DROPLET TRACKING VELOCIMETRY (DTV): AUTOMATED MEASUREMENT OF DROPLET MOTION AND SHAPE USING DIGITAL IMAGE PROCESSING

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ABSTRACT

This paper presents droplet tracking velocimetry (DTV), an image processing algorithm for analyzing the time dependent behavior of droplets in digital videos. DTV provides information beyond particle image velocimetry (PIV), giving not only the droplet's velocity and trajectory, but also its size, shape deformation, pixel intensity, and other characteristics important for understanding dynamic behavior and for bioassays. While prior algorithms have been shown to analyze static droplet images, this is the first which tracks droplet characteristics over multiple frames. We apply DTV to several case studies from industry and academia, using the published videos without modification. The results demonstrate that DTV can serve as a useful tool for researchers in droplet microfluidics.

KEYWORDS: droplets, particle image velocimetry, particle tracking velocimetry, image processing, detection

INTRODUCTION

One of the practical challenges in droplet microfluidics is the physical characterization of droplet populations. Emerging droplet-based high throughput assays require the measurement of droplets' physical properties including size distribution [1], shape deformation [2], as well as migration trajectory and velocity [3-4]. Particle image velocimetry (PIV) has long been used for analyzing single-phase flow [5], and has also been used to study microflow patterns within droplets [6]. However, there is currently no method to track the time-dependent movement and shape of droplets as a whole. PIV, which generally relies on opaque or fluorescent flow tracers, is difficult to apply to translucent droplets. Colloidal techniques like dynamic light scattering (DLS) can provide average size of droplet populations, but generally do not measure properties at the individual droplet level.

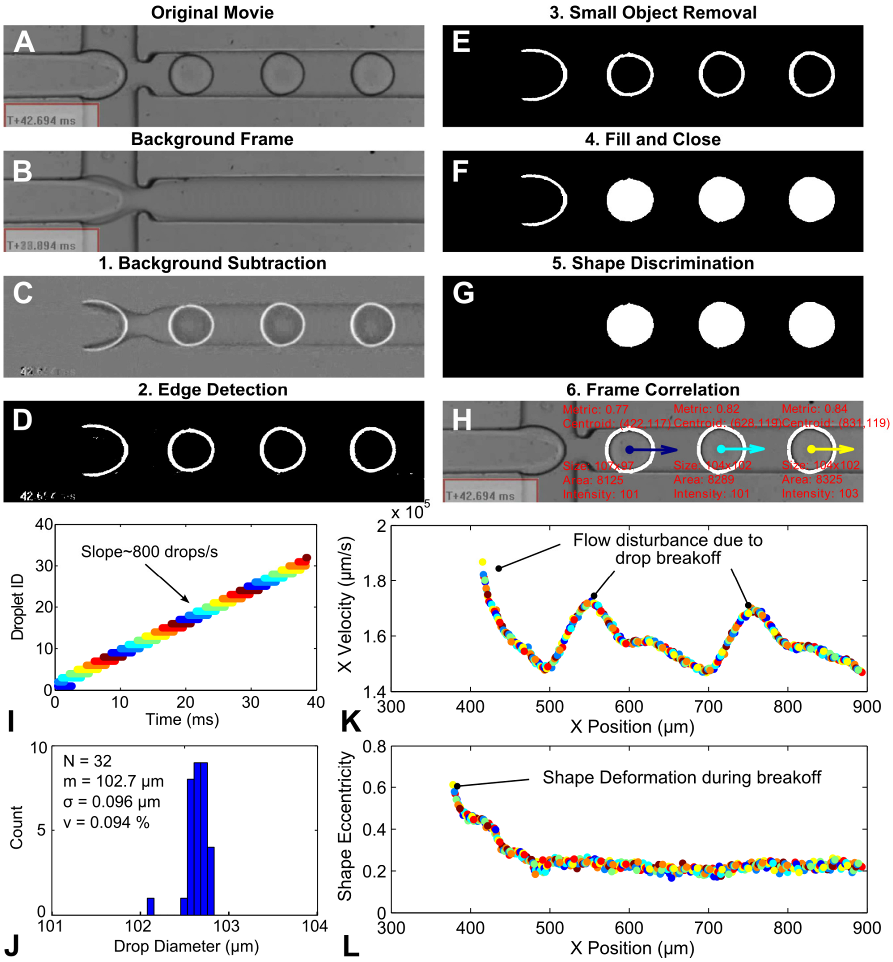

Fig 1: (A-H) DTV image processing steps. (I) Measurement of drop generation frequency. (J) Histogram of drop sizes (N=32). (K) Droplet velocity at different positions along the channel, revealing flow perturbations. (L) Shape deformation vs. position, showing the distance required for a detached drop to obtain a spherical shape.

CONCEPT

DTV is a digital image processing algorithm which tracks droplet size, trajectory, velocity, deformation, and many other parameters through frame-by-frame video analysis. DTV does not require tracer particles or a traditional PIV setup; instead, it exploits the dark boundary surrounding the droplet which occurs naturally due to surface curvature and the difference in refractive index between the two phases. It also utilizes a background subtraction scheme to improve its accuracy. The use of image processing on static images has been shown, for example, in [1], which used a Hough transform to identify circular droplets. DTV, by contrast, can identify and track droplets over multiple frames, and the algorithm can also be applied to non-spherical drops (ie plugs).

Fig. 1 illustrates the DTV processing steps applied to a video of a Fluigent drop generator. They include: (1) *Background frame generation*. The ideal background frame is simply an image of an empty channel. If such an image is not available, a background frame can be auto-generated by averaging several frames, or similar statistical combinations. (2) *Background subtraction and image inversion*. Subtracting the sample image from the background creates a grayscale image with a white boundary around droplets. (3) *Edge Detection*. The grayscale image is converted to binary using a threshold or edge detection filter. (4) *Small object removal*. Contiguous objects with area smaller than a user-defined threshold are removed. This is helpful for filtering noise or erroneous objects. (5) *Morphological fill*. The droplet boundaries are closed, forming well-defined, filled regions often referred to as 'blobs' in machine vision. (6) *Shape Discrimination*. Regions not meeting a user-defined circle metric are removed, and the remaining are recorded as droplets. (7) *Frame correlation*. The algorithm attempts to link each droplet to a match in prior frames based on user-defined criteria. DTV repeats the above steps for each movie frame, tabulating a database of droplets. For each droplet at each timepoint, DTV records 18 parameters, including (1) droplet ID (an identifier for each unique droplet), (2) frame number, (3) time, (4) x coordinate, (5) y coordinate, (6) x velocity, (7) y velocity, (8) total velocity, (9) area, (10) major axis length, (11) minor axis length, (12) equivalent diameter, (13) orientation, (14) shape eccentricity, (15) circle metric, (16) mean pixel intensity, (17) maximum pixel intensity, and (18) minimum pixel intensity. The database can be used to report on individual droplets, or for statistical analysis of droplet populations. The software overlays the boundary and velocity vector of each droplet for illustrative purposes, and provides plots and data export for quantitative analysis.

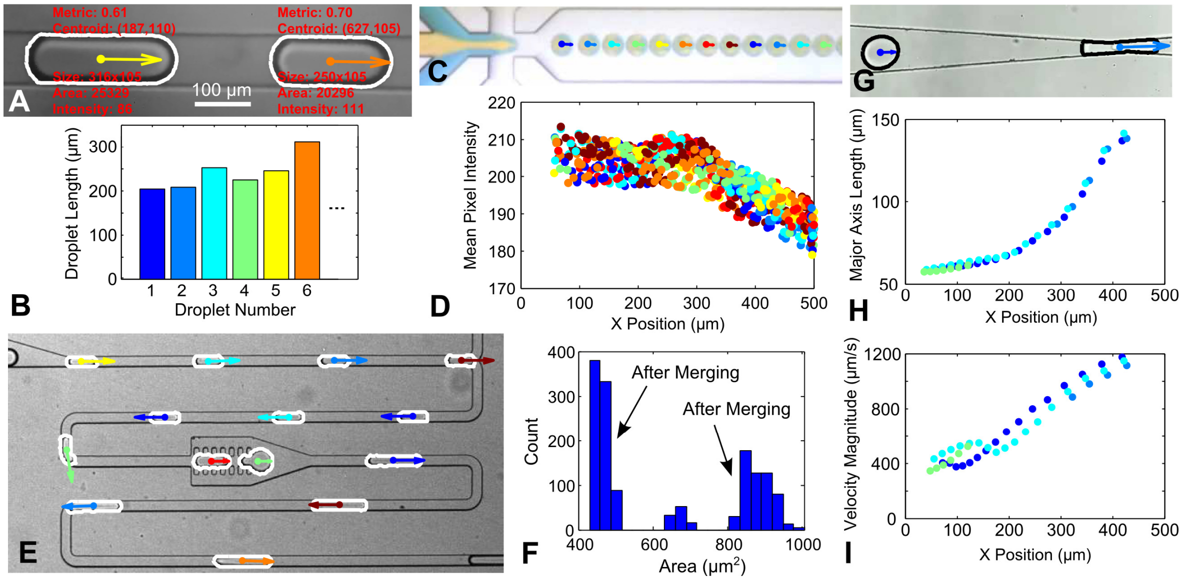

Fig. 2: Examples illustrating the capabilities of DTV. (A-B) Measuring the length of non-circular plugs (C-D) Quantifying droplet mixing by tracking pixel intensities in each droplet. (E-F) Measuring size distributions in a drop merging device. (G-I) Quantifying the elongation and increased velocity of a drop at a channel narrowing.

RESULTS

To demonstrate its utility, DTV is applied to a wide variety of case studies reported in both academia and industry. By tuning parameters in the image processing steps, DTV can be applied to a variety of videos without modifying them. Typical results for the Fluigent drop generator are shown in Fig. 1I-L. The drop generation frequency can be extracted by taking the slope of the graph showing droplet ID vs. time. Size distributions are reported in histograms. Interestingly, DTV can reveal subtle flow fluctuations which are difficult to measure experimentally. In this case, the velocity plot reveals periodic disturbances to the flow due to repeated drop breakoff upstream.

Other case studies are shown in Figs. 2-3. Fig. 2A demonstrates the ability of DTV to recognize non-circular plugs. This example records variation in plug length over time. Fig. 2C-D illustrates quantitative analysis of a drop mixing chip from Dolomite microfluidics. The mixing of the blue and yellow dyes within drops can be measured by tracking the mean pixel intensity in each drop as a function of channel position. If used with monochrome fluorescent images, this technique can be useful for cell and enzyme assays in droplets. Fig. 2E-F illustrates the velocity and size analysis of droplets in a pillar-induced drop merging chip [7]. Here, DTV is used to track the plug areas over 231 frames. After two adjacent droplets merge in the pillar structure, the merged droplet is identified as a new entity. A histogram shows two primary distributions representing the plugs before and after the mixing junction. A third population in an intermediate size range can also be seen. This video also demonstrates the ability of DTV to track motion through curved channels.

In Fig. 3, DTV is used to analyze droplet trajectories and changes in shape during tensiophoresis, the migration of droplets in an interfacial tension gradient [3-4]. Tensiophoresis utilizes microgradients in surfactant concentration to sort droplets without chemical labels or on chip actuators. As droplets travel from the low to the high surfactant stream due to capillary migration, the non-uniform capillary pressure across the droplet causes it to deform. DTV can be used to quantify both velocity and deformation during drop migration. (The DTV software was originally developed for analyzing tensiophoretic migrations). Since the migration velocity scales inversely with protein concentration [4], DTV can help provide an indirect measure of protein concentration in a droplet.

Fig. 4 demonstrates the scalability of DTV. Here, the algorithm is used to track the movement of >200 droplets in a meandering channel [8]. The heat maps show the horizontal and vertical velocities of the droplets over 30 frames and >5000 measurements. In large scale analysis, resolution and video quality are of critical importance. Each droplet should span a sufficiently large number of pixels in order for the software to accurately identify it and quantify its properties. Extrapolating the results from this low resolution video suggests that with high definition (HD) video, DTV will be able to detect 100's of drops (and potentially >1000 drops) per frame.

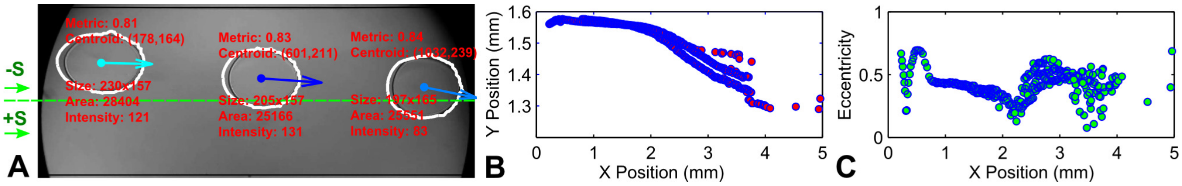

Fig. 3: DTV used to quantify drop migration velocities and deformation during tensiophoresis [3-4].

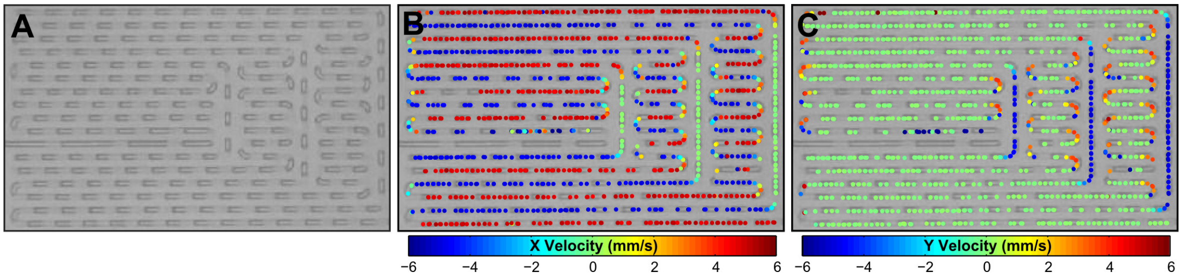

Fig. 4: High-throughput plug velocity analysis. (A) Original movie [8], with >200 plugs in a meandering channel. DTV is used to measure plug velocity over 30 frames. (B-C) Heat map of the horizontal and vertical velocity.

CONCLUSIONS

In each of the above case studies, DTV gathers comprehensive information on droplet characteristics which are not easily measured using existing techniques. When coupled with high speed and high resolution cameras, DTV can provide precise tracking measurements valuable to researchers in multiphase and digital microfluidics. As with any image processing algorithm, DTV becomes more accurate with increasing video resolution; however, the tradeoff is the increased analysis time. The current algorithm can attain 2-20 frames per second. Real time analysis can potentially be achieved if performance can be improved with efficient compiled algorithms, or with the help of hardware accelerators such as graphic processing units (GPUs) or field programmable gate arrays (FPGA).

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