

# Volumetric Measurements by Image Segmentation on Centrifugal Microfluidic Platforms in Motion

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## Graphical User Interface Manual

The volumetric measurements GUI (*volumes.m*) was designed using MATLAB 2013b and requires the installation of the Image Processing Toolbox to function. The main GUI file and all related functions in the archive must be located within the same folder for proper operation of the GUI.

### Interface basics

The left side of the interface is comprised of an axis object on which images are displayed during the course of program use. The right side of the interface contains the necessary functions to perform volume measurements from images of microfluidic devices. To make the process more user friendly, higher order functions are greyed out until pre-requisite steps are performed.

The method used in this interface assumes the following features about the source images:

- The microfluidic platform outline is black (or dark).
- The imaged area inside the liquid aliquots is lighter than the platform outline.
- The aliquot forms an enclosed shape with segmentable edges (platform outline and meniscus).

### Loading images

Images can be loaded into the interface with the **Load images** button. A dialog box will open asking the user to select the image files to load. The images do not have to be in the same folder as the interface. Multiple images can be loaded simultaneously. In the case of multiple simultaneous images, the GUI assumes that the **image stack is a set of replicates of the same image**. While all images are processed at the same time, only the first of the list is displayed on the left axis object as all others are considered to be replicates. To measure volumes in images that are not replicates, it is recommended that each image be loaded separately for individual processing.

### Grayscale conversion

Conversion to grayscale of the loaded images can be accomplished using one of 3 methods. Following conversion, the first image of the list is displayed on the left axis. The three conversion methods are:

- Direct channel selection (**Red**, **Green** or **Blue** radio buttons).
- Standard grayscale conversion algorithm (**Convert to Grayscale** radio button)
  - The algorithm used is derived from the standard NTSC luminance computation:
$$0.2989 \times R + 0.5870 \times G + 0.1140 \times B$$
- Automatic channel selection (**Automatic** radio button).
  - When selecting the automatic option, the user will be prompted to click on a point inside the volume to be measured. The program will then draw a 50 by 50 pixel window around

the click and find the image channel with the highest intensity to ensure maximum contrast between the black background and the aliquot to be measured.

Once a method is selected, the **Select Channel** button performs the desired conversion operation on all loaded images.

## Image processing

Several image processing steps are available for application to grayscale images before segmentation. With the exception of cropping, all processing operations are selected via the appropriate check box and are performed immediately before segmentation when the **Process files** button is pressed.

- **Crop Images**
  - Cropping allows the selection of a region of interest if required by the user. When the **Crop Images** button is pressed, an interactive rectangle object appears on the image in the left axis object. This object can be dragged around until a desired region is selected, after which the user must right click inside the rectangle and select **Crop Image**. All loaded images are then cropped using the same coordinates and the first image of the list is displayed on the left axis.
- **Median Filter**
  - Applies a 3 by 3 median filter on images to reduce noise.
- **Unsharp Filter**
  - Applies a 3 by 3 unsharp filter to sharpen image features.
- **Adjust contrast**
  - Increases image contrast through intensity stretching. This process increases the dynamic range of the image by stretching a specific intensity range over all available intensity levels.

## Segmentation

Segmentation of the processed images can be accomplished through two methods. The segmentation process creates a binary image where edges are identified as 1s and the background as 0s.

- **Laplacian of Gaussian operator**
  - Discrete Laplacian filter convolved with a Gaussian smoothing filter used to obtain the gradient magnitude of an image. Highly sensitive to edges but also to noise.
- **Canny Operator**
  - Image gradient approximation followed by non-maximum suppression and edge tracing through hysteresis. Less sensitive to noise but also to edges in areas of low contrast.

Three parameters can be specified for each method:

- **Threshold**
  - Multiplier used to adjust the automatically-computed threshold for edge tracing.

- Only pixels having a larger gradient and/or gradient magnitude than the threshold are selected, so increasing the threshold will reduce the number of identified edges to retain only the strongest ones.
- **Gaussian filter sigma**
  - Both segmentation techniques apply a Gaussian filter to smooth out image noise.
  - The standard deviation (sigma) of the filter can be selected by the user. A larger value increases the amount of blur which can assist segmentation when noise is present.
- **Object cleaning (Remove all objects with less pixels than \_ )**
  - After segmentation, all connected objects are identified and objects having a smaller size than the user determined limit are deleted. This cleans up the image for easier processing.

Segmentation occurs when the **Process files** button is pressed.

## Volume Measurement

Following segmentation, the volumes of the images aliquots can now be measured.

- **Select Calibration Area**
  - After clicking the **Select Calibration Area** button, the user is prompted to select the calibration aliquot by clicking anywhere inside its outline. The nearest 4 objects to the click are then retained, bridged, morphologically filled and displayed on the image with a label identifying the area as the calibration area.
- **Select Volumes to Measure**
  - After clicking the **Select Volumes to Measure** button, the user is prompted to select aliquots to measure. An unlimited number of aliquots can be selected simultaneously.
  - After selection of each aliquot, the same process is applied as described in the calibration section above to obtain a filled area. Each new volume is labeled for ease of use.
  - To end the selection process, the user must press the **Enter** key.
- **Measure Volumes**
  - When the **Measure volumes** button is pressed, the areas of all selected aliquots are computed and ratioed against the calibration aliquot to obtain their volumes. The volumes are displayed in the **Volumes** table underneath the **Measure volumes** button, along with the mean and relative standard deviation of the same volume across different files.

## Test Image

A test image ('test image.png') is included in the archive. The top chamber contained a 10  $\mu$ L aliquot, while the other chambers were injected with a 20 and 40  $\mu$ L aliquot, respectively. This image can be used to verify the different parameters of the GUI and practice its use.

## Tips

To obtain appropriate segmentation, it is important to find the right **Threshold** and **Standard deviation** settings for your application. In the case of:

- Incompletely segmented images (not a closed outline)
  - Your **Threshold** is set too high or the **Standard deviation** is set too low.

- Noisy images
  - Enable median filtering to reduce the bulk of the noise.
  - Increase the ***Threshold*** until all but the desired outlines remain.

## Contact

If you have any questions about the GUI, its use or the method behind it, please contact Alexei Kazarine at [alexei.kazarine@mail.mcgill.ca](mailto:alexei.kazarine@mail.mcgill.ca)