueous test solution (ATS)
containing 0.03% of Triton-X in distilled deionized water (DDW) to demonstrate the application of the technique to clear liquids. Measurements of pure DDW aliquots were not readily reproducible owing to the thinness of the meniscus, which provided less than ideal contrast at the resolution of our camera and could result in incomplete segmentation. Triton-X was added to reduce the surface tension of water, which renders the meniscus slightly thicker and easier to segment. This solution was also utilized to conduct an evaluation of the technique when applied to varying chamber aspect ratios and platform rotational frequencies. The effect of liquid colour on the performance of the technique was investigated using solutions of commercial food colouring (Club House Food Colour Preparation, McCormick Canada, London, ON, Canada) of three primary colours (red, blue and yellow) prepared in distilled deionized water (DDW) containing 0.03% Triton-X and mixed in equal proportions to obtain three secondary colours (orange, purple and green). The final red and blue solutions had optical densities of ~1.5 at their respective spectral maxima while the yellow solution had an optical density of ~0.5. To verify the effectiveness of the technique on liquids of varying surface tension, experiments were conducted using organic solvents of varying surface tensions: hexadecane (Caledon Laboratories Ltd., Georgetown, ON, Canada), ethylene glycol, methanol and ethanol (Sigma-Aldrich Canada Co., Oakville, ON, Canada).

Experimental procedure

An initial set of experiments was conducted to assess the figures of merit of the technique and evaluate potential sources of error. These experiments were conducted using the ATS described in the reagents section. A preliminary experiment was carried out to evaluate the error associated with the imaging and image processing of an artificial ‘ideal’ platform. This platform was constructed by printing a 1:1 design drawing of disk A on paper and inserting it between two transparent DVDs to keep it flat. All eight wedges of the artificial platform were imaged five times each and the areas of each chamber were measured and averaged over the replicate images.

The precision figure of the technique applied to real platforms was then determined by injecting a 10 μL aliquot of ATS into each chamber of a replicate of disk A with a 10.0 μL microsyringe (701 RN SYR, Hamilton Company, Reno, NV, USA). The wedges of the platform were imaged individually for five replicate images each and the areas corresponding to each injected aliquot were measured and averaged over the replicate images.

An accuracy experiment was conducted with two replicates of disk A to measure a series of injected pre-metered volumes using a 10 μL calibration aliquot. All injections in this experiment were carried out with a 50.0 μL microsyringe (705 RN SYR, Hamilton Company, Reno, NV, USA). A 10 μL aliquot of ATS was injected into the calibration chamber of every wedge on both platforms. On the first platform, 20 μL and 40 μL were injected respectively into the 4 and 5 mm radius measurement chambers. The same process was repeated for the second platform using 30 and 50 μL instead. The wedges of each platform were imaged separately for five replicate images each and the areas of the aliquots in each chamber were measured. Aliquot volumes were obtained by using the ratio of their areas against that of the calibration aliquot located on the same wedge.

The effects of chamber shape and aspect ratio on method precision were investigated using replicates of disks B–D. Aliquots of 10 μL of ATS were injected into every chamber of each platform. Each of the sixteen wedges per platform was imaged five times. Areas were computed for every image and averaged over replicate images to obtain one area per aliquot.

The effectiveness of the technique applied to platforms operated at varying rotational frequencies was evaluated using the filled replicate of disk D utilized in the aspect ratio experiments. Each of the sixteen wedges of this platform was imaged five times for a series of rotational frequencies from 200 to 1400 RPM in 200 RPM increments. Areas for all liquid aliquots were measured and averaged over the respective replicate images.

To explore the influence of liquid surface properties on the performance of the technique, experiments were conducted using 10 μL aliquots of organic solvents injected into every chamber of a disk A replicate. Separate replicates were utilized for each of the tested solvents: ethylene glycol, hexadecane, methanol and ethanol. Each platform wedge was imaged five times immediately after injection into and sealing of wedge chambers to avoid solvent evaporation. The areas for all liquid aliquots were measured and averaged over the respective replicate images.

An application of the technique to six coloured solutions (red, blue, yellow, orange, purple and green) was demonstrated using a replicate of disk A. A 50 μL aliquot of each coloured solution was injected into separate 5 mm radius measurement chambers using a 50.0 μL microsyringe. A clear 10 μL aliquot was injected into corresponding calibration chambers with a 10.0 μL microsyringe to serve as a reference. Each coloured aliquot was imaged five times and processed to obtain a volume per replicate image that was averaged to obtain one volume per aliquot.

Results and discussion

In an ideally fabricated platform, the measured volume uncertainty ΔV_m can be propagated from eqn (1):

$$\Delta V_m = \sqrt{\left(\frac{\Delta V_m}{\delta V_{cal}} \times \Delta V_{cal}\right)^2 + \left(\frac{\delta V_m}{\delta A_m} \times \Delta A_m\right)^2 + \left(\frac{\delta V_m}{\delta A_{cal}} \times \Delta A_{cal}\right)^2}$$

(2)

where ΔV_{cal} is the uncertainty on the pre-metered calibration volume and ΔV_m and ΔV_{cal} are the localization uncertainties on the areas of the measured aliquot and the calibration aliquot, respectively. Since both areas are simultaneously measured using the same method, it can be assumed that...
their localization precisions, \( \frac{\Delta L_m}{L_m} \) and \( \frac{\Delta L_{cal}}{L_{cal}} \) are equivalent so that they can be generalized to an area localization precision \( \frac{\Delta A}{A} \). Since the localization uncertainty is dependent on camera resolution, optical magnification of the imaging system and reliability of the fabrication technique, it is best determined experimentally within a given test system. As such, the figures of merit of the technique were characterized by a set of experiments designed to evaluate potential sources of error.

To evaluate the ideal localization precision of the imaging system, the technique was first applied to area measurements of chambers on an artificial “ideal” platform (Fig. 2A–D). The RSD associated with the imaging and processing steps was computed from area measurements performed on each chamber of the platform. This “ideal” localization RSD was calculated to be 0.4% and found to be independent of chamber size at the magnification utilized for the experiment.

The experimental localization precision on real platforms was obtained from measurements of 10 \( \mu L \) aliquots injected into every chamber of a disk A replicate with an example measurement demonstrated in Fig. 2E–H. An experimental localization RSD was calculated by evaluating the variations in area for the same liquid aliquot between five replicate images per aliquot. This RSD was found to be 0.3%, which is comparable to the results obtained from the artificial platform experiment, demonstrating a high precision for the localization component of the technique.

The precision \( \frac{\Delta V_m}{V_m} \) of the technique for real liquid aliquots was quantified through the variation of aliquot areas within the same wedge as well as between different wedges for pre-metered aliquots of the same volume injected into all chambers of a disk A replicate (Table 1). First, an RSD was calculated for areas obtained from aliquots injected into different sized chambers located on the same wedge. This RSD was found to be 1% and appears to be limited by the precision of the syringe used to perform the injections, which has a manufacturer established precision of 1% per injection at 80% of the barrel capacity (i.e. roughly 0.08 \( \mu L \) for the 10 \( \mu L \) syringe). This indicates that the main limitation of the technique appears to be the uncertainty on the calibration volume (\( \Delta V_{cal} \)). RSD values were then calculated for areas of aliquots injected into same sized chambers on different wedges. These RSDs were found to be roughly 3% and did not appear to vary with chamber size. This variability appears to be caused by the irreproducibility of the xenon-arc strobe light illumination during platform operation. This irreproducibility causes a variation of 1% in the average intensity of the images of the different wedges. While the localization of the blacked-out chamber edge does not vary with varying light levels, the localization of the meniscus edge depends on the thickness of the meniscus, which fluctuates with illumination intensity. As such, a variation in the illumination intensity causes an increase or decrease in the areas obtained for aliquots in same-sized chambers, rendering measurements obtained from different wedges less reproducible. This effect demonstrates the need for the calibration aliquot to be located on the same image as a measured aliquot in order to obtain the best precision possible.

Accuracy figures were computed following the measurement of pre-metered aliquots (20, 30, 40 and 50 \( \mu L \)) on two test platforms. A 10 \( \mu L \) aliquot of DDW was injected as a calibration standard into the calibration chamber on every wedge. The volumes of the two other aliquots were measured and compared to the known volumes. The mean relative error between the injected and measured volumes was found to be on the order of 3%. This error can be explained by the fact that the meniscus volume is not taken into account during the measurement.

As the segmentation step relies on a closed outline, only the inner side of the meniscus is included, creating an offset that is not included during area measurement. This offset, which represents the meniscus volume, appears to be nearly constant for all aliquots resulting in its impact decreasing with increasing aliquot volume. The propagation of this effect through the ratio operation of the technique results in a linear deviation in measured volume that is proportional to aliquot volume. A linear least squares calibration model of measured volumes against injected volumes (Fig. 7) showed excellent linearity \( (R^2 = 0.9997) \) and a standard error of prediction of ~0.2 \( \mu L \) (2%), indicating that such a model can easily correct this deviation. It is important to note that this effect was not present for the previously demonstrated precision measurements as all aliquots were of identical volume, resulting in a constant aliquot volume to meniscus volume ratio.

Additional experiments were conducted to demonstrate the applicability of the technique to chambers of varying shapes. Differing chamber shapes present two distinct features in regards to localization uncertainty: a chamber outline of varying size and a variable meniscus length. Since the former is easily segmented due to the black contrast layer, its influence on the measurement uncertainty could be considered negligible. To evaluate the influence of the former, identical aliquots were injected into replicates of disks B–D which contained rectangular chambers of varying aspect ratio and

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**Table 1** Precision experiment data*<sup>a</sup>

<table>
<thead>
<tr>
<th>Precision</th>
<th>RSD (%)</th>
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</thead>
<tbody>
<tr>
<td>Ideal localization</td>
<td>0.4</td>
</tr>
<tr>
<td>Real localization</td>
<td>0.3</td>
</tr>
<tr>
<td>All chambers on a wedge</td>
<td>1</td>
</tr>
<tr>
<td>3 mm radius chambers</td>
<td>3</td>
</tr>
<tr>
<td>4 mm radius chambers</td>
<td>2</td>
</tr>
<tr>
<td>5 mm radius chambers</td>
<td>3</td>
</tr>
</tbody>
</table>

* Figures of merit obtained from the precision experiment. \( n = 8 \) for the ideal localization RSD. \( n = 6 \) for all other RSDs.
size. Each wedge of each platform was imaged individually and areas were obtained for all aliquots. An RSD between aliquots located on the same image in different sized chambers was computed for every aspect ratio (Table 2). These RSDs were found to be 1% on average and did not show variation with aspect ratio, indicating that the localization uncertainty on the meniscus appears to be comparable to the localization uncertainty of the chamber outline. This figure is also similar to those obtained during the precision experiments using disk A, which also presented a variation in meniscus length for same volume aliquots located in chambers of different radii. This demonstrates that the technique provides precise volume measurements that can be reliably scaled to chambers of varying size and shape.

In order to quantitatively monitor liquid flow during each unit operation, it is important that the measurement technique be scalable to a wide range of rotational frequencies. To demonstrate this scalability, experiments were conducted using the filled replicate of disk D from the previous experiment. Images of each platform wedge were taken at rotational frequencies varying from 200 to 1400 RPM in 200 RPM increments. An RSD was calculated for the areas obtained for aliquots in different-sized chambers for every frequency. The RSDs for the different rotational frequencies did not present any variation and were found to be 2% on average, indicating that the technique can be applied to a wide range of rotational frequencies.

While many analytical procedures make use of aqueous solutions of samples, certain sample preparation methodologies require the use of organic solvents or high concentrations of detergents. The varying surface properties of these liquids present an additional challenge to the volume measurement technique due to their influence on the imaged meniscus. To evaluate the effect of surface tension on the precision of the volume measurement technique, experiments were conducted using aliquots of four organic solvents with varying surface tensions: ethylene glycol, hexadecane, methanol and ethanol. A separate replicate of disk A was allocated for each solvent. Every chamber of a platform was filled with a 10 μL aliquot of a selected solvent and each wedge was imaged individually. The areas of each aliquot were obtained and RSDs were computed for each solvent between aliquot areas located in different-sized chambers on the same wedge. The RSDs obtained for ethylene glycol and hexadecane were 1% and 5%, respectively. Ethanol and methanol were found to be incompatible with the adhesive utilized in the fabrication of our platforms as their injection resulted in leaks during platform rotation. As such, no RSDs could be computed for these two solvents.

In a comparison between the figures found for ethylene glycol and hexadecane, method precision was found to be inversely proportional with surface tension. This effect could be caused by the change in contact angle of the meniscus, which decreases with surface tension. Imaged meniscus thickness appears to be inversely proportional to surface tension due to increased wetting of the top and bottom polycarbonate layers. Due to this interaction, it appears that at low surface tensions, the meniscus volume and thickness scales with chamber size, decreasing method precision. To mitigate this influence, careful choice of the substrate must be made to reduce wetting of the platform surface by the liquid. However, reduced wetting will result in a thinner meniscus which requires a higher resolution or larger magnification for successful segmentation. This effect was responsible for the difficulty in obtaining measurements for pure DDW aliquots and prompted the addition of trace amounts of detergent. It should be noted that another possible contribution to the difference in precision is the density of the solvents being used. Since the applied centrifugal force depends on density, it is conceivable that the imaged meniscus could be thicker for a less dense liquid. However, given the difference in thickness obtained between pure DDW aliquots and the detergent-spiked DDW aliquots, it is more likely that the difference is due to variation in surface tension.

Analytical processes often employ spectroscopic detection techniques for precise determination of analytes. As spectroscopic analyses often employ brightly coloured analytes, successful image-based volume measurements of such compounds must be flexible in regards to analyte colour. To ensure the applicability of the volume measurement technique to coloured liquids, a study was performed using six solutions of food colouring dyes: red, yellow, blue, green, orange and purple (Fig. S1f). A 50 μL aliquot of each dye was injected into a measurement chamber of a disk A replicate along with a 10 μL calibration aliquot in corresponding

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**Table 2 Aspect ratio experiment data**

| Chamber aspect ratio | RSD  
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1:1</td>
<td>2%</td>
</tr>
<tr>
<td>1:1.8</td>
<td>1%</td>
</tr>
<tr>
<td>1:2.3</td>
<td>1%</td>
</tr>
</tbody>
</table>

*Figures of merit obtained from the aspect ratio experiment (n = 10 for the 1:1 and 1:1.8 while n = 14 for the 1:2.3 RSD. Outliers due to injection issues/disk failure were discarded).*
calibration chambers. Each dye aliquot was imaged five times, segmented and processed to obtain a volume for every colour. The RSD between measured dye volumes was computed and found to be 1%, indicating that the colour of the analyte did not have any impact on the measurement process.

In order to accommodate darker liquid colours, an additional approach can be implemented by modifying the initial grayscale conversion process. Since the white background is uniform in all three colour channels (red, green and blue) for clear-coloured liquids, a specific colour channel can be selected directly instead of using a grayscale conversion algorithm. This selection was automated following a single click inside the coloured liquid aliquot area by the user. A 50 by 50 pixel area was selected around the clicked location and the mean intensity of those pixels was computed for all three colour planes. The colour channel with the maximum mean intensity was then retained. This ensures that the segmentation occurs on the intensity plane with the highest contrast to the black background, which has a value of 0 in every colour channel. The use of a clear calibration aliquot can enable the simultaneous measurement of volumes of several different coloured analytes in a single image. Measurement of highly opaque liquids, such as blood, could be accomplished by employing a white masking layer instead of black, ensuring complete segmentation of the chamber and aliquot edges.

The effect of several enhancement techniques including median filtering, contrast enhancement and unsharp masking of the images was explored to help reduce noise in the images and increase the intensity difference at edges to improve segmentation. It was found through the precision experiment data that the best precision was obtained without any enhancement. This supports the previous hypothesis that the largest contribution to the measurement uncertainty $\Delta V_{\text{cal.}}$ is the propagation of the calibration volume uncertainty $\Delta V_{\text{cal.}}$

The requirement for a pre-metered aliquot for calibration could be avoided through an alternate calibration method if the interior height of platform chambers can be accurately measured. A reference length or scale bar could be applied on the platform to obtain a pixel to area conversion factor. In combination with the knowledge of interior chamber height, this factor could allow for direct volume measurements on an image without the use of a calibration aliquot. However, a practical implementation of this approach could be challenging due to the requirement for accurate knowledge of interior chamber heights, which can vary between platform replicates depending on the employed fabrication technique.

The measurable volume range on a single platform is primarily determined by the height ($z$) of the platform, as it controls the area that can be imaged for a chosen volume. A decrease in $z$ could lead to more sensitive determinations of smaller volumes, while an increase in $z$ can allow the measurement of larger aliquots. However, a larger $z$ precludes the simultaneous measurement of smaller volumes due to a minimum area that must be occupied by the liquid before the occurrence of a meniscus that can be segmented. In absolute terms, the measurable volume range is also highly dependent on camera resolution and optical magnification, which should render it scalable for a variety of applications. Due to this flexibility, the optimization for a volume range should be conducted according to the application specific needs and instrumentation available to experimenters.

**Conclusions**

An image-segmentation based volume measurement technique was developed and demonstrated to be an effective tool for volume measurements on CM platforms in motion. The use of edge-based segmentation renders the technique applicable to aliquots in features of varying size, shape and aspect ratio with no loss in precision. The demonstrated technique was shown to be flexible towards the needs of experimenters, requires minimal user input and does not utilize any additional instrumentation other than a digital camera and stroboscope, components that are inexpensive and readily available in most CM laboratories. Although our experimental setup utilized standard commercial instrumentation to allow general exploration of the method’s capabilities, a more compact and application-based system can be envisioned that utilizes a smaller stroboscope directed underneath the platform, shining through a diffuser. While the technique’s performance was found to be influenced by liquid-substrate surface interactions, this effect can be mitigated through careful selection of platform substrate to suit the desired measurement.

Direct volume measurements offer the potential for the development of CM analytical methods that require precise volume knowledge without the need for metering chambers, saving valuable real estate on CM platforms. Such measurements should allow the possibility to evaluate new design features directly and quantitatively through real time flow monitoring. The ability to measure volumes without relying on discrete introduction via metering chambers could be highly valuable for use in continuous-flow platforms where such introduction may not be desirable.

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**References**