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

Microfluidic Centrifugation Assisted Precipitation based DNA Quantification

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Supplementary data for

1) Section 1: Lab on DVD principle

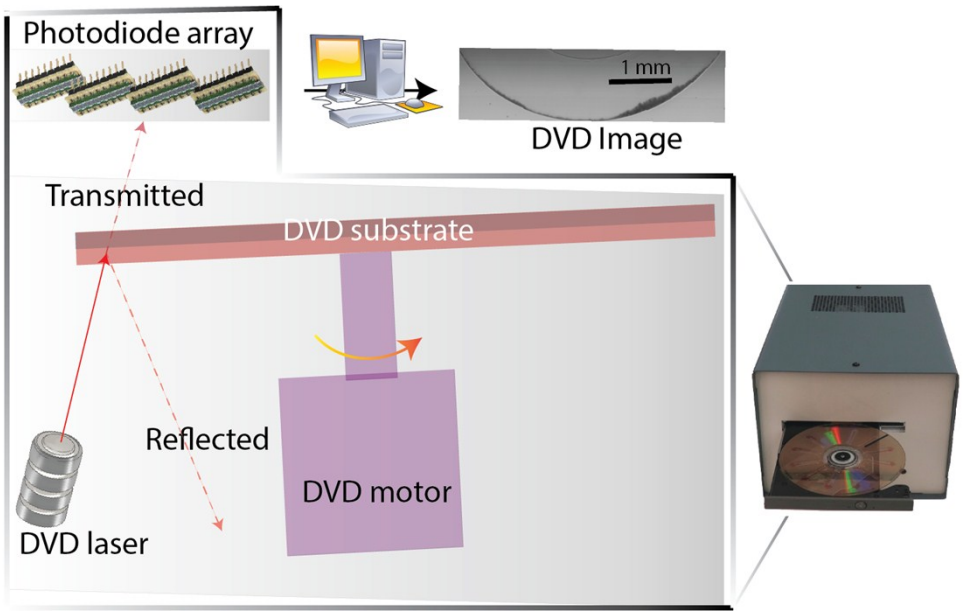

Fig.S1 Assembly of the modified DVD platform: spinning DVD motor, DVD laser, photodiode array, DVD substrate connected to image generation software on a computer.

2) Section2: MATLAB code for DNA quantification

```
%-------------------------------------------------------------------------
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% 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
```
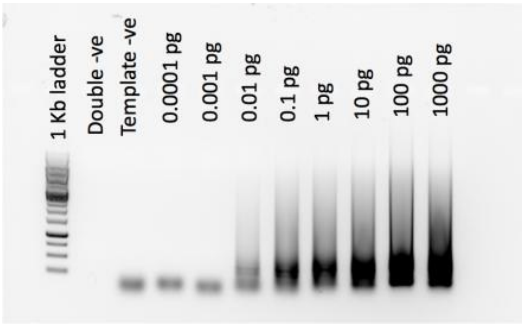

```
% The U shape border on the blank image has a threshold between 85 and 95
%  when generated by the Lab on DVD player
% If the code sees the precipitate on the image then only it proceeds ahead
% to the next step. Separate threshold range for U shape border in CMOS
% sensor images.

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 0.1ng/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



FigS2. 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 sample was of 5 μl volume taken out from the DVD after LAMP amplification, for evaluation.

4) Section 4: LAMP products

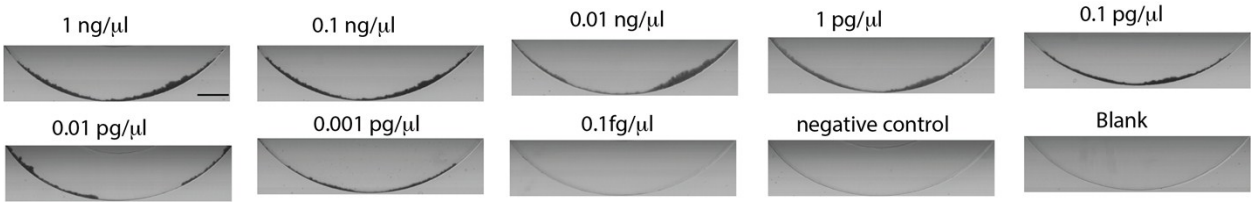


Fig.S3 Images generated from DVD with ten serial 10 fold dilutions of plasmid DNA starting with a template concentration of 0.2ng/μl to 0.02fg/μl in 5 μl volume negative control and blank with no DNA or amplification mix. Scale bar: 1 mm.

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.