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Image-based Analysis of Droplets in Microfluidics

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***Abstract*—In order to design a microfluidic device that can produce monodispersed encapsulated enzymes as droplets, it is essential to be able to evaluate the system during its development. An automated method to determine the size of the droplets as well as a method to tag and track droplets as they move in the system is desirable for system evaluation. We apply the Hough transform for circles to determine droplet size. Most of the droplets in the images are detected, and the best results are obtained at 20x magnification. We also test the ability of the ImageJ 'particle tracker' plugin to determine the behaviour of the droplets as they move in microfluidic systems. It is effective in tracking droplets that travel less than 50 pixels between frames.**

I. Introduction

Microfluidics, which emerged in the 1980s, is the manipulation of fluids flowing through channels with dimensions as small as tens to hundreds of micrometers. Microfluidic systems use very small amounts of samples and reagents, are of low cost, have a low analysis time and can perform separations and detections with high resolution and sensitivity [1]. Another advantage of microfluidics is the manipulation of multiphase flows which enables the generation and manipulation of droplets within the system. This characteristic of microfluidics allows for the generation of encapsulated enzymes within the system as droplets. Encapsulated enzymes are used in bioreactors to remove waste metabolites and to correct inborn metabolic deficiencies [2] [3].

Imaging has been used to analyse microfluidics systems for applications ranging from blood type determination [4] to the investigation of samples of tissues and cells [5]. Image analysis of droplets provides important information, such as droplet size, used to generate usable encapsulated enzymes. We apply the Hough transform for circles to this end.

When designing a microfluidic system to produce droplets, it is very useful to understand the behaviour of the droplets as they move through different parts of the system and how their characteristics change when parameters such as fluid flow rate is varied. We test the ability of an image analysis method to accurately determine the behaviour of the droplets in microfluidic systems.

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II. Methods

A. Materials and Image Acquisition

The study made use of images obtained from experiments conducted by the CSIR in Pretoria on microfluidic systems. The experiments involved the formation of droplets and examination of their movement within the channels of the systems. The microfluidic systems were designed and manufactured by the Materials Science and Manufacturing Department of the CSIR. Liquids pumped through the microfluidic systems resulted in droplet formation. The microchip was placed under an Olympus CKX41 microscope. Images and video were captured using a Casio EX-F1 digital camera mounted on the microscope. The images were calibrated using the known geometries of the microfluidic systems determined in the design phase of the fabrication. For a 20x magnification image, 273 pixels were equal to 50μm. Images were captured of droplets in microfluidic systems at three magnifications (4x, 10x and 20x) for droplet size determination. Video footage of droplets moving through various microfluidic systems at different flow rates was captured at 10x magnification for droplet tracking. All image processing was done using ImageJ.

B. Droplet Size Determination

The Hough transform for circles was applied because of the circular nature of the droplets. The images were pre-processed using a Sobel edge detector to highlight changes in pixel intensity. Vertical and horizontal derivatives are generated from two 3 × 3 convolution kernels. The final image is given by combining the derivatives, using the square root of the sum of the squares. The images were converted to binary by analyzing the histogram of the image and setting an automatic threshold level. A test threshold (mean image intensity) is taken and the pixels at or below the threshold are assigned to the background class and those above are assigned to the foreground (object) class. The four corner pixels of the image are assumed to be background. The average of the foreground and background pixels is calculated, the threshold is incremented to the mean of the two class means and the process is repeated. The threshold is found when

$$Threshold > (average\ background\ + average\ objects) \div 2$$

The Hough transform [7] is a technique used to find curves in images and can be extended to find circles by replacing the equation of a curve with the equation of a circle in the detection process. It tolerates noise and occlusion. The Hough transform uses an array, called an accumulator, to detect the existence of a curve. The dimension of the accumulator is equal to the number of

unknown parameters. A circle with radius R and centre (a,b) can be described by the following parametric equations:

$$x = a + Rcos(\theta)$$
$$y = b + Rsin(\theta)$$

The transform finds the parameters (a,b,R) to describe circles in the image. The Sobel gradient calculates the gradient of image intensity at each point in the image. The result will show the likelihood of any point in an image representing an edge, and its likely orientation. If an edge is detected, the parameters of the edge will be calculated and the value of the accumulator cell in which the parameters fall will be incremented. The cells with the highest values indicate the most likely lines or circles.

C. Tracking of droplets

The tagging and tracking of droplets provides information to evaluate the design of microfluidic systems. The information may be used to determine the behaviour of the droplets as they move through different parts of the system and to determine how this behaviour changes as the flow rates of the fluids are varied. The tracking method, namely the 'particle tracker' plugin in ImageJ, is based on a feature point tracking algorithm [6]. In the case of this study the feature points were the bright drops and the neighbourhood was the dark background. The tracking process does not require previous mathematical modelling of the motion; it self-initialises, it can handle temporary occlusions and droplet appearance and disappearance from the image.

The image stacks were pre-processed with a median filter to reduce the noise in each frame and Gaussian blur to smooth each frame. A sharpening filter, consisting of spatial convolution with a kernel was applied to sharpen the edges of the droplets in each frame after application of the Gaussian blur. The Otsu method [8] was used for thresholding. It assumes the image contains only foreground and background pixels and then separates the foreground pixels from the background pixels to minimize their intra-class variance.

The 'analyze particle' command in ImageJ was used to select the droplets in each frame as regions of interest and separate them from the rest of the image. The 'analyze particle' command counts and measures objects in binary or thresholded images. It scans the image until it finds the edge of an object, outlines the object and then fills it, and then moves on to find the edge of a new object. The size (area) range of particles of interest can be specified and particles outside this range are ignored. This is particularly useful if it is not possible to eliminate all noise and artifacts during pre-processing. If all the particles of interest fall into a certain circularity range as with the images in this study, the circularity can be set to a certain range to eliminate the measurement of features which are not particles. The 'wand tool' in ImageJ is used in the 'analyze particles' plugin to outline objects. It selects thresholded pixels forming a continuous area by tracing an edge until it returns to the starting point to create a selection. The 'measure' command in ImageJ used in the 'analyze particles' plugin is used to obtain area and shape descriptors - the plugin measures the area of the particles as well as their circularity and roundness. The roundness is calculated by:

$$Roundness = 4 \times \frac{[Area]}{\pi \times [Major\ axis]^2}$$

The circularity, which ranges from 0 (infinitely elongated polygon) to 1 (perfect circle), is calculated by:

$$Circularity = 4\pi \times \frac{[Area]}{[Perimeter]^2}$$

The area and shape descriptors of the droplets provide a filter parameter to eliminate features which are not droplets so that only droplets remain as regions of interest. The regions of interest –droplets- are then converted to masks.
The tagging and tracking of droplets was achieved by using the 'particle tracker' plugin in ImageJ [6]. The algorithm consists of two parts: feature point detection and trajectory linking. The first part of the algorithm detects feature points in every frame of the video footage so that they can be linked in to trajectories later on. The feature point detection consists of the following four steps:

- Image restoration
- Estimation of point locations
- Refinement of point locations
- Non-particle discrimination

In the image restoration step the image is corrected for imperfections. Two different effects are accounted for during this step. The first is long wavelength modulations of the background intensity caused by non-uniform sensitivity among the camera pixels or uneven illumination. This is corrected for by assuming the feature points are small compared to the background variations. The second is discretization noise from the digital camera. The discretization noise from the camera is modelled uniformly Gaussian with a correlation length of λ_n = 1 pixel. The correlation length gives a measure of the range in which fluctuations in one area of the image influence those in another area. Estimating the feature point locations is achieved by finding the local intensity maxima in the filtered image that has been restored. The refinement of the point locations will reduce the standard deviation of the measured positions of the feature points. The non-particle discrimination part of the feature point detection is used to discard spurious detections which can be dust or particle aggregates.

In the second part of the algorithm, a linking algorithm finds points that correspond to the same particle in succeeding frames and links the particle positions into trajectories. The feature point detection algorithm is applied to each frame to produce a matrix for each frame containing the location of the points detected. The number of matrices produced is equal to the number of frames in the video.

Trajectories are formed by finding a set of relations between the locations of the points in succeeding frames so that a cost function is minimized. The algorithm is extended to consider several frames in each linking step to make provision for particle occlusion.

The aim of this part of the study was to tag and track droplets as they move in microfluidic systems. The first part of the 'particle tracker' algorithm detects feature points in each frame of the video, namely droplets. The user defined parameters for detection are: radius, cutoff and percentile. The radius parameter is the approximate radius of the droplets in the images given in pixel units. This parameter was chosen as slightly larger than the average droplet radius but smaller than the smallest inter-droplet separation. The cutoff parameter is a score for non-droplet discrimination [5]. The percentile parameter determines which bright pixels are accepted as droplets. All local maxima in the upper *r*th percentile of the image intensity distribution are potential droplets. These three parameters are used to detect droplets in each frame. All the video sequences contain droplets of similar size and are recorded at the same magnification. Therefore a radius of 8 pixels, a cutoff of 0 and a percentile of 0.1 were used for the droplet detection in this study.

The user defined parameters for trajectory linking are: displacement and link range. The displacement parameter is the maximum number of pixels that a droplet is allowed to move between two successive frames. The link range is the number of frames that is considered to determine the best possible association between droplets in succeeding frames. The link range is determined by first tracking the droplets with a default link range of 2. After viewing the results, the link range is adjusted to improve the linking of particles from one frame to another. In sequences where droplets travel long distances between frames, a larger link range should be used. The link range is useful to overcome droplet occlusion and droplet disappearance and appearance from frames. The parameter values will be different for each sequence.

III. Results and Discussion

A. Droplet Size Determination

The aim of this part of the study was to develop an automated method of droplet size determination. During image acquisition, 18 images were captured containing 3591 droplets for analysis. The images were acquired at three different magnifications; 4x, 10x and 20x magnification to determine at which magnification the method would work best.

The first test conducted using the Hough transform was to determine how many of the droplets could be detected, as a proportion of droplets in the image. The droplets were manually counted to determine the number of droplets in each image. Only complete droplets were counted. The results were summed for each magnification and are given in Table I.

TABLE I. Results of Droplet Detection

	Manual count	**Hough Transform**	**% difference**
4x	3219	4444	38.1
10x	326	403	23.6
20x	46	48	4.3

The Hough transform detects more droplets than were counted manually. This is because during the manual count, only complete droplets were counted and fractions of droplets were ignored. The Hough transform finds the parameters of the droplets even if they are not complete as shown in Fig. 1. The Hough transform does however give each circle it finds a strength value; the circles with high strength values are most likely to be complete droplets. We used a cutoff strength value of 100, 300 and 500 for images at 4x, 10x and 20x magnification respectively to differentiate complete droplets from partial droplets. The cutoff value is dependent on the quality of the images and the magnification at which the images were captured at. This is because the strength value is determined by how many pixels vote for the centre of a circle. Larger circles, at 20x magnification, will have more pixels voting because the circumference of the circle is larger. All the droplets considered for size measurement had a strength value of higher than 100, 300 and 500 for images at 4x, 10x and 20x respectively.

The second test conducted was to determine how the area of the Hough transform-segmented droplets compares with manually segmented ones. Each droplet was individually outlined and the number of pixels in it was calculated to give the area. The Hough transform outputs the radius of a droplet and the area is calculated using the formula for the area of a circle. Only a proportion of the droplets were manually selected because of the large number present in the images. The results are given in Table II.

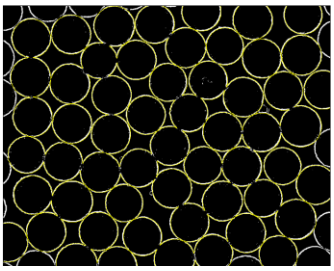

Figure 1: Example of droplet detection for the Hough transform.

TABLE II. Comparison of Hough Transform Area Measurements with Manual Measurements

	Average % difference	**Maximum% difference**	**Minimum% difference**
4x	4.7	22.4	0
10x	2.0	5.6	0.02
20x	1.0	2.2	0.2

The Hough transform performs very well when compared to manual measurement. It performs best with images at 20x magnification with the lowest maximum percentage difference.

B. *Droplet Tracking*

With a radius of 8 pixels, a cutoff of 0 and a percentile of 0.1, every droplet was detected in every frame. Fig. 2 gives an example of a droplet trajectory over 70 frames. For this video series a distance parameter of 15 pixels and a link range of 4 frames were used. Fig 3 gives an example of a droplet trajectory over 100 frames. In this figure it is noted that the trajectory jumps to another droplet instead of following the droplet under consideration. This phenomenon is very common when the droplets travel more than 50 pixels between frames.

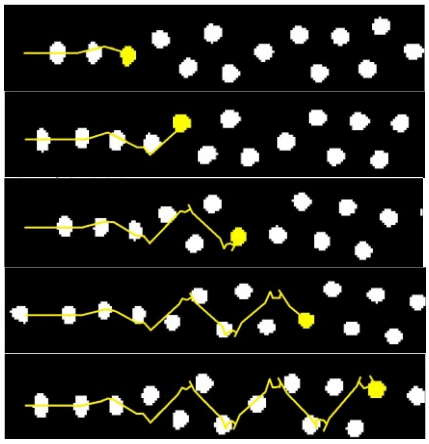

Figure 2: Example of a droplet (yellow) trajectory over 70 frames.

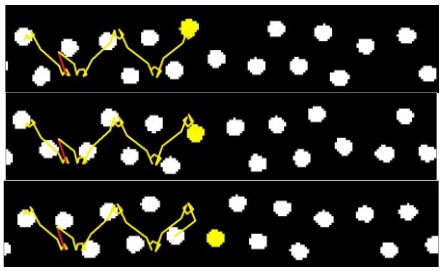

Figure 3: Example of a droplet (yellow) trajectory over 100 frames showing trajectory jump in red.

IV. CONCLUSION

The Hough transform has been used to determine the size of droplets for droplet distribution determination [9] and characterization of multiphase dispersions [10].The Hough transform detects most of the droplets in the images. The best results are obtained at 20x magnification, i.e. when the droplet size is largest. An improvement of the image quality at this magnification could provide even better results. The disadvantage of analyzing droplets at this magnification is that fewer droplets per image can be analyzed because of the high magnification. It therefore could be advantageous to analyze droplet at a lower magnification when more droplet data is needed in a short time.

Tracking algorithms [11] [12] similar to the 'particle tracker' algorithm have been used to track droplets. These algorithms use steps similar to the 'particle tracker' algorithm, although the way in which some of the algorithms are implemented is not well documented. The 'particle tracker' algorithm has the advantage of ease of implementation in a readily available image analysis package. It is effective in tracking droplets that travel less than 50 pixels between frames to determine their movement patterns. Increasing the frame rate at which the video sequence is captured will decrease the distance all the droplets travel between frames, which could prevent failure. The user has to input the user defined parameters as discussed above and can filter the trajectories to the frames of interest. The user can decide which particles or areas are of interest and the method outputs the results. The method thus can be customized to the user's needs. The results from this study provide information about the characteristics and behavior of droplets in microfluidics systems. This information can be used to aid system designers in manufacturing a microfluidic system that can produce monodispersed encapsulated enzymes – droplets - of a predefined size.

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