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

# Point-of-care blood coagulation assay enabled by printed circuit board-based digital microfluidics

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## Supplementary Figures

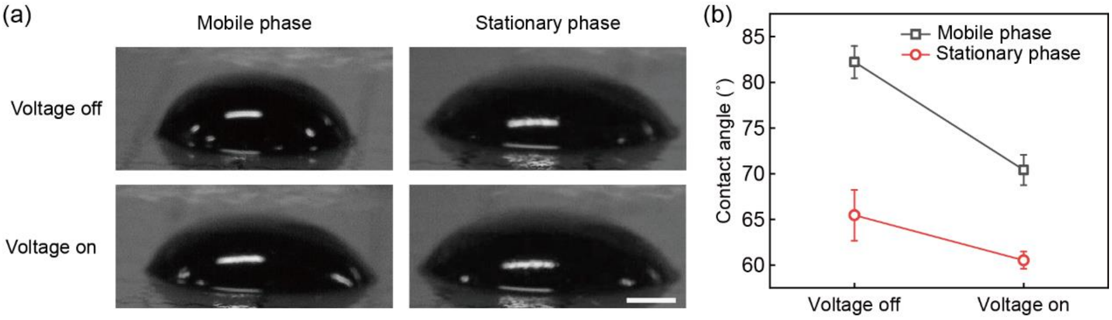


**Figure S1**. Contact angles of the blood drops on the electrode array in the mobile and stationary phase with voltage (230 V) off and on. (a) Micrographs showing the side view of the blood drops on the electrode array. Scale bar, 1 mm. (b) Bar plot of the measured contact angles. Data represent mean ± SD with n = 3.

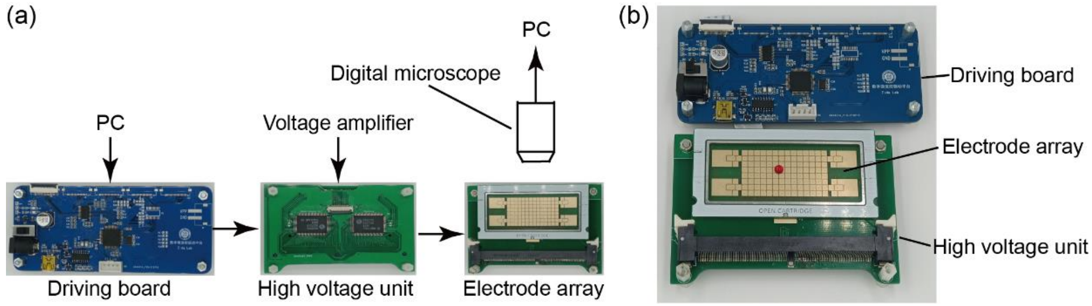


**Figure S2**. (b) Hardware diagram of the platform. A computer interfaces and programs the driving board, which sends voltage signals to the high voltage unit, which takes in high voltage from the voltage amplifier and directly controls the voltages of each electrode on the electrode array. A digital microscope captures the motion of the sample, and the images are then processed by a computer. (b) Photograph of key components assembly of the platform. The driving board connects to the high voltage unit through a flexible printed circuit (FPC) connector, and the high voltage unit connects to the electrode array through a card edge connector.

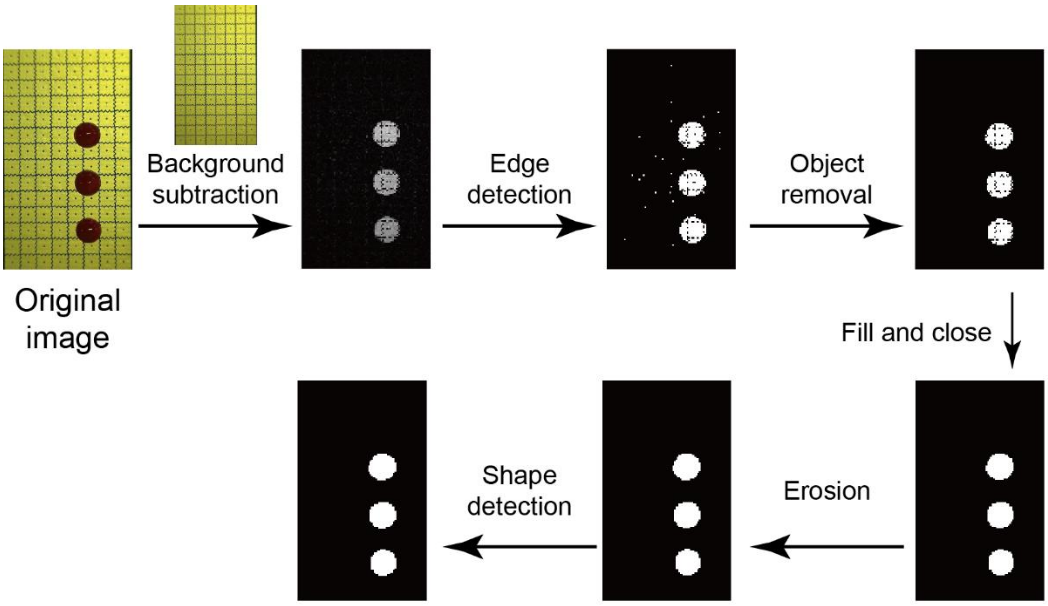


**Figure S3**. The image analysis pipeline for the calculation of the velocity of blood drops. A synthesized background image is subtracted from each frame of the video, and edge detection, object removal, fill & close, erosion, and shape detection are subsequently applied on the resultant image to generate a binary image with blood drops segmented. Droplet Morphometry and Velocimetry (DMV) software[1] is used for image processing.

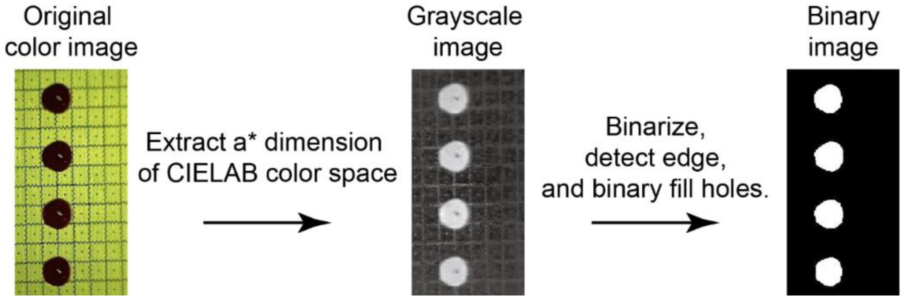


**Figure S4**. The image analysis pipeline for the calculation of the length of blood drops. The original image in RGB color space is converted to CIELAB color space, and the a* dimension is extracted and converted to a grayscale image. The resultant grayscale image is then binarized before edge detection and binary fill holes are performed to generate a binary image with blood droplets segmented. The length of the blood drop is defined as the length in the stretching (horizontal) direction. The code sample is included in this Supplementary Information.

## Supplementary Text

Code sample for the analysis of the droplet length in MATLAB.

```
% Define parameters
filename='*.avi'; % input the file name
dropletsNum = 2; % the number of droplets to be detected
[ROI_x1, ROI_y1, ROI_x2, ROI_y2] = deal(1, 1, 1280, 960); % region of interest
BW_THRESHOLD = 0.6; % threshold for binarization
Minimum_Droplet_Size = 1000; % in pixels. Smaller objects droplets will be removed.
Minimum_Droplet_Distance = 50; % in pixels
startTime = 0; % in seconds
endTime = 30; % in seconds

% Load video
Video_in = VideoReader(filename);% VideoReader object
framesNum=floor(Video_in.FrameRate * Video_in.Duration); % total frame number
frameRate=Video_in.FrameRate; % frame rate

% Initialize output CIELAB video
[~, filename_clean,~] = fileparts(filename);
Video_out= VideoWriter(strcat(filename_clean, '_CIELAB')); % output video in avi format
Video_out.FrameRate = frameRate;
open(Video_out);

% Initialize output video with droplet length indicated for quality control
Video_out_length= VideoWriter(strcat(filename_clean, '_length')); % output video in avi format
Video_out_length.FrameRate = frameRate;
open(Video_out_length);

% Initialize log file
log_id = fopen(strcat(filename_clean, '_log.txt'),'w');

% Initialize matrices
droplet_length = zeros(framesNum, dropletsNum);
droplet_loc = zeros(framesNum, dropletsNum);

for id = 1 : framesNum
    videoFrame = read(Video_in,id);
    videoFrame = videoFrame(ROI_y1:ROI_y2, ROI_x1:ROI_x2,:);
    fprintf(['Processing Frame No. ' num2str(id) '\n']);

    % preprocessing
    videoFrame_lab = rgb2lab(videoFrame); % Convert to LAB space
    videoFrame_gray = mat2gray(videoFrame_lab(:,:,2));% Convert to grayscale
    writeVideo(Video_out, videoFrame_gray); %Write frame to final video file
```

```
        videoFrame_BW = imbinarize(videoFrame_gray, BW_THRESHOLD);% Convert to BW image

        %Use region properties to remove small objects.
        cc = bwconncomp(videoFrame_BW);
        stats = regionprops(videoFrame_BW, 'Area');
        idx = find([stats.Area] > Minimum_Droplet_Size ); % Minimum_Droplet_Size is defined in the
Parameter section.
        videoFrame_object_removal = ismember(labelmatrix(cc),idx);
        se = strel('disk',10);        % 'se' is for image close operation
        videoFrame_close = imclose(videoFrame_object_removal, se); % Image close operation
        videoFrame_fill = imfill(videoFrame_close, 'holes');      % Image fill
        % Calculate droplet length
        length_x = sum(videoFrame_fill, 2);
         [length_temp, loc_temp] = findpeaks(length_x, 'MinPeakDistance',
Minimum_Droplet_Distance);
        if size(length_temp, 1) == dropletsNum
            droplet_length(id,:) = length_temp;
            droplet_loc(id,:) = loc_temp;
         else
            disp("Unexpected number of droplets detected!");
            fprintf(log_id, ['Unexpected number of droplets detected at Frame No. ', num2str(id),
'!\n']);
        end
        % Write Quality Control video
        videoFrame_fill2 = videoFrame_fill;
        videoFrame_fill2(loc_temp, :) = 1;
        writeVideo(Video_out_length, mat2gray(videoFrame_fill2));
        % Show images - uncomment when necessary
        % figure; imshow(videoFrame); title('Original image');
        % figure; imshow(videoFrame_gray); title('Grayscale');
        % figure; imshow(videoFrame_BW); title('Binary');
        % figure; imshow(videoFrame_object_removal); title('Object removal')
        % figure; imshow(videoFrame_close); title('Closed');
        % figure; imshow(videoFrame_fill); title('Filled');
end
% Write data file
writematrix(droplet_length, strcat(filename_clean,'_droplet_length.txt'), 'Delimiter','tab');
writematrix(droplet_loc, strcat(filename_clean,'_droplet_loc.txt'), 'Delimiter','tab');

% Write log file
fprintf(log_id, ['Video name: ', filename,'\n']);
fprintf(log_id, 'Region of interests (ROI_x1, ROI_y1, ROI_x2, ROI_y2):\n');
fprintf(log_id, '%d\t%d\t%d\t%d\n', [ROI_x1, ROI_y1, ROI_x2, ROI_y2]);
fclose(log_id);
```

```
% Close video file
close(Video_out);
close(Video_out_length);
```

## Supplementary Videos

**Video S1**. Captured video from the experiment and the corresponding temporal curves of velocity and length of blood drops.

**Video S2**. Video showing the motion of blood drop at different phases and the results of drop segmentation.

**Supplementary Table**

**Table S1.** Comparison of this assay with other works.

| Tools | Droplet microfluidics | Acoustic waves | | Laser speckle | Micropost array | Electrical impedance | | Digital microfluidics |
|---|---|---|---|---|---|---|---|---|
| **Reference** | [2] | [3] | [4] | [5] | [6] | [7] | [8] | This work |
| **Sample type** | Whole blood | Blood plasma | Blood plasma | Whole blood | Whole blood | Whole blood | Whole blood | Whole blood |
| **Assay time** | 40 min | 70 s | 4 min | 10 min | 40-60 min | 30 min | 40 min | 20 min |
| **Multiplexity** | Single test | Multiple test | Single test | Single test | Multiple test | Single test | Single test | Multiple test |
| **Detection target** | Viscosity tracing | Particle mobility | Frequency response | Speckle intensity fluctuations | Clot stiffness | Electrochemical impedance spectrum | Electrical impedance | Velocity tracing and clot stiffness |
| **Sample volume** | 100 μL | 1.5 μL | 1 μL | 40 μL | 17 μL | 17 μL | 17 μL | 17 μL |

## References


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