Microfluidic Centrifugation Assisted Precipitation based DNA Quantification

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Supplementary data for

1) Section 1: Lab on DVD principle

Fig.S1 Assembly of the modified DVD platform: spinning DVD motor, DVD laser, photodiode array, DVD substrate connected to image generation software on a computer.

2) Section2: MATLAB code for DNA quantification

%----------------------------------------------------------------------------
% Code to illustrate DNA quantification.
captionFontSize = 14;
% Read in a standard image
ImageName = 'Image1.png';
InfoImage = imfinfo(ImageName);
B= InfoImage.FileSize;
F= 9085414/B;
DNAImage = imread(ImageName);

%Image size standardisation

% grayscale image
DNAImage = rgb2gray(DNAImage)
% Step 1: Image check to distinguish between blank and precipitate
figure
hold on
imshow(DNAImage)
DNAImage = imcrop(DNAImage)

subplot(2, 2, 1);
imshow(DNAImage);
% Maximize the figure window.
set(gcf, 'units','normalized','outerposition',[0 0 1 1]);
drawnow;
caption = sprintf('Original Image');
title(caption, 'FontSize', captionFontSize);
axis image;
% Histogram generation and display.
[pixelCount, grayLevels] = imhist(DNAImage);
%bar(grayLevels, pixelCount, 'FaceColor', 'r');

% Binary image generation, thresholdValue = 95 for DVD images

thresholdValue = 95;

binaryImg = (DNAImage < thresholdValue);
Sum2=0
for k=85:thresholdValue
    Sum2=Sum2+ pixelCount(k)*((255-grayLevels(k))/255)
end

binaryImg = imfill(binaryImg, 'holes');

% Image to concentration conversion Factor 115 determined experimentally from
% the images. For the highest concentration
% 129 ng/ul this factor gives a precipitation area of 1290 a.u. This gives
% a resolution of 1 for every 0.1 ng/ul change in DNA concentration.
% This factor is separate for Camera images.

Sum= Sum2/115
subplot(2, 2, 2);
bar(pixelCount);
%title('Histogram of DNA image', 'FontSize', captionFontSize);
xlim([0 255]); % Scale x axis manually.
grid off;

% Display the threshold as a line on histogram
hold on;
maxYValue = ylim;
line([thresholdValue, thresholdValue], maxYValue, 'Color', 'b');
% Place a text label on the bar chart showing the threshold.
annotationText = sprintf('Thresholded at %d gray levels', thresholdValue(end));

% Display the binary image.
subplot(2, 2, 3);
imshow(binaryImg);
title('Binary Image, obtained by thresholding', 'FontSize', captionFontSize);

% For blank image elimination
if (Sum2>20)

DNAImage = imadjust(DNAImage,[0.45 0.55],[]);
figure
imshow(DNAImage)

% Step 2: Second part if image is not blank
% Crop the image to keep the region containing the DNA precipitate
DNAImage = imcrop(DNAImage)

% Display the grayscale image.
% Image adjustment: it works for most images from the DVD. This contrast step
% makes the DNA look prominent over its background which is necessary for its
% quantification.
subplot(2, 2, 1);
imshow(DNAImage);
% Maximize the figure window.
set(gcf, 'units','normalized','outerposition',[0 0 1 1]);
drawnow;
caption = sprintf('Original Image');
title(caption, 'FontSize', captionFontSize);
axis image;
% Histogram generation and display.
[pixelCount, grayLevels] = imhist(DNAImage);

% Binary image generation
s=nonzeros(pixelCount)
A=max(s)

Y = quantile(s,[0,0.8])
thresholdValue = find(pixelCount>Y(2),1);

binaryImg = (DNAImage < thresholdValue);
Sum2=0
for k=1:thresholdValue+10
    Sum2=Sum2+ pixelCount(k)*((255-grayLevels(k))/255)
end

binaryImg = imfill(binaryImg, 'holes');

% Factor 115 (Multiply F by suitable value for Camera images) below is to ensure 129 ng/ul in 10 ul volume, this factor gives a precipitation area of 1290 a.u. This gives a resolution of 1 for every 0.1ng/ul change in DNA concentration. So, in essence the value of the precipitation level by adjusting F should be fixed as the product of known concentration and %volume of sample.

%Sum= (Sum2*(F)/115)/10.46 +14
%Calculating unknown quantity of DNA
Sum= Sum2*F/115
%Calculating precipitation level
subplot(2, 2, 2);
bar(pixelCount);
%title('Histogram of DNA image', 'FontSize', captionFontSize);
xlim([0 20]); % Scale x axis manually.
ylim([0 1800])
grid off;

% Display the threshold as a line on histogram
hold on;
maxYValue = ylim;
line([thresholdValue, thresholdValue], maxYValue, 'Color', 'b');
% Place a text label on the bar chart showing the threshold.
annotationText = sprintf('Thresholded at %d gray levels', thresholdValue(end));

% Display the binary image.
subplot(2, 2, 3);
imshow(binaryImg);
title('Binary Image, obtained by thresholding', 'FontSize', captionFontSize);

subplot(2, 2, 4);
imshow(DNAImage);
title('Outlines, from bwboundaries()', 'FontSize', captionFontSize);
axis image;
hold on;
end
3) Section 3: Gel Electrophoresis

Fig S2. A qualitative evaluation of the LAMP assay with Gel Electrophoresis method with eight 10 fold diluted concentrations of target DNA and two control samples with no DNA (Template –ve) and no DNA template and primers (Double –ve), respectively. Each of these samples was of 5 µl volume taken out from the DVD after LAMP amplification, for evaluation.

4) Section 4: LAMP products

Fig S3 Images generated from DVD with ten serial 10 fold dilutions of plasmid DNA starting with a template concentration of 0.2ng/µl to 0.02fg/µl in 5 µl volume negative control and blank with no DNA or amplification mix. Scale bar: 1 mm.

Supporting video captions

Movie S1

The supplementary video shows the aggregation process of DNA-GelRed complex upon insertion in a U-shaped channel of a modified DVD substrate. The entire process of precipitate formation is demonstrated starting with formation of mini flake like aggregates to slow precipitation upon centrifugation. The video is captured in real time, hence the entire process takes about 30 seconds.