Title of the project

Integrated microfluidic droplet-digital nucleic acid quantification

Category (translational/bioengineering/biodesign): Bioengineering

Investigators (IISc and clinical institutions)

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Statement of research

Viral load quantification is being recommended by health agencies for diseases like HIV and HCV for diagnosis and disease management. Yet, we lack cheap, portable sensitive devices to detect and quantify viruses from clinical samples. We propose an integrated microfluidic droplet-digital isothermal nucleic acid quantification point-of-care device to address this need. Employment of Recombinase polymerase amplification (RPA), fastest isothermal amplification method working at close to room temperature allows in fast detection and reduces the complexity of using thermal cyclers for on-chip amplification. Pico-litre sized droplets generated inside the microfluidic chip act as a microbioreactor for on-chip isothermal amplification of viral nucleic acids. Viral nucleic acids are distributed in droplets with amplification reagents and fluorescent marker following Poisson distribution. This gives a digital fluorescence signature after amplification which is later quantified for nucleic acids. All unit operations required for droplet digital amplification and quantification are integrated on the chip decreasing the associated instrumentation for separate amplification and droplet generation. These include uniform distribution of biomolecules inside the aqueous droplets, mixing of amplification reagents with the sample inside the droplets and amplification and detection of nucleic acids.

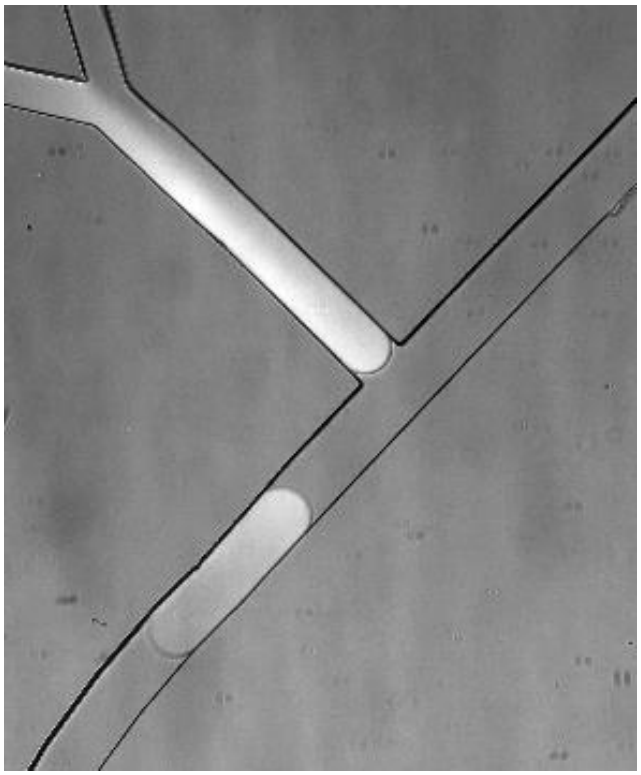

Fig: Droplet generation inside microchannels. Fluorescent dye and reagents mix with the nucleic acid solution at the Y junction and droplets are generated at the T-junction due to pinch-off of the aqueous phase