Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Volumetric Measurements by Image Segmentation on Centrifugal Microfluidic Platforms in Motion

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Figures S-1 and S-2 present pictures of two of the platforms utilized during experiments. Figures S-3 to S-5 display example measurements obtained during the aspect ratio, solvent and rotational frequency experiments, respectively. Tables S-1 to S-3 present summaries of the data obtained during the aspect ratio solvent and rotational frequency experiments. Figures S-6 to S-13 show an example of use of the included MATLAB GUI.

Figure S-1: Picture of the colour analysis platform following injections of 50 µL aliquots of three primary and three secondary colour dyes.

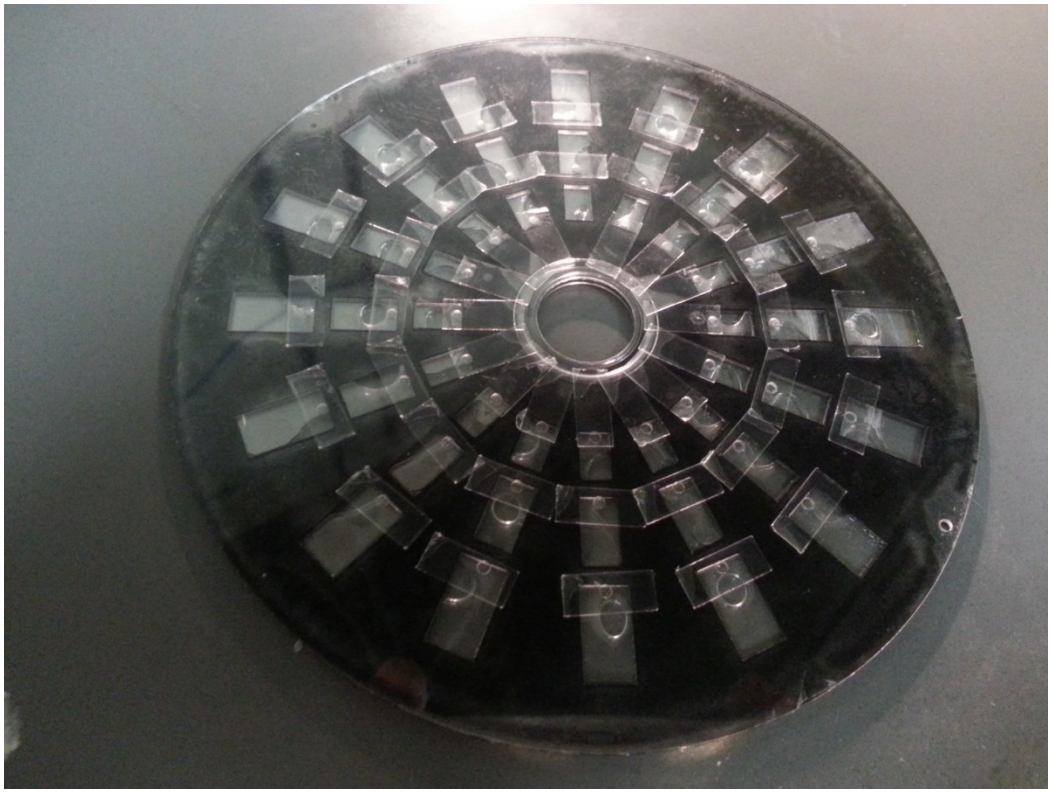

Figure S-2: Picture of the 1:1.8 aspect ratio test platform following injection of 10 µL aliquots into every chamber.

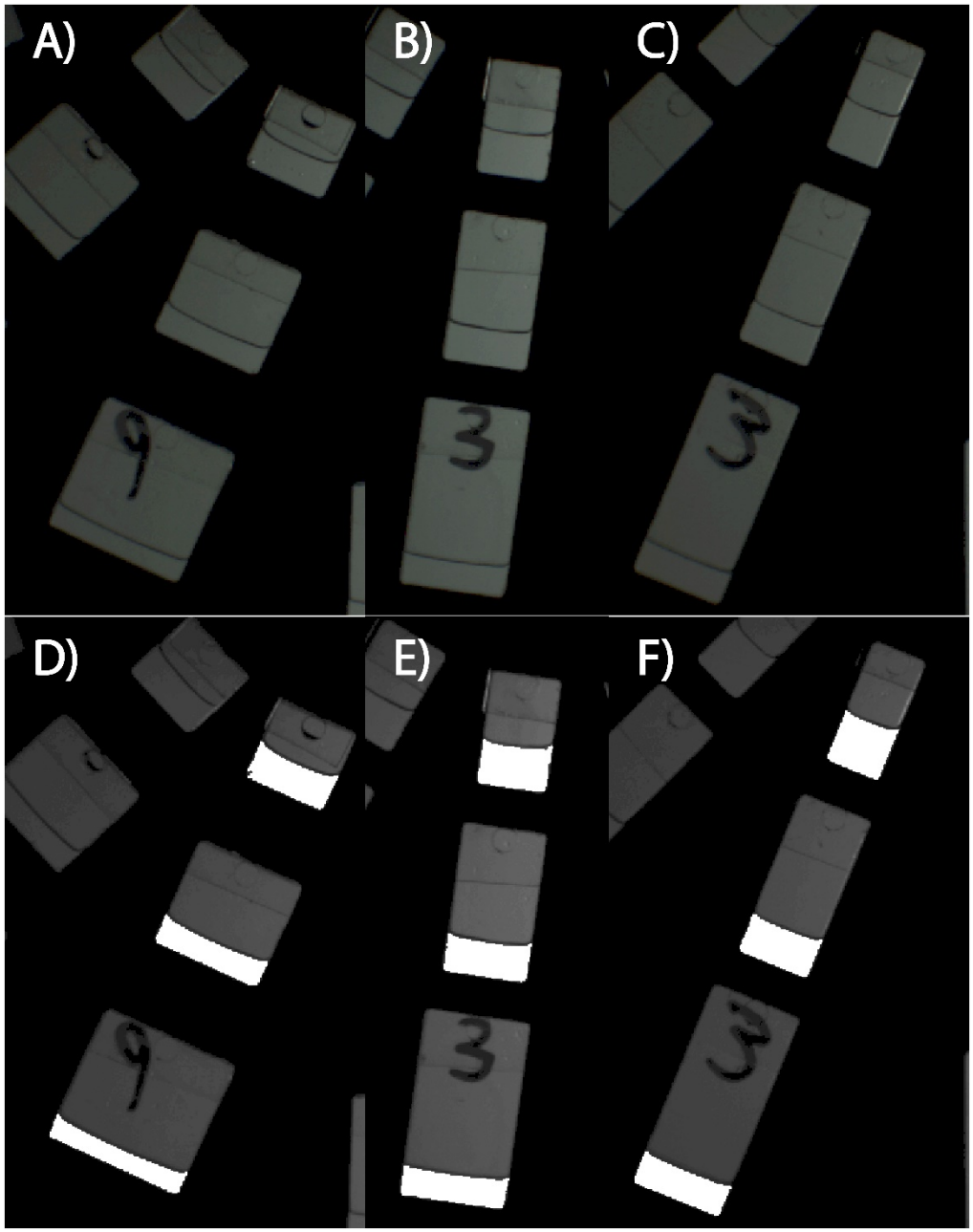

Figure S-3: Images obtained during the aspect ratio experiments. A) Raw image of the 1:1 aspect ratio platform. B) Raw image of the 1:1.8 aspect ratio platform. C) Raw image of the 1:2.3 aspect ratio platform. D)-F) Images A-C segmented with highlighted aliquot areas.

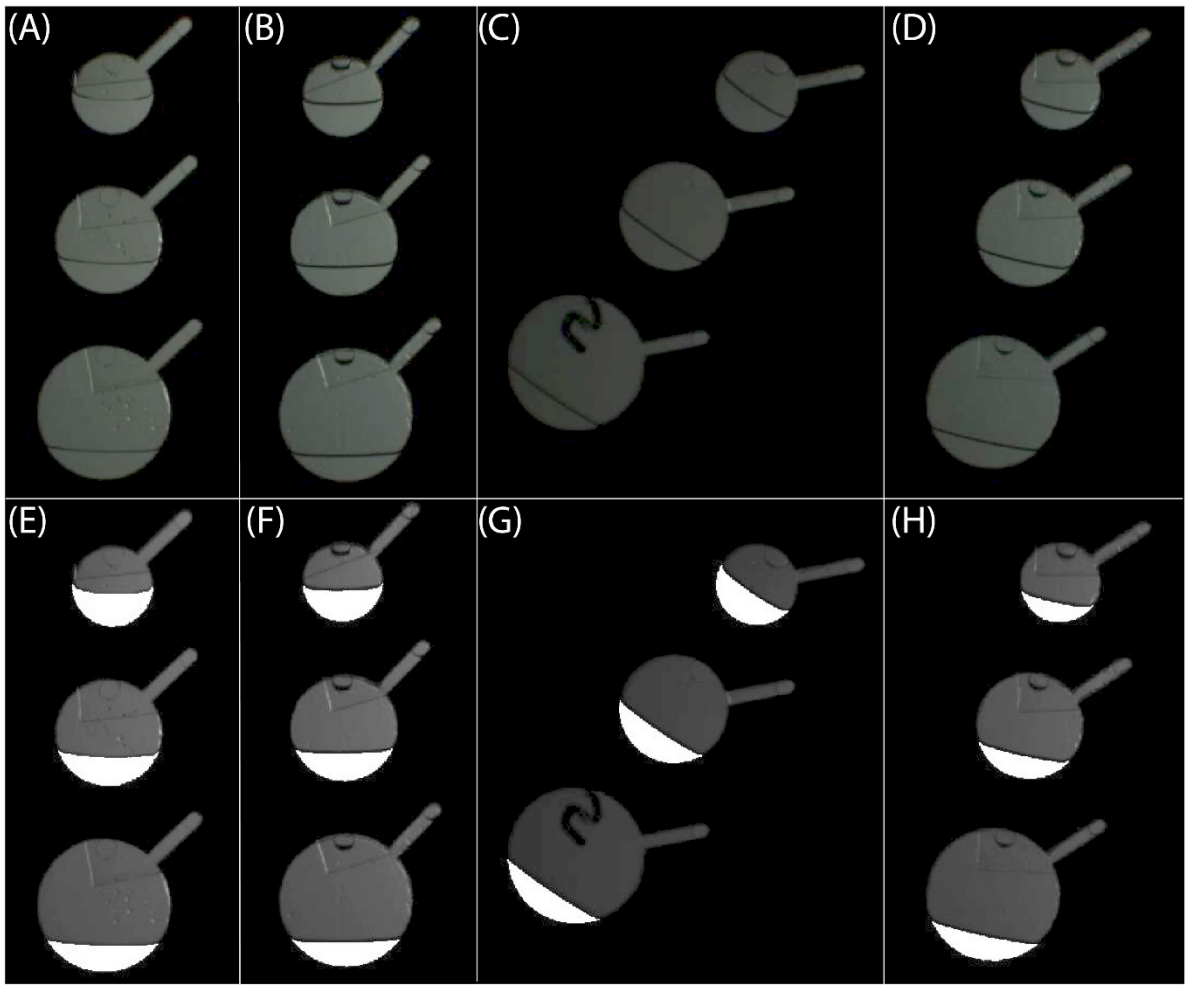

Figure S-4: Experimental images obtained during the solvent experiment. A) Raw image of ethylene glycol aliquots. B) Raw image of hexadecane aliquots. C) Raw image of methanol aliquots. D) Raw image of ethanol aliquots. E)-H) Images A-D segmented with highlighted aliquot areas.

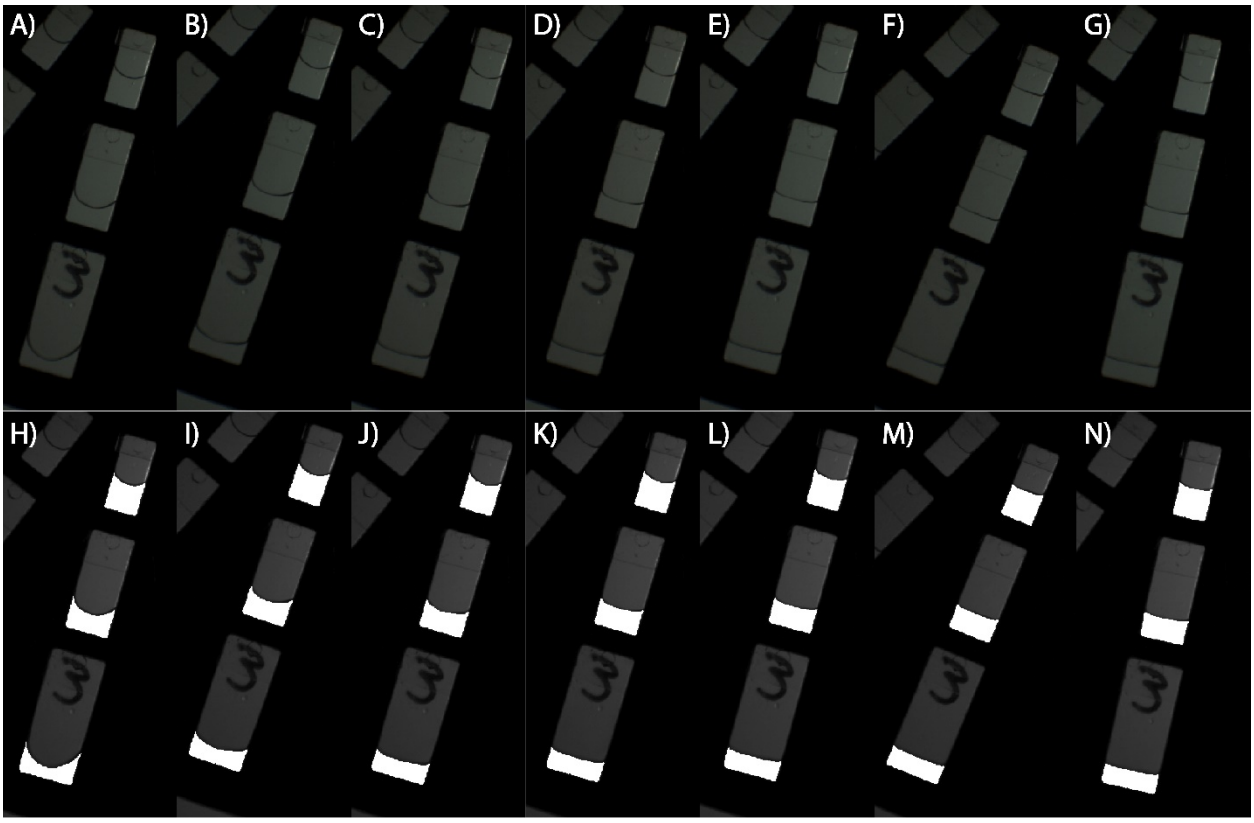

Figure S-5: Experimental images obtained for aliquots of DDW inside a test platform rotated at A) 200 RPM B) 400 RPM C) 600 RPM D) 800 RPM E) 1000 RPM F) 1200 RPM and G) 1400 RPM. H)-N) Images A-G following segmentation with highlighted aliquot areas.

Table S-1: Accuracy experiment data		
Volume (μL)	Relative error on accuracy (%)	RSD (%)
20	2	1
30	2	1
40	4	1
50	3	4
Mean	3	2

Table S-1: Solvent Experiment Data		
Solvent	Surface Tension at 25°C (mN/m)	RSD (%)
Ethylene Glycol	47.99	1.00
Hexadecane	27.05	5.00
Methanol	23.23	N/A
Ethanol	23.22	N/A

Table S-3 : Rotational Frequency Experiment Data	
Rotational Frequency (RPM)	RSD (%)
200	2
400	2
600	2
800	2
1000	2
1200	1
1400	2

MATLAB GUI

This section presents a series of figures depicting the graphic user interface (GUI) developed to implement the volume measurement technique. The GUI was developed using MATLAB. As the GUI was designed to be utilized on high resolution monitors (1920 by 1080 pixels), resizing of figure elements to fit this appendix resulted in a loss of detail. Details regarding code functioning can be found within the comments of the volume.m file within the included archive.

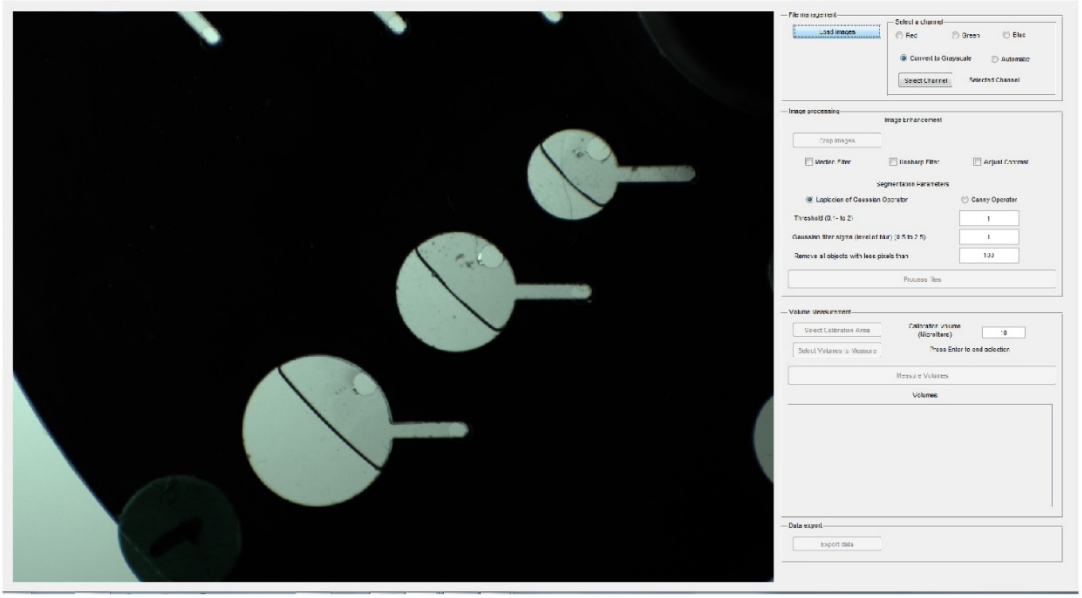

Figure S-6: Loading and display of a set of replicate raw images into the main interface. The first image of the series is displayed. It should be noted that the software assumes that all images loaded simultaneously are replicates.

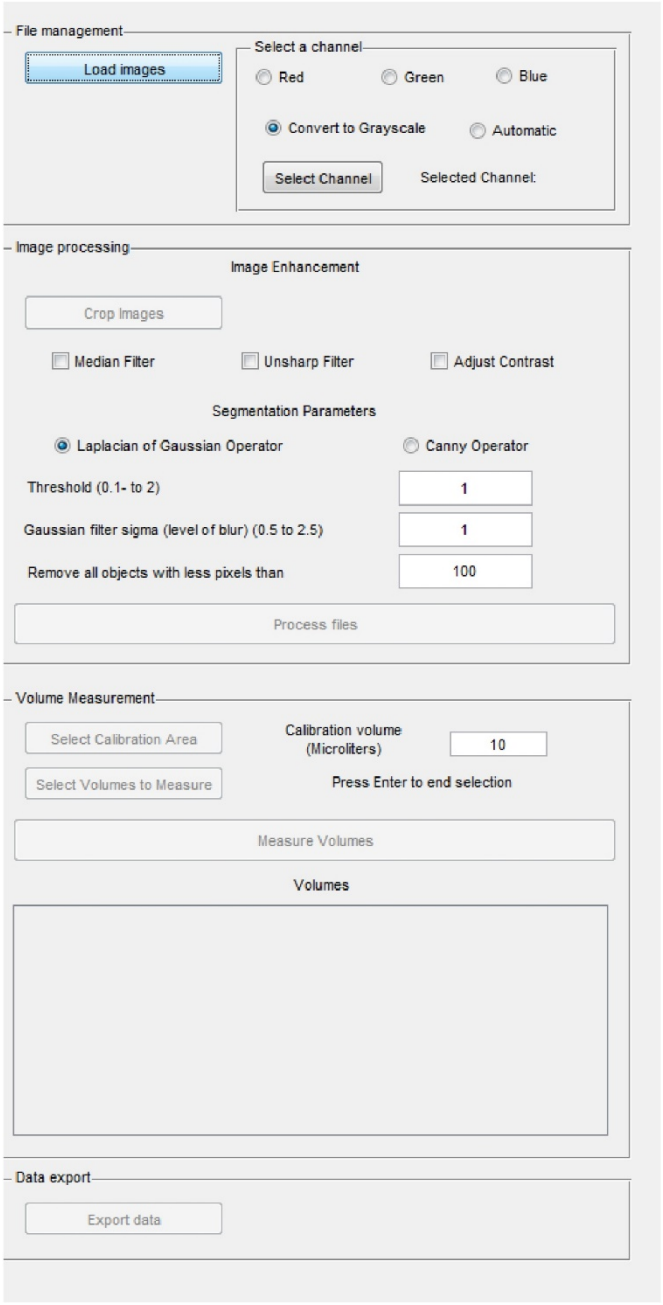

Figure S-7: Expanded image of the interface depicted on the right of Figure S-6 demonstrating the main menu of the GUI. After loading a set of images, conversion to grayscale is accomplished through one of the methods presented above. The user can either select a specific colour channel for use, apply a standard Grayscale conversion algorithm or use the "Automatic" selection method. The "Automatic" channel selection requires selection of a pixel inside the chamber to establish which colour channel of the image presents the brightest intensity for a 50 by 50 pixel window around the selected pixel.

Figure S-8: Conversion and display of raw images into grayscale. The first replicate is displayed following conversion.

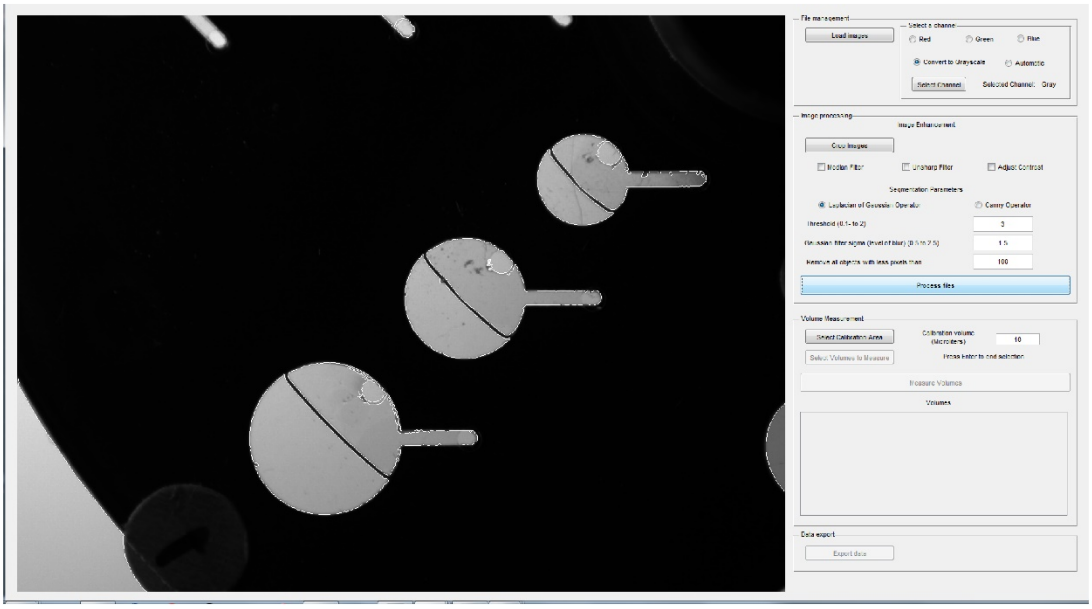

Figure S-9: Segmentation of grayscale images. All images are segmented using the selected settings and the first replicate is then displayed.

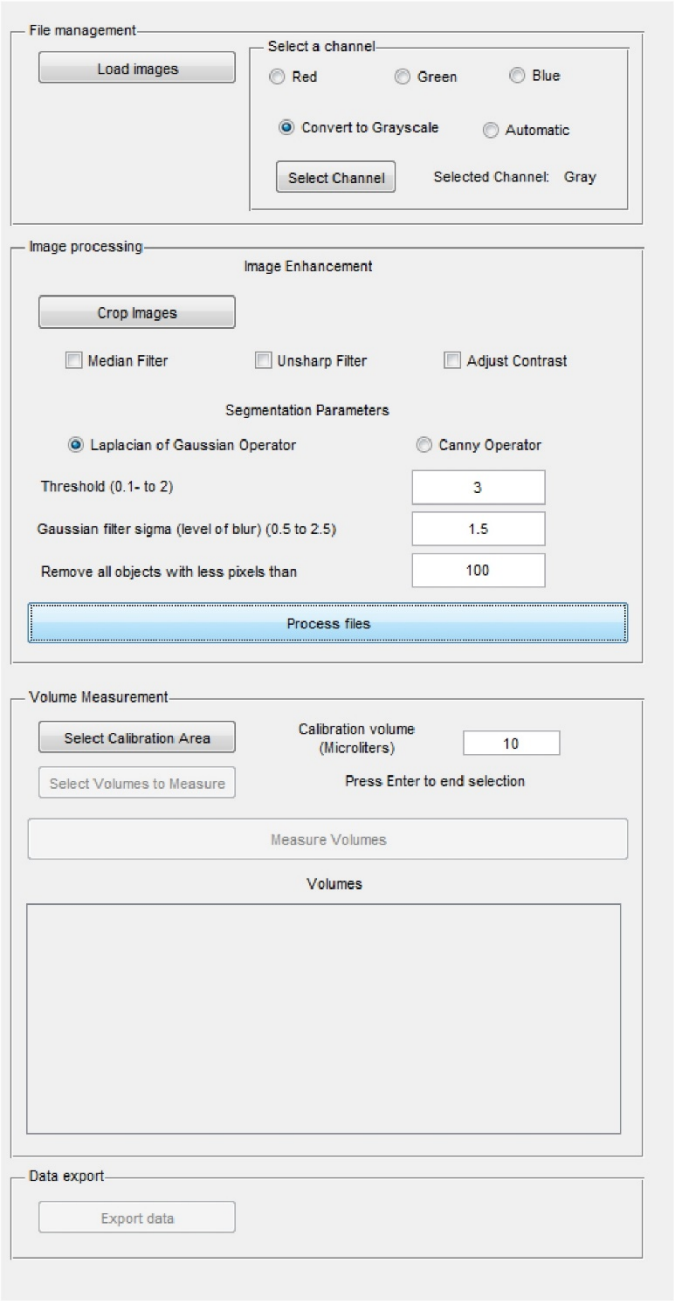

Figure S-10: Zoomed image of the interface depicted on the right of Figure S-9 demonstrating the main menu of the GUI. Segmentation parameters are modified by the user for optimal segmentation. The threshold parameter multiplies the automatically-generated threshold by the user entered number. Here, the default threshold is multiplied by 3 rendering the edge-selection process more selective. The standard deviation of the Gaussian component of the Laplacian-of-Gaussian operator is then selected. Finally, a cleanup is performed for segmented objects that are below a certain pixel limit which is specified by the user.

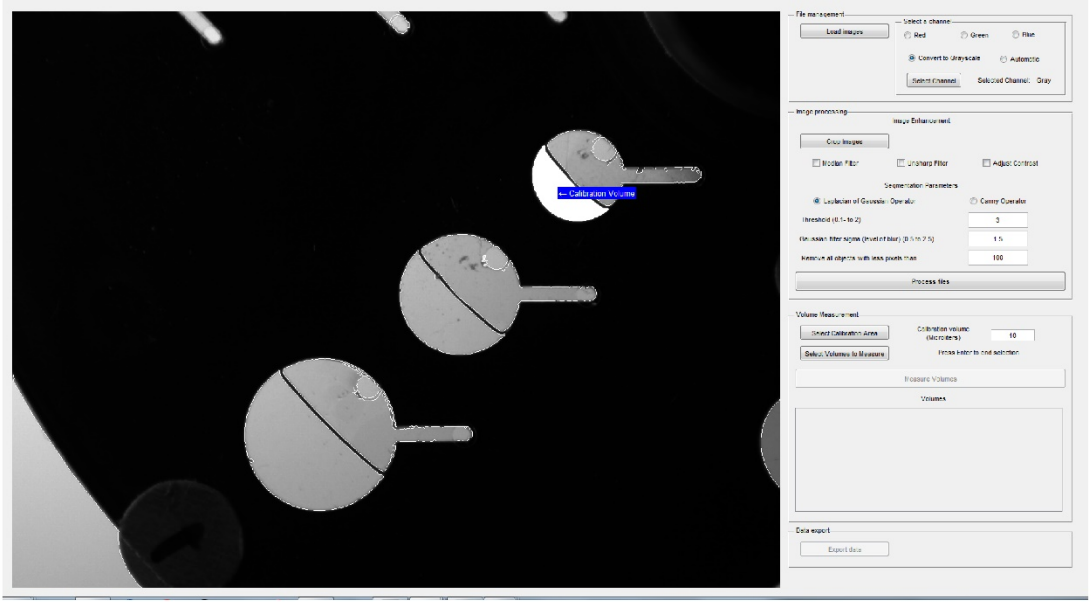

Figure S-11: Selection of the calibration volume by the user. The same coordinates are applied to all replicate images of a set.

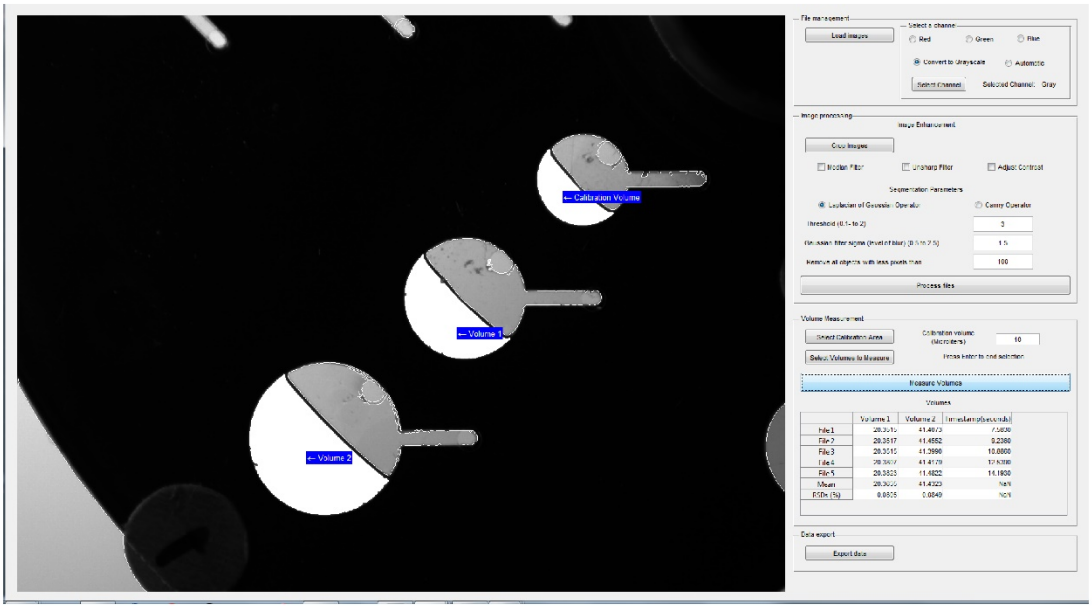

Figure S-12: Selection of the aliquots to measure by the user. The same coordinates are applied to all replicate images of a set. The measure button is then pressed to obtain the volume measurements.

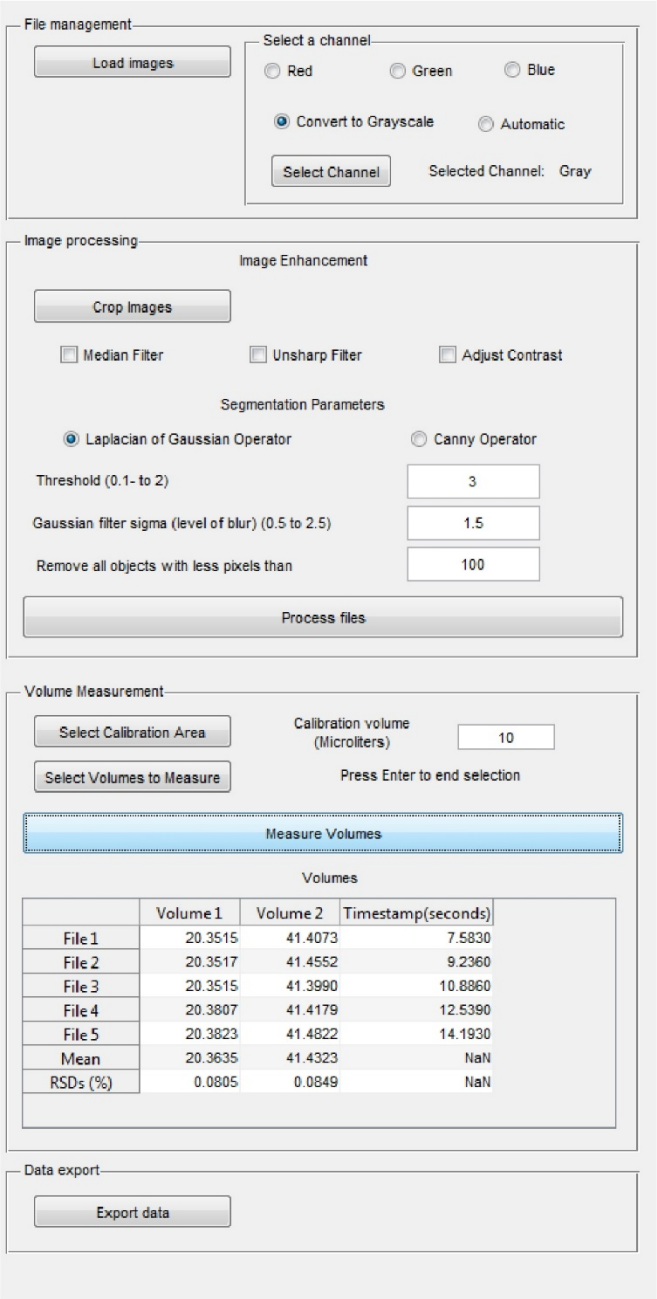

Figure S-13: Zoomed image of the interface depicted on the right of Figure S-12 demonstrating the main menu of the GUI. The volume of the calibration aliquot is entered by the user with the default being 10. The volume for each aliquot is then calculated and presented to the user for each file. Replicate statistics and the timestamp of each image are then presented for convenience.