Direct DNA Analysis with Paper-Based Ion Concentration Polarization

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by,

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Introduction

- DNA analysis is essential for diagnosis and monitoring of many diseases.
- Conventional DNA testing relies on methods generally limited to the laboratory e.g. PCR.
- The flow cytometry based sperm chromatin structure assay (SCSA) is the standard for sperm DNA integrity assessment.
- Nanostructured sensors show potential for scalable analysis of DNA e.g. nanopores, single and arrayed nanoparticles, nanostructured microelectrodes.
- These nanostructures have also been integrated into microfluidic and nanofluidic systems, with demonstrated performance comparable to conventional DNA testing technologies. However, the nanofabrication in silicon and glass is a barrier to widespread application.

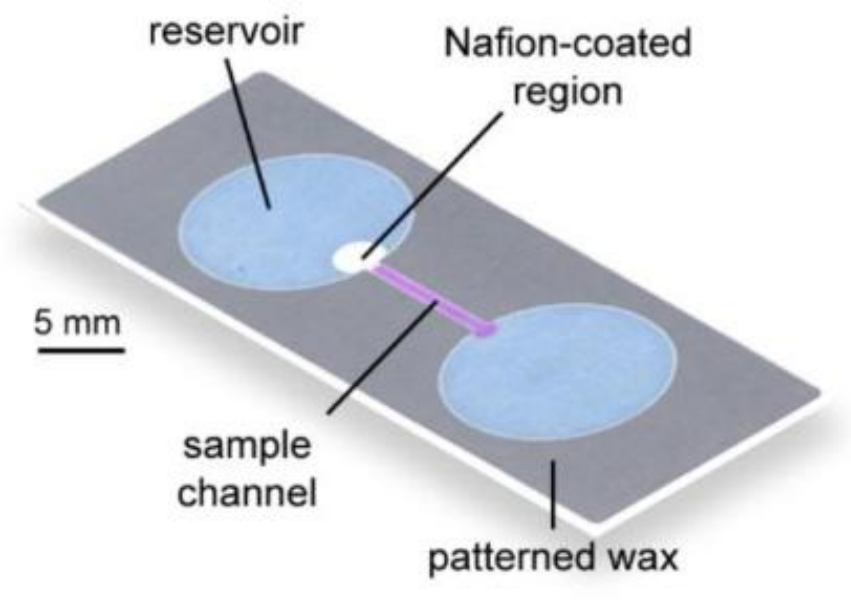
Introduction

- This paper demonstrates direct DNA analysis in paper-based devices, uniquely enabled by ion concentration polarization (ICP) effects at the interface of patterned nanoporous membranes in paper.
- Paper has emerged as an inexpensive, versatile, and scalable platform for diagnostics, with microfluidic paper-based analytical devices (μPADs) as a central format.
- The paper based ICP approach in this paper has been utilised to detect hepatitis B and assess male fertility.
- Hepatitis B virus (HBV) DNA targets in human serum are simultaneously preconcentrated, separated, and detected in a single operation with a limit of detection (LOD) of 150 copies/mL.
- We clinically assess the DNA integrity of sperm cells in raw human semen samples by preconcentrating and separating denatured DNA from intact DNA.
- The percent DNA fragmentation results from the paper-based ICP devices strongly correlate ($R^2 = 0.98$) with the conventional sperm chromatin structure assay (SCSA) and inform the same clinical outcomes.

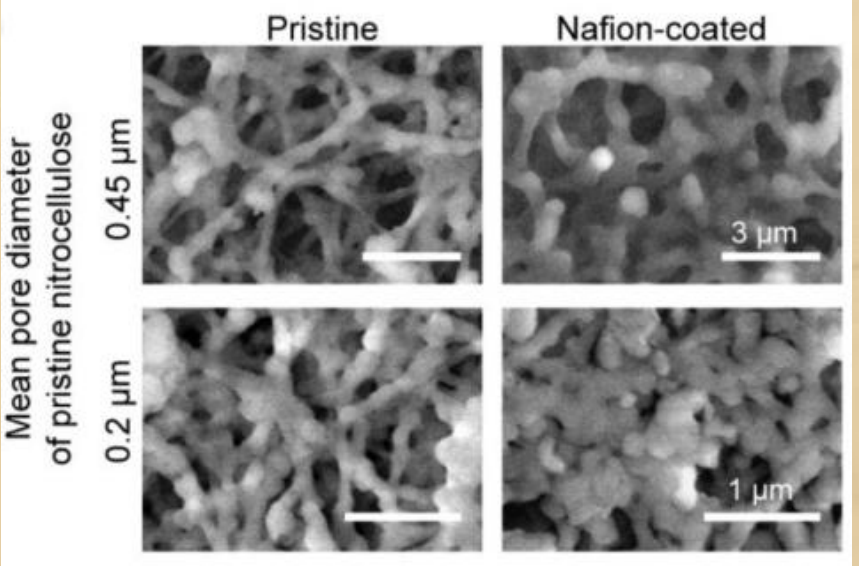
Experimental

Design

- The paper-based ICP device is nitrocellulose paper with a sample channel and reservoirs defined by patterned wax, and a region coated with cation-selective nanoporous Nafion
- Nanoporous Nafion is the main functional component in the device enabling nanoscale electrokinetic transport of ions for inducing ICP.
- Nafion-coated regions of nitrocellulose paper with original mean pore diameters of 0.2 and 0.45 μm are shown in environmental scanning electron microscopy images.
- Device designs were created in Microsoft PowerPoint and printed on nitrocellulose paper using a solid wax printer.
- 0.5 μL of Nafion was manually pipetted to each device and prewetted with DI water.



Device schematic with reservoirs (blue), sample channel (purple), and Nafion-coated region (white)



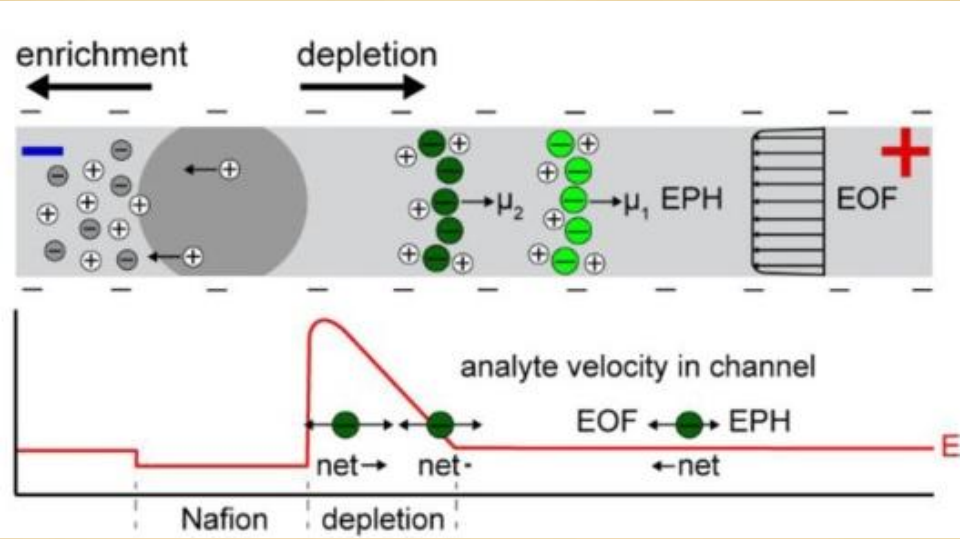
Environmental scanning electron microscopy images of nitrocellulose paper with and without Nafion coating

Experimental

Operation of the Paper-Based ICP Device

•To operate the device, reservoirs are prewetted with DI water, and sample is added to the sample channel and voltage is applied to induce ICP.

• Cations selectively migrate through the nafion coated region toward the cathode causing an ion enrichment zone at the cathodic nafion interface.



Schematic of ICP enrichment and depletion effects under applied voltage. Net movement of analytes is governed by electrophoretic migration (EPH) and electroosmotic flow (EOF), inresponse to the local electric field strength, E.

•The efflux of cations causes anions to vacate the region due to electrical neutrality, forming an ion depletion zone. The depletion zone propagates and repels anions toward the anode.

• The net movement of anions in the channel is dictated by electrophoretic migration (EPH) toward the anode and electroosmotic flow (EOF) toward the cathode.

• Anions experience different electrokinetic forces along the channel as a result of local changes to the applied electric field and ionic concentration from ICP.

• EOF is dominant downstream of the depletion zone, causing net movement of analytes toward the depletion boundary in the cathodic direction. EPH is higher than EOF in the depletion zone, causing anions to migrate toward the depletion boundary in the anodic direction.

• The balance of these two opposing effects focuses ions at the depletion boundary.

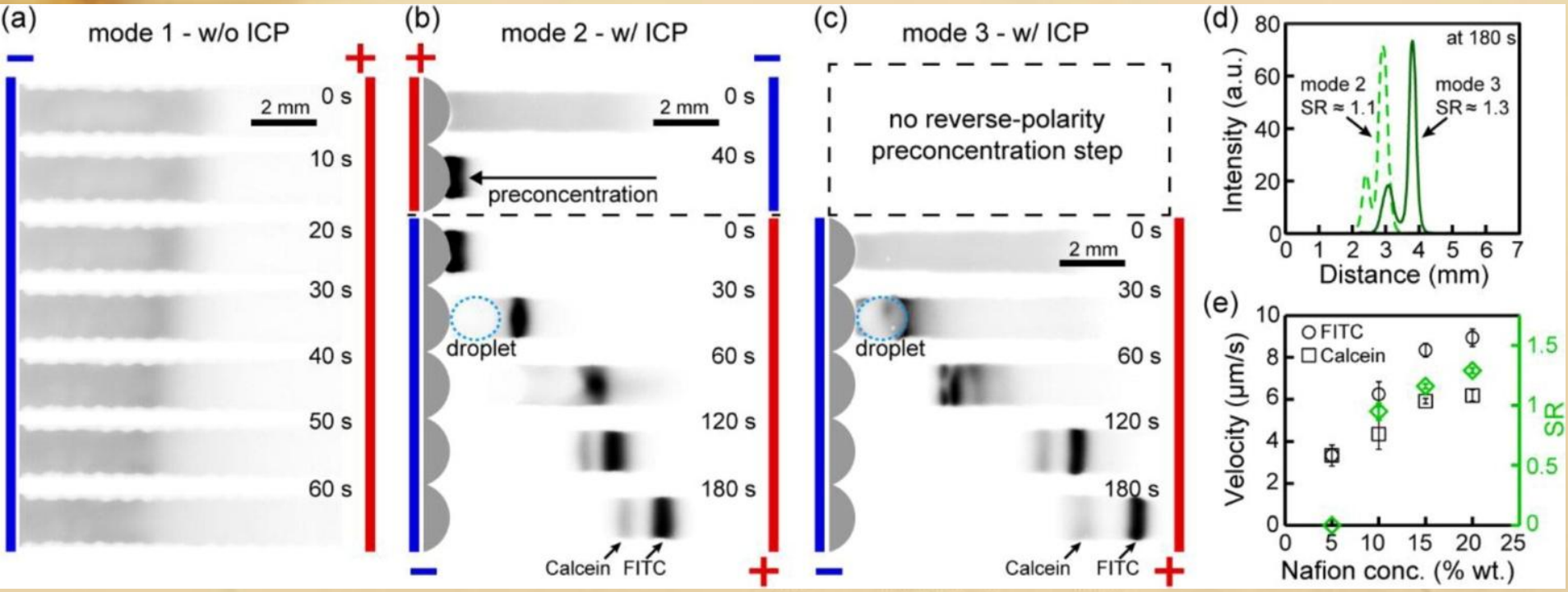
• For a system with multiple anionic analytes, each analyte migrates at a different net velocity due to variations in their electrophoretic mobility, enabling separation following preconcentration.

Experimental

- Fluorescein and calcein were used as fluorescent tracers
- A Low Mass DNA ladder was used to demonstrate DNA separation in the device.
- Hepatitis B analysis used synthetic hepatitis B virus
- Human semen samples from patients and healthy donors incubated at 37 °C for 30 min to allow liquefaction. Computer-assisted sperm analysis (CASA) was used to obtain standard semen parameters in accordance with World Health Organization guidelines.

Results

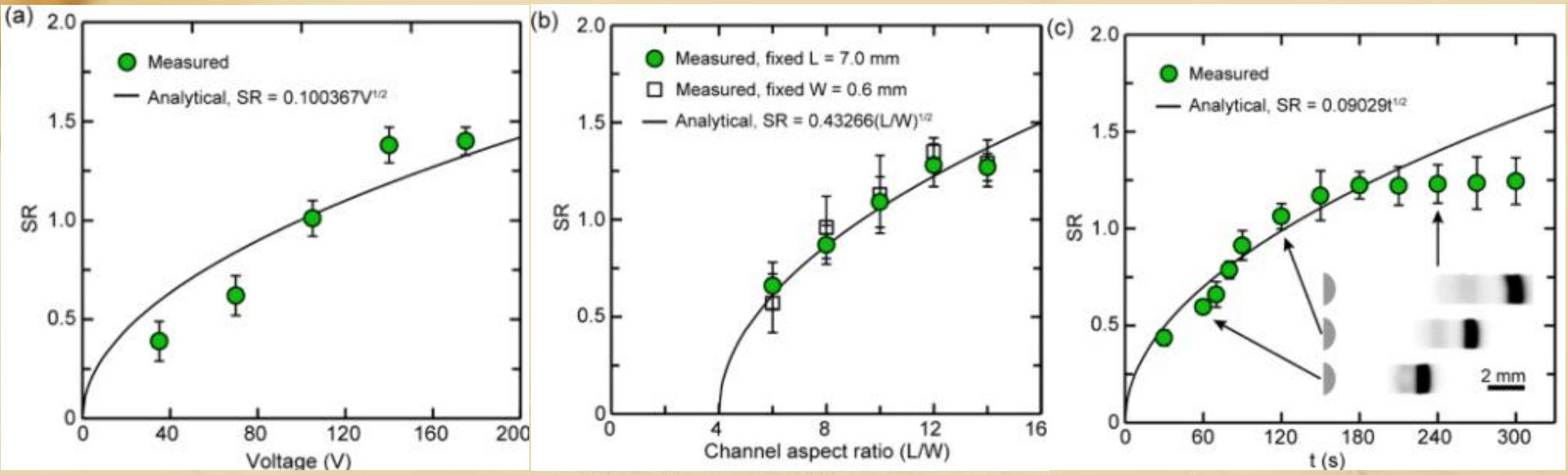
Preconcentration and separation in the paper-based ICP device



(a) Operating mode 1–transport without ICP. (b) Operating mode 2–preconcentration and separation with switching-polarity ICP. (c) Operating mode 3–direct preconcentration and separation with ICP. (d) Separation resolution (SR) in modes 2 and 3. (e) Effect of Nafion concentration (% wt.) on the separation process. Data points are the average of $n = 3$ measurements with error bars as one standard deviation. Images have been contrast adjusted for presentation (similarly in each case).

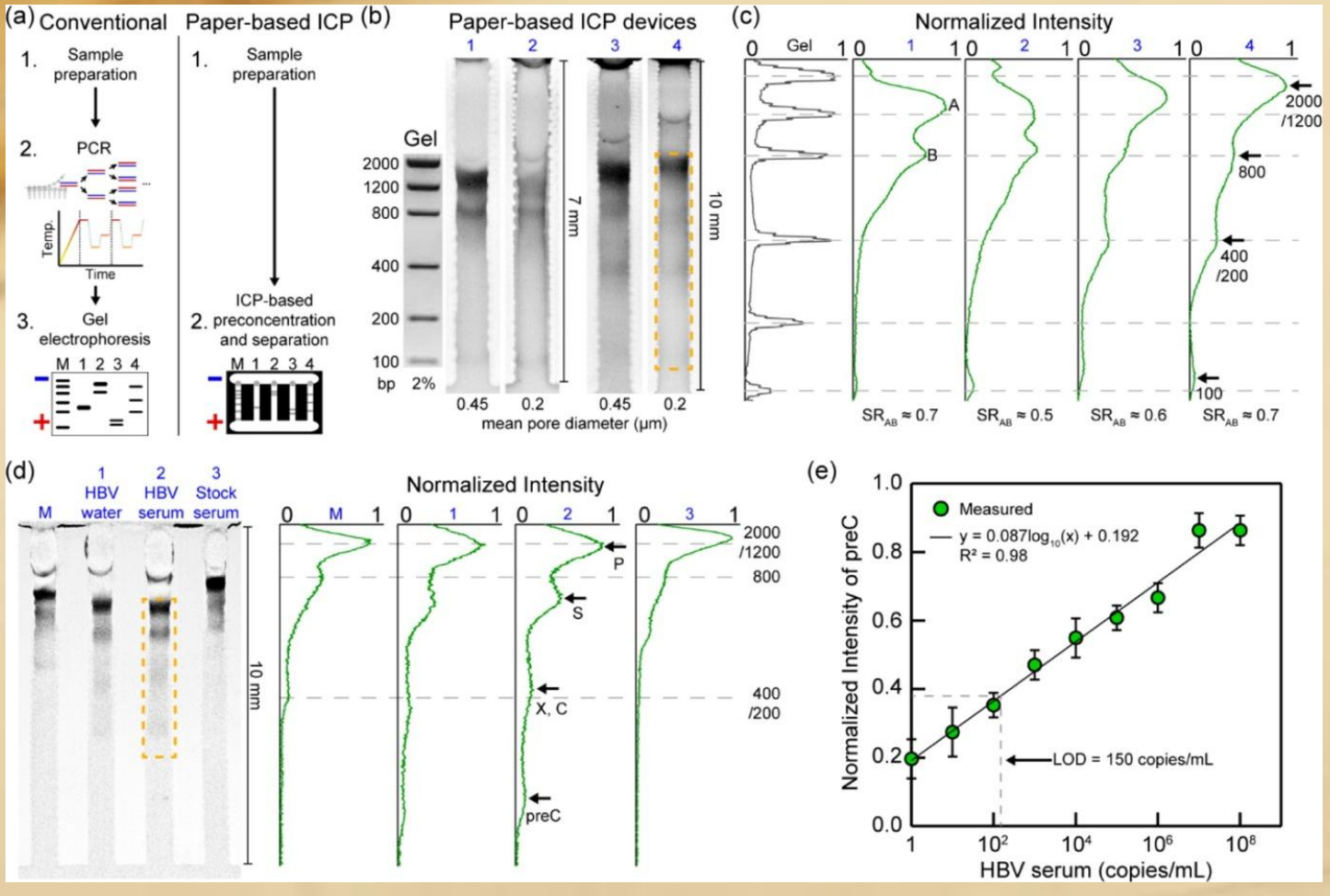
Results

Characterization of separation parameters for the paper-based ICP device



(a) Separation resolution (SR) as a function of applied voltage, using devices of fixed geometry (channel length of 7 mm and width of 0.6 mm). (b) SR as a function of channel aspect ratio (i.e., length/width), with a fixed field strength of 200 V/cm. (c) SR as a function of residence time, using an applied field of 200 V/cm and devices with channel aspect ratio of 12. SR improves with increasing residence time up to 180 s, where band broadening from Joule heating becomes limiting (inset images). Plotted points are the average of $n = 3$ measurements with error bars as one standard deviation. Images have been contrast-adjusted for presentation.

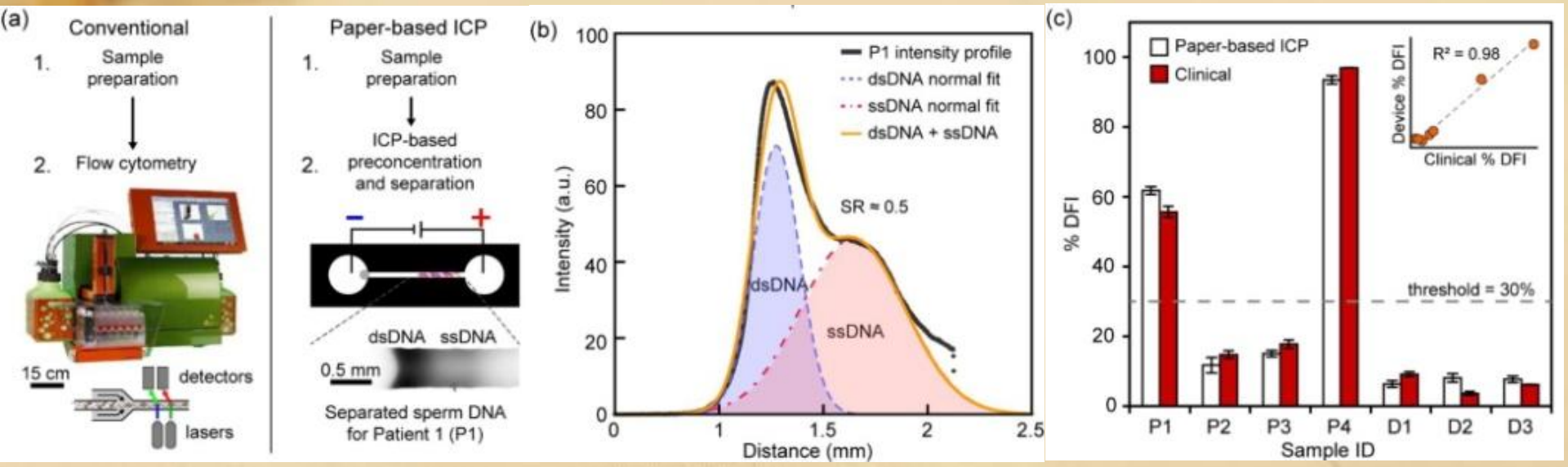
PCR-free hepatitis B analysis



(a) Comparison of conventional and paper-based ICP approaches for HBV DNA analysis. (b) Separation of a DNA standard containing dsDNA fragments ranging from 100 to 2000 bp (6 fragments in total), using 7 and 10 mm channel devices of two different mean pore diameters, 0.2 and 0.45 μm. The separations are compared to an ideal case in 2% agarose gel (image from Invitrogen). (c) Intensity profiles are measured for the gel and the four paper-based ICP cases using the region of interest (ROI) outlined by the yellow dashed box in (b).

(d) Multiplexed analysis of the DNA standard, HBV DNA in water, HBV DNA in serum, and stock serum in a multichannel device. The intensity profile for each channel is measured using the ROI box. (e) A limit of detection (LOD) of 150 copies/mL is achieved by measuring the peak intensity of the precore (preC) fragment at serially diluted HBV DNA concentrations in serum. The LOD is the HBV DNA concentration at three standard deviations from a base intensity of 0.20. Each data point is the mean of the normal fit to the intensity profile of the preC fragment, with error bars as one standard deviation. Images have been contrast adjusted for presentation (similarly in each case).

Clinical Assessment of Human Sperm DNA Integrity



(a) Comparison of conventional and paper-based ICP approaches. Contrast adjusted image showing preconcentration and separation of sperm DNA for patient P1.

(b) Intensity profile of the separated DNA. The percent DNA Fragmentation Index (% DFI) is calculated using areas under the normal fits.

(c) % DFI results for patients and donors. Inset shows correlation ($R2$ = 0.98) between paper-based ICP and clinical results. A % DFI threshold of 30% is used to determine clinical outcome. Error bars for the device results represent one standard deviation of the normal fits. Clinical results are the average of two measurements with error bars as one standard deviation.

Conclusion

- Direct DNA analysis by leveraging electrokinetic transport at the interface of patterned nanoporous membranes in nitrocellulose paper.
- Paper-based ICP devices were used to detect HBV DNA in human serum and assess sperm DNA integrity in raw human semen
- For hepatitis B testing, a LOD of 150 copies/mL was achieved with no prior viral load amplification, sufficient for early diagnosis of HBV.
- Multiplexed analysis of HBV was also demonstrated in a four channel device.
- Paper-based ICP enables inexpensive (materials cost <1 USD per test) and rapid (~10 min) DNA analysis in a simple format with potential for widespread application.


Thank
you...
Don't Fear Moving Slowly Forward,
Fear Standing Still.....