Biosensors

B. Tech.

Course No.: EEL 3050

L-T-P [C]: 3–0–2 [4]

Prof. AJAY AGARWAL

ELECTRICAL ENGINEERING

IIT JODHPUR

Lecture 29 dated 04th Nov. 2024

Early Lab-on-Chip (LOC):

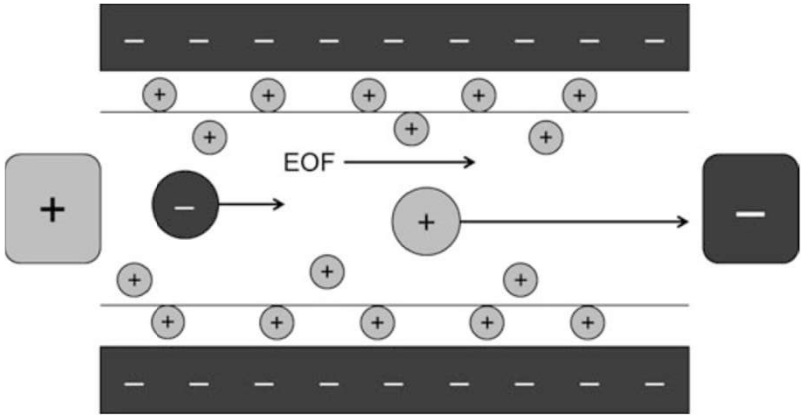
- The original LOC were realized to miniaturize liquid chromatography (LC) & capillary electrophoresis (CE),
  - to achieve **better sensitivity** and
  - **enhanced separation** performance.
- The LOC development begun with the aim to form smaller-diameter capillary for LC & CE, by microchannels.
- Electrophoresis is a laboratory technique used to separate DNA, RNA or protein molecules based on their size & electrical charge.
- LC is a lab technique that separates & identifies substances in a liquid sample by passing it through a column, filled with a material that separates the substances

Principle of **Capillary Electrophoresis** (CE)

- Biomolecules flow through a capillary channel under an applied voltage.
- Depending on their polarity & charge, each biomolecule flows at a different speed

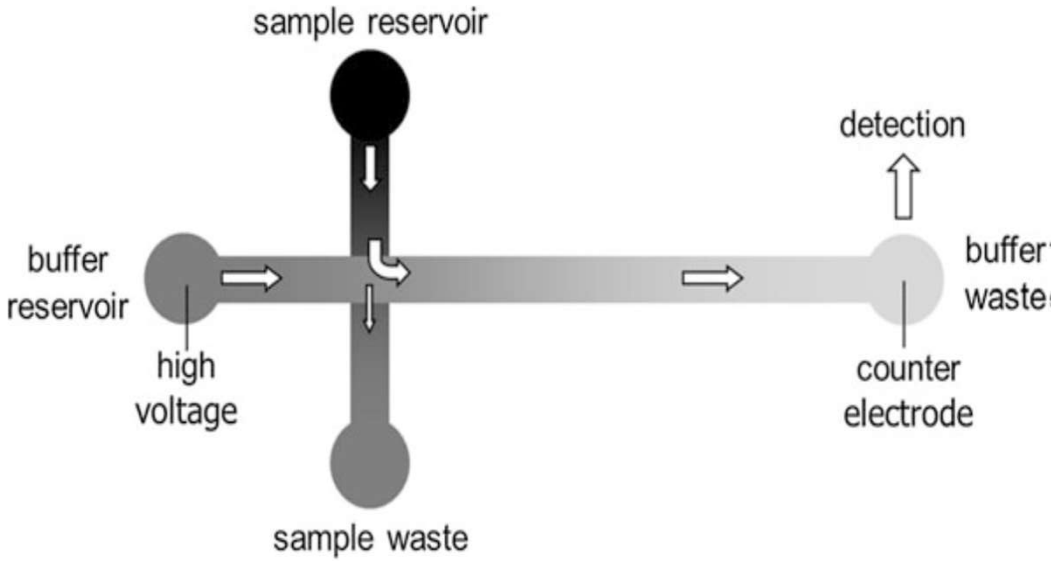
Principle of **Capillary Electrophoresis** (CE)

- High voltage (+) is applied to the beginning of a long capillary column & ground (–) to the end of it.
- The column itself is **negatively charged**, & an electric double layer (EDL) is formed near at the inner wall of a column.
- The cations in 1st layer are firmly bound to the wall, while those in 2nd layer are free to move



- Because a high voltage is applied along the capillary column, the free cations are pulled towards the anode, & this movement generates the bulk flow of liquid, called electroosmotic flow (EOF).
- If +ve or –ve charged biomolecules (e.g., proteins) are introduced into stream of EOF, they are separated by differences in their electrophoretic mobility.
- +ve charged biomolecules are pulled faster than those pulled by bulk flow, while -ve charged biomolecules are pulled more slowly than those pulled by bulk flow.
- The separation of biomolecules is achieved this way

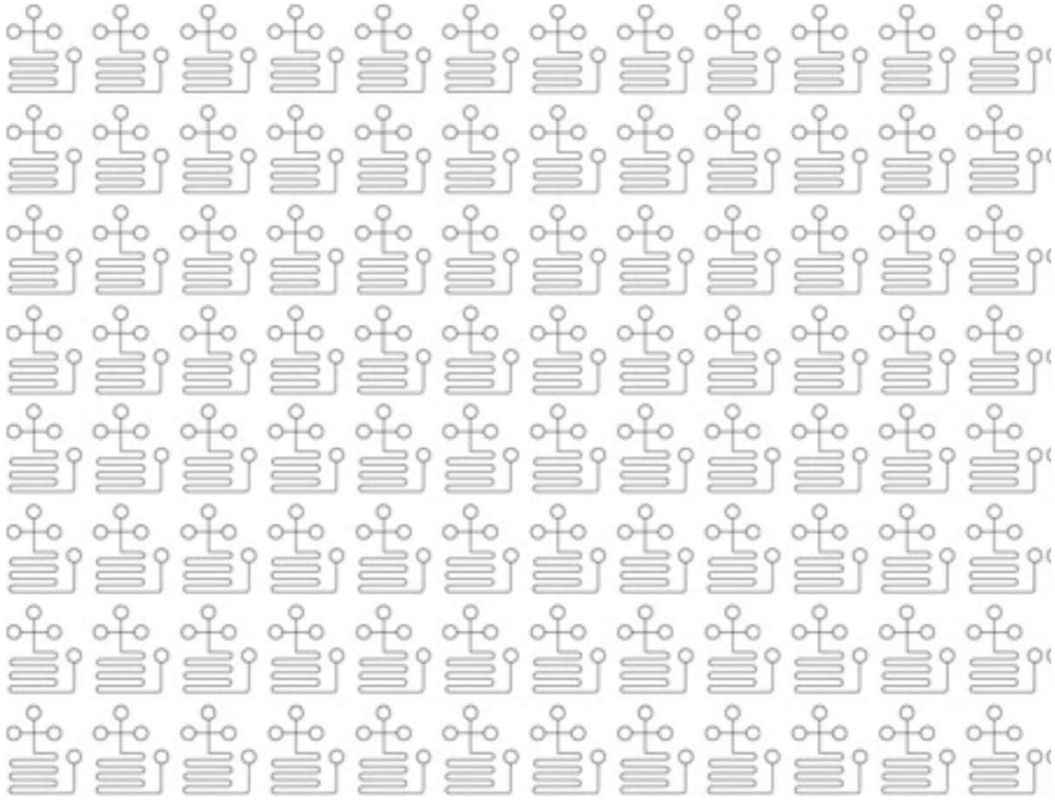
- Detection is typically achieved by using UV/ Vis spectrophotometry because many proteins show absorbance in UV and/ or visible wavelengths (i.e., without using **fluorescent** dyes or **radioisotopes**).
- The capillary column can be transformed into microchannels **with** a couple of wells for applying voltage, introducing sample and making spectrophotometric detection, i.e., in an LOC platform.



The typical layout for conducting capillary electrophoresis in LOC

- This single CE layout can be duplicated, for example, 96 times (12 × 8), for high-throughput analysis

Multiplexed (12 × 8 = 96) capillary electrophoresis LOC



Biosensors

B. Tech.

Course No.: EEL 3050

L-T-P [C]: 3–0–2 [4]

Prof. AJAY AGARWAL

ELECTRICAL ENGINEERING

IIT JODHPUR

Lecture 30 dated 06th Nov. 2024

Assignment – 3 (Points: 10)

- Propose 2 Non-invasive methods of Blood Glucose estimation.
- Explain any one method in detail
  - Glucose concentration co-relation of prosed sample & blood
  - What kind of Biosensor can be used for the proposed method
  - Its principle of operation, schematics etc.
  - Advantages of the proposed method
  - Add references …

- Do it in a team of 5 students. Write who has done what.
- Submit report & ppt

LOCs for Point-of-Care Testing (POCT):

- A significant amount of time is often required for clinical diagnostics, including immunoassays, from a few hours up to a week.
  - **Immunoassays** is a test that uses the binding of **antibodies** to **antigens** to identify & measure certain substances.
- The primary cause of this time lag is not just the assay time, but it is also the distance between the point of subject care & a laboratory facility, and subsequent delivery time.
- This time lag is a serious problem, when early detection of disease is important.
- Thus, the concept of point-of-care diagnostics has emerged.
- Point-of-care testing (POCT) generates results quickly so that treatment during acute care can be implemented, leading to improved clinical & economic outcomes.

Biosensors

B. Tech.

Course No.: EEL 3050

L-T-P [C]: 3–0–2 [4]

Prof. AJAY AGARWAL

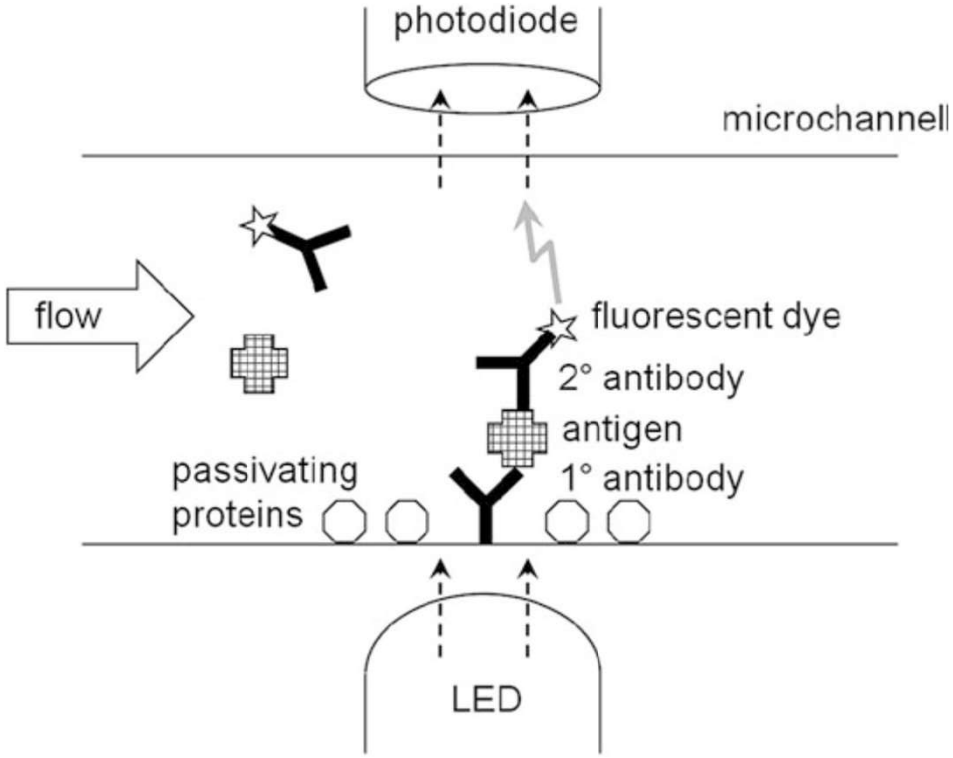
ELECTRICAL ENGINEERING

IIT JODHPUR

Lecture 31 dated 07th Nov. 2024

POCT:

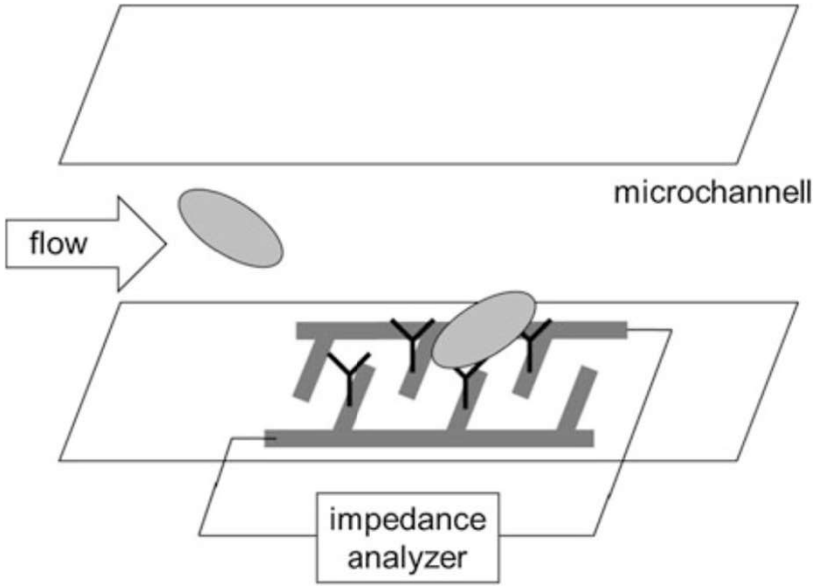
- POCT **decreased** the turnaround time of analysis to a few minutes.
- Tests that were sent to an outside lab started shifting to point-of-care.
- POCT in LOC is generally achieved by incorporating **immunosensors** into **microchannels**.
- The **sandwich** immunoassay in a microchannel format, with light source (LED) and light sensor (photodiode) attached to it.



Optical immunosensor LOC

Interdigitated Microelectrode LOC:

- The interdigitated microelectrode (IME) immunosensor, a very popular form of impedance-based **immunosensor**, also in a microchannel format.
- More advanced immunosensors can also be implemented in LOC platforms; for example, SPR, QCM, electrochemical ELISA, etc.

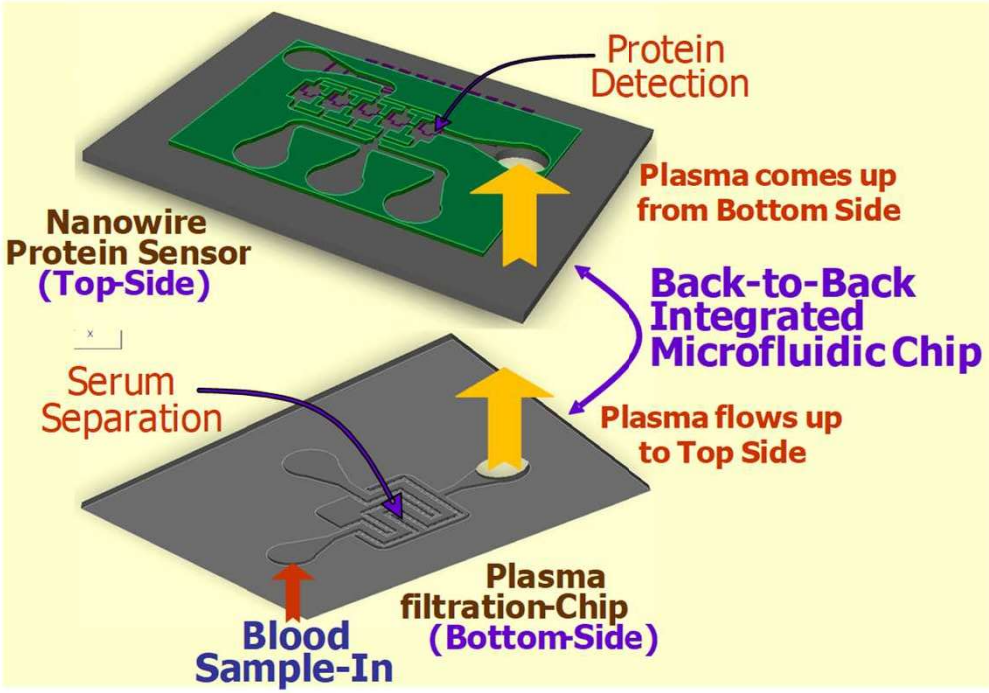


IME (interdigitated microelectrode) immunosensor LOC

- QCM measures resonance frequency changes, f, of an **oscillating quartz crystal**,
- SPR measures changes of the surface plasmon resonance angle, Θ.

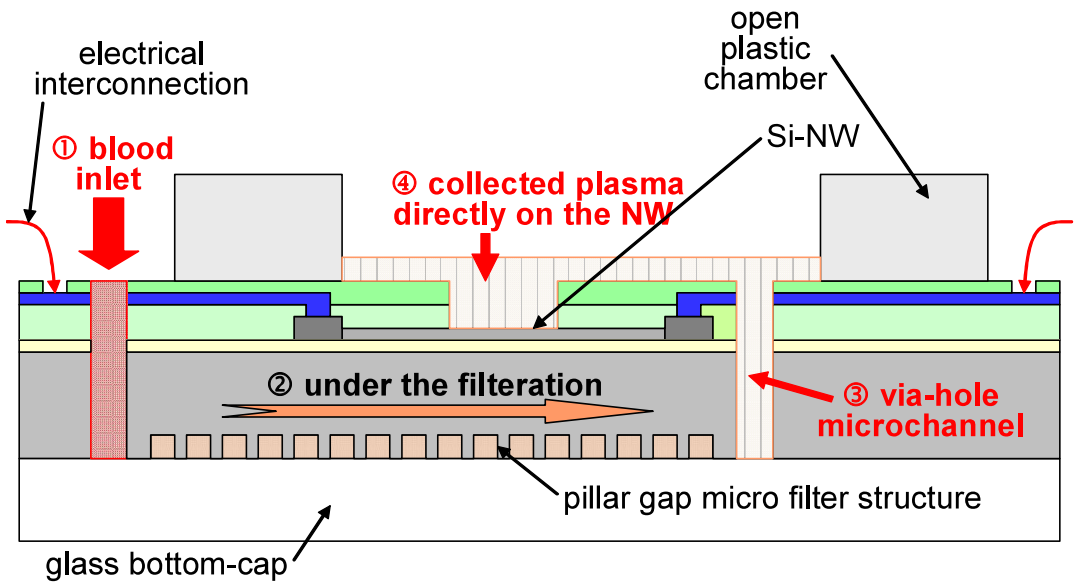
Lab-on-Chip system

Conceptual drawing of back-to-back integration of Si-NW biosensor for protein detection with microfiltration chip for plasma separation

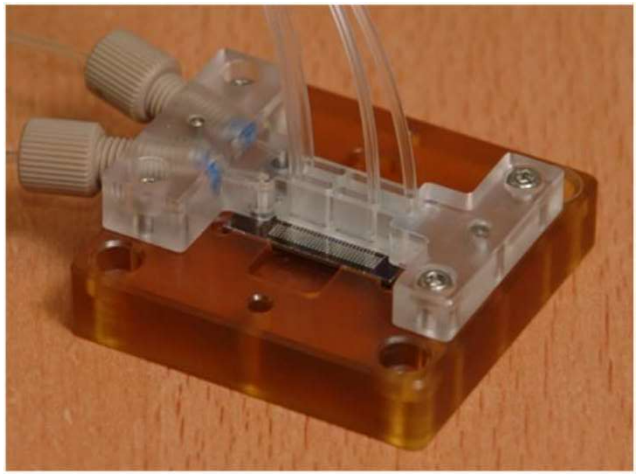


TG Kang, **Ajay Agarwal**, et al., μTAS2007, 3-7 Oct 2010, Groningen, The Netherlands.

Lab-on-Chip system



Working principle of sample flow in the back-to-back integrated microfluidic device..

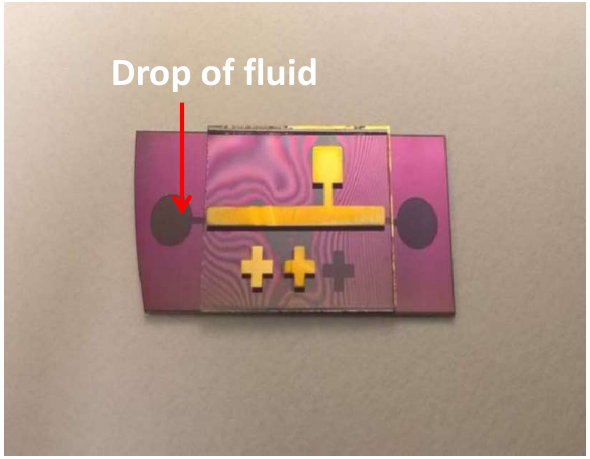


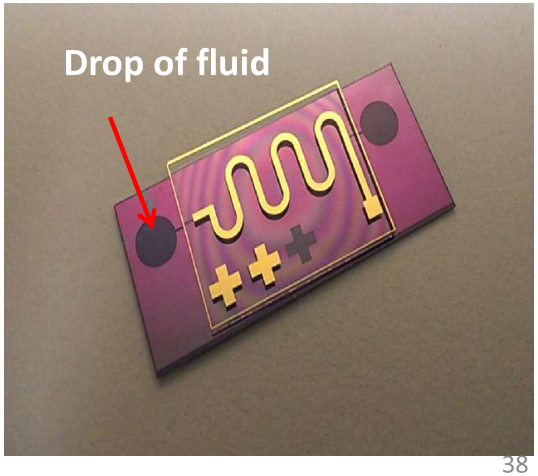
Customized plastic housing for back-to-back integrated microfluidic device

Micro-Viscometer

Key Features:

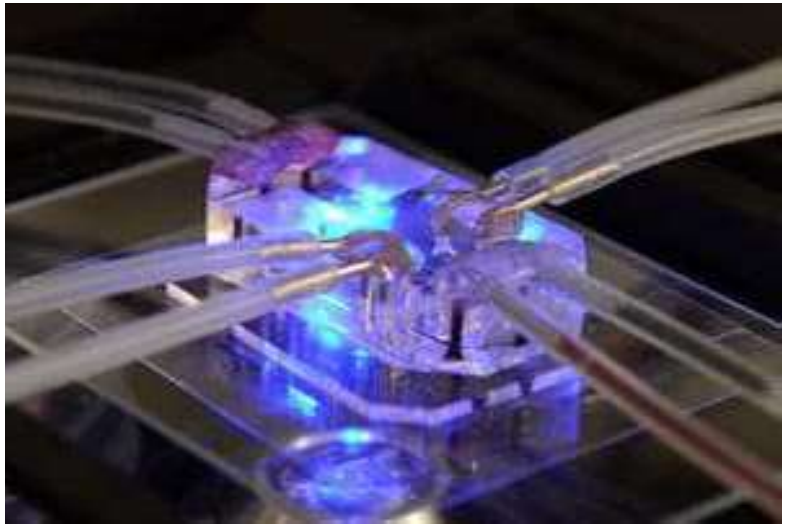
- Sample Volume: < 500 μl
- Viscosity Range: 0.1cP - 100cP
- Measurement time: <10 sec. to 5 minute; depending on viscosity
- Highly Repeatable Viscosity measurement (< 5% variation)
- Easy to use





Organs-on-chips:

- Organs-on-chips are 3D cell culture microdevices, aiming to reproduce the key functions of living organs on a chip.
- These microfluidic devices are more efficient than conventional cell culture techniques since they are able to mimic microenvironments as well as their influence on organ function.



- Organs-on-chips use microfluidics & microfabrication technologies to **better** replicate the functionalities of living organs.
- Among them we can find models like gut on a chip, heart on a chip, liver on a chip, lung on a chip, tumor on a chip, muscle on a chip, multiple organs on a chip etc.

Biosensors

B. Tech.

Course No.: EEL 3050

L-T-P [C]: 3–0–2 [4]

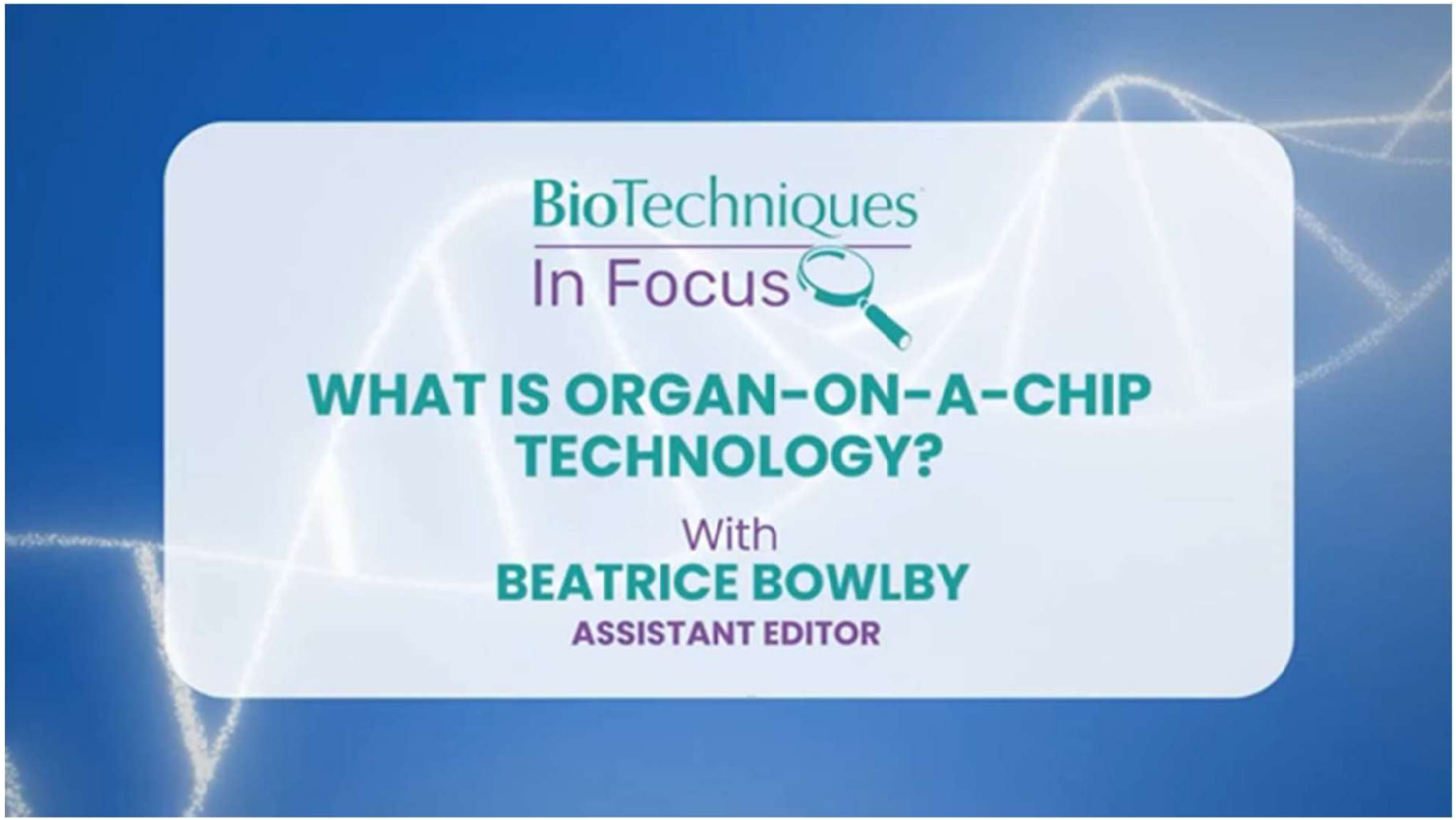
Prof. AJAY AGARWAL

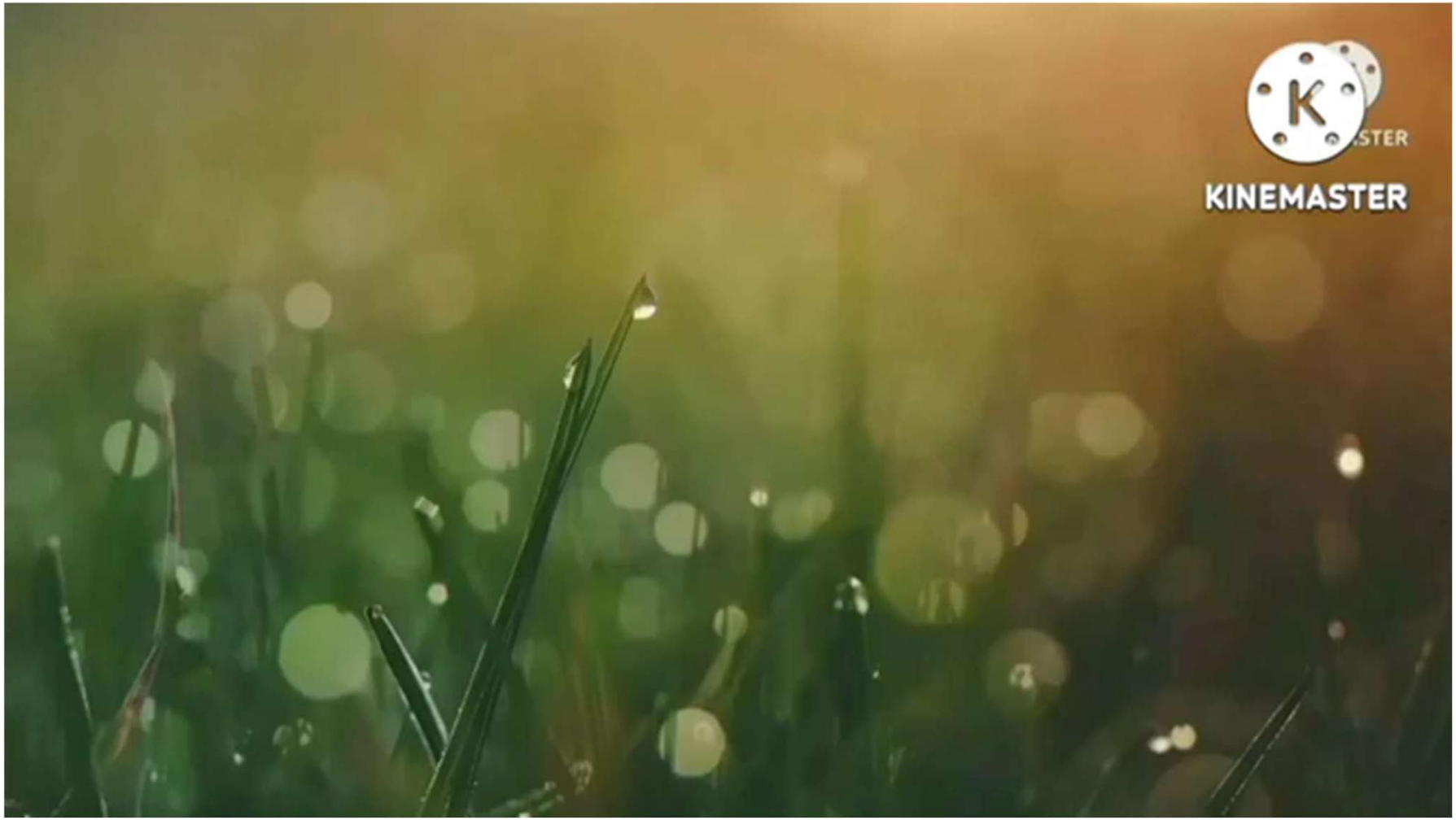
ELECTRICAL ENGINEERING

IIT JODHPUR

Lecture 32 dated 09th Nov. 2024

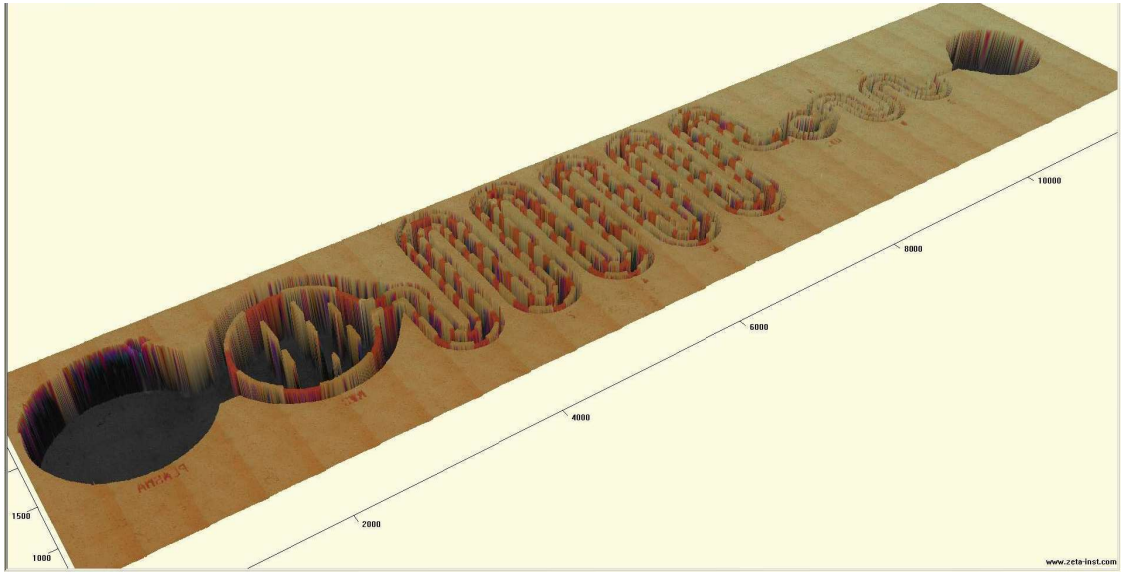
Organ-on-chip: https://youtu.be/8tlHd5pYHOY





https://youtu.be/whjBR9CafXI

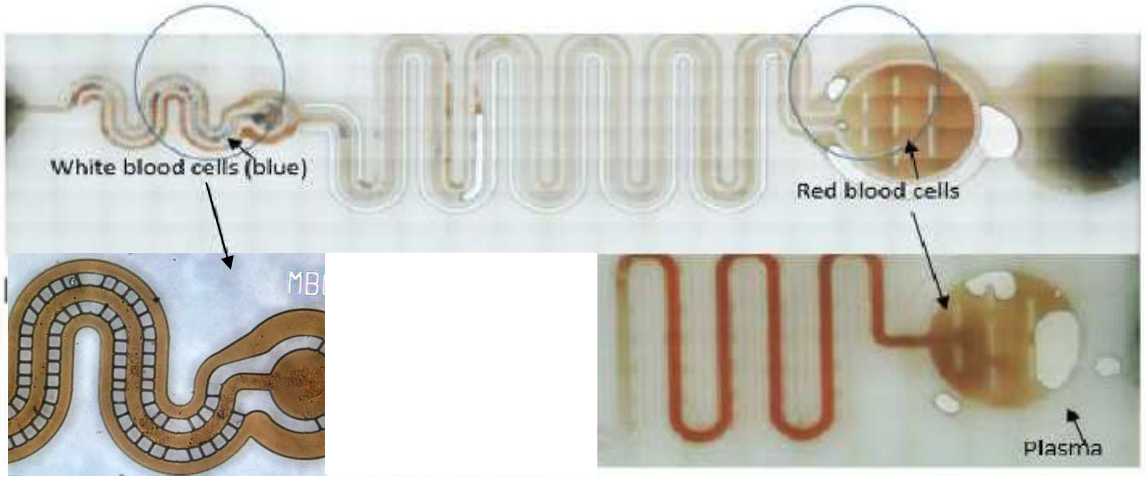
Lab-on-a-Chip (LOC) for *Particles Separation and Counter*



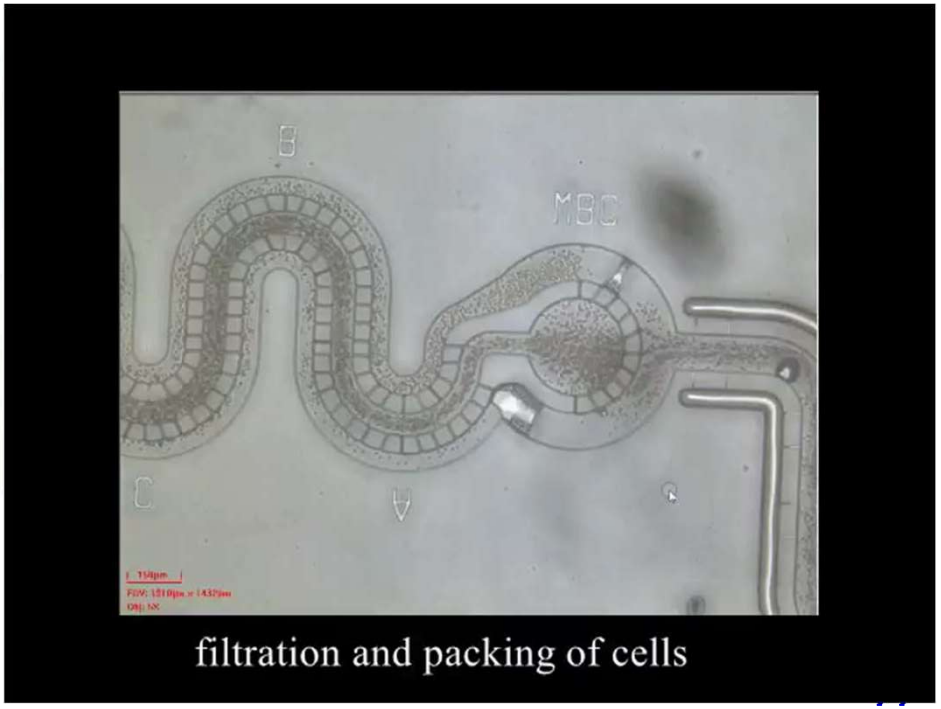
Ajay Agarwal and Balyan Prerna, Indian Patent No. 2592/DEL/2013, May 06, 2016

Filtration of Blood cells

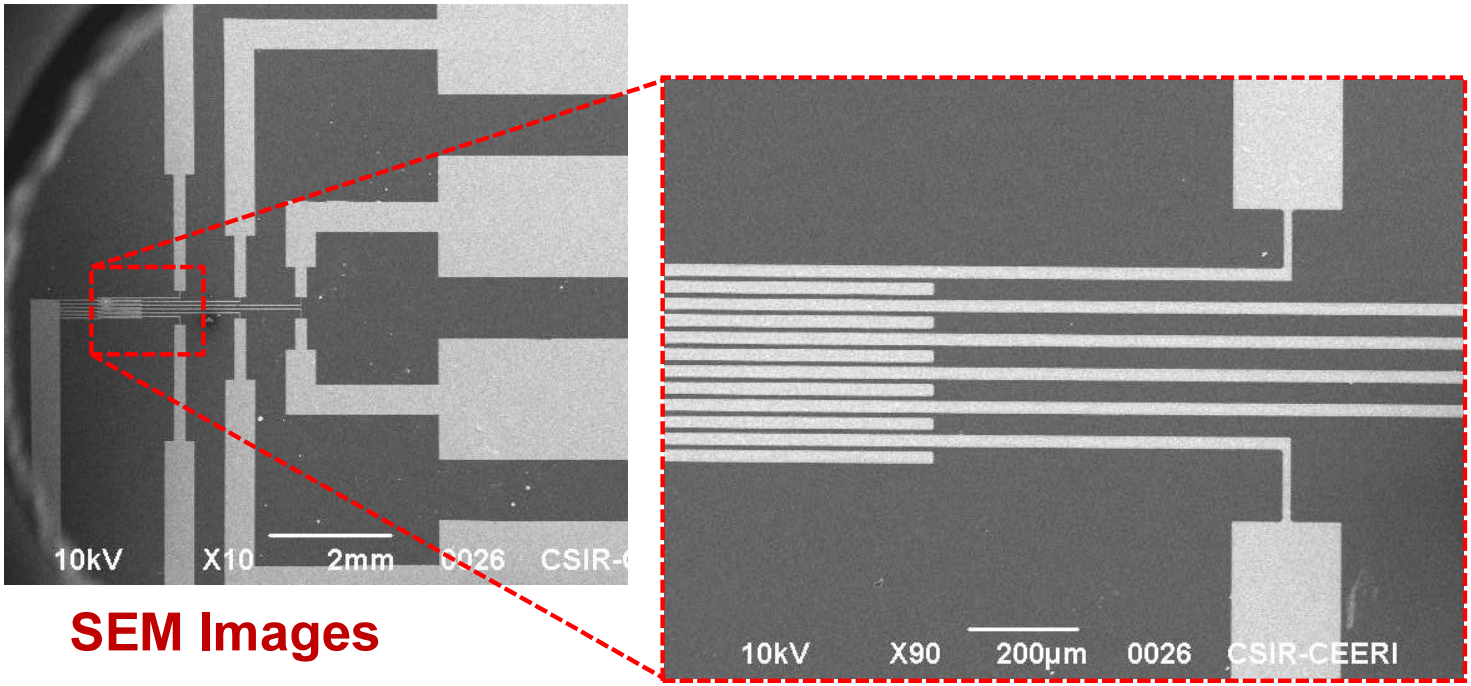
- *Separation of WBCs and RBCs from blood.*
- *Most of the WBCs were retained in the WBC separation and collection region, however some amount of cells tends to escape due to deformable nature of the cells.*



Separation of blood cells



Micro-electrodes array



SEM Images

Electro-chemical sensor

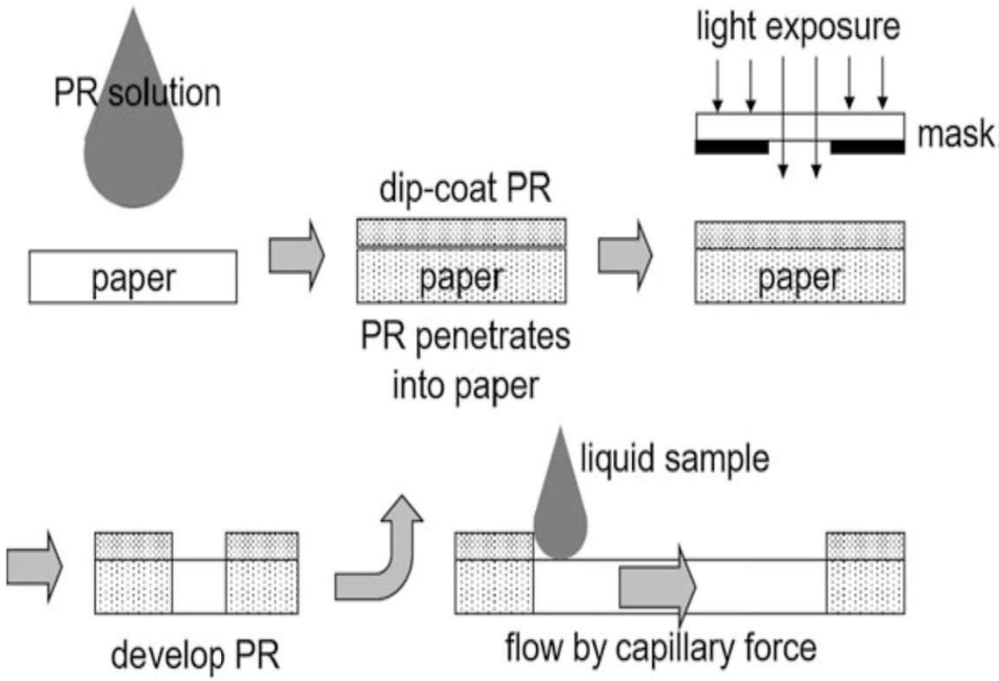
Suman Singh, **Ajay Agarwal,** et al., **Chemical Engineering Journal,** 313, (2017), 283–292

Paper-Based LOCs:

- Paper (chromatography paper) has increased in popularity as an alternative to silicon-based materials for fabricating LOCs.
- The paper-based LOCs are
  - **lower** in **cost**,
  - much thinner,
  - easier to fabricate, &
  - easier to use than conventional LOCs.
- The fabrication & use of such devices are referred to as paper microfluidics.
- Most paper-based LOCs are used for sensing & biosensing applications, they are referred as **microfluidic paper analytic devices, or μPADs.**

Paper-Based LOCs fabrication:

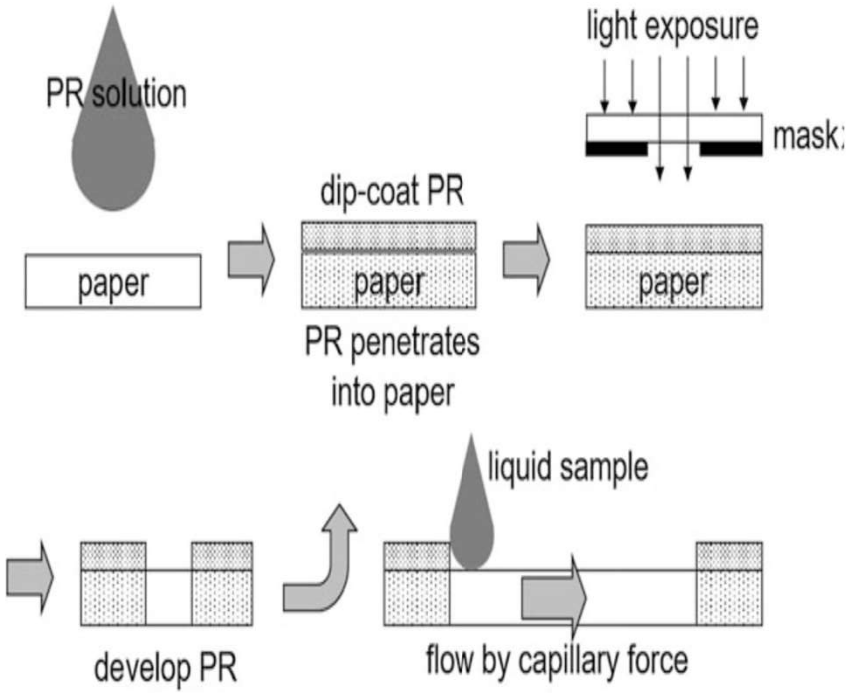
- The paper is pre-coated with thin film of photoresist (PR) through spin-coating or by dipping the paper into the PR solution (dipping is preferred for simplicity).
- Since papers are permeable to most solutions, **PR penetrates** into the paper.
- While the paper is generally hydrophilic **due** to its **cellulosic fiber composition**, most PRs are **hydrophobic** (meaning water-hating) & thus cause both the **surface & inner paper fibers** to become quite **hydrophobic**.



Fabrication of a paper-based LOC

Paper-Based LOCs fabrication:

- After the drying, the **mask is placed** on the PR-coated paper substrate, and both are irradiated with ultraviolet (UV) light.
- UV radiation causes positive PR to become chemically **unstable** & can be removed by a chemical developer.
- While negative PR hardens under UV radiation & the non-hardened PR is removed by a developer.
- Either way, an area is free of PR
- Sample liquid can flow through the area that is free of hydrophobic PR, while it cannot flow through the area that is covered and filled with hydrophobic PR.



Fabrication of a paper-based LOC

Paper-Based LOCs:

- Paper-based LOCs do not require a cover slide, thus requiring no bonding procedure.
- For further simplicity, wax may also be printed directly on the paper, eliminating the need for spin-coating/dipping or a mask.
- The fundamental difference in paper-based LOCs: its microfluidic channel is not hollow but is filled with paper fibers.
- Once a **liquid sample is loaded** to its inlet, the liquid spontaneously flows through its fibers via **capillary action** (also referred as wicking), thus does not require **electroosmotic flow** or **external pumping**.
- This capillary action (or **wicking**) is perhaps the major **advantage** of paper-based LOCs over other conventional LOCs.

Paper-Based LOCs:

- The fibers in paper also **slow the flow**, & in **more viscous liquid** samples, the liquid **may not flow** at all (e.g., whole blood).
- Depending on applications, this can be beneficial, where we want flow obstruction for actively filtering certain components from the liquid sample (for example, filtering blood cells from whole blood).
- More sophisticated filtration can be achieved by utilizing a chromatographic **filter** paper, with known **pore size** (narrowly distributed) & known **functional groups** (**nitro- or sulfone-**) that are added to the cellulose fibers.
- Different chemical & biological assays have been performed using paper-based LOCs: glucose, cholesterol, lactate, alcohol, heavy metals, enzymes, proteins, etc., utilizing both optical and electrochemical detection.

Any **Questions**?