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Laser-direct-write fabrication of paper-based point-of-care diagnostics

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Outline

- Paper-based microfluidics – POC diagnostics
- Our patterning approach – Laser-direct-write
- Patterning flow-paths and enabling flow-control in them
- Patterning for multiplexed detection on LFDs
- Patterning for improved sensitivity and limit-of-detection of LFDs
- Conclusion

Introduction - Paper-based devices

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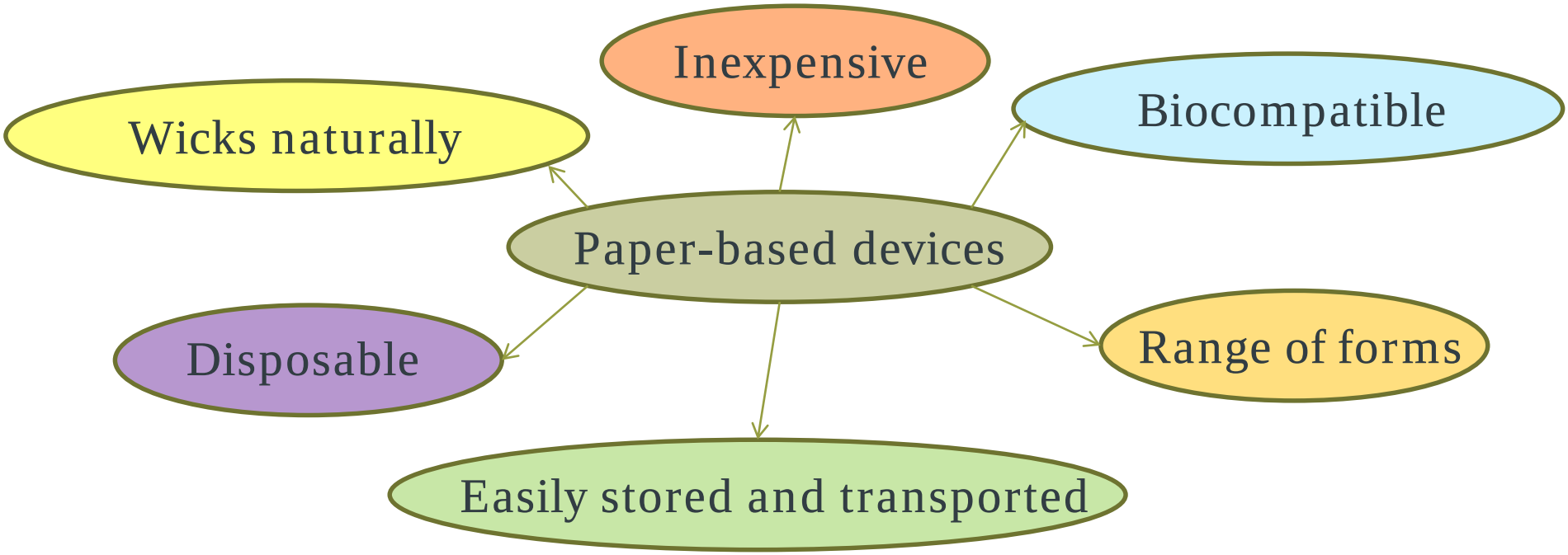
Low-cost alternatives to conventional POC diagnostic tools



Initial target application – POC diagnostic sensors that satisfy

- WHO defined 'ASSURED' criteria – Affordable, User-friendly, Equipment-free and Deliverable
- Aimed at requirements in countries with low-resourced settings

Introduction - Paper-based fluidics

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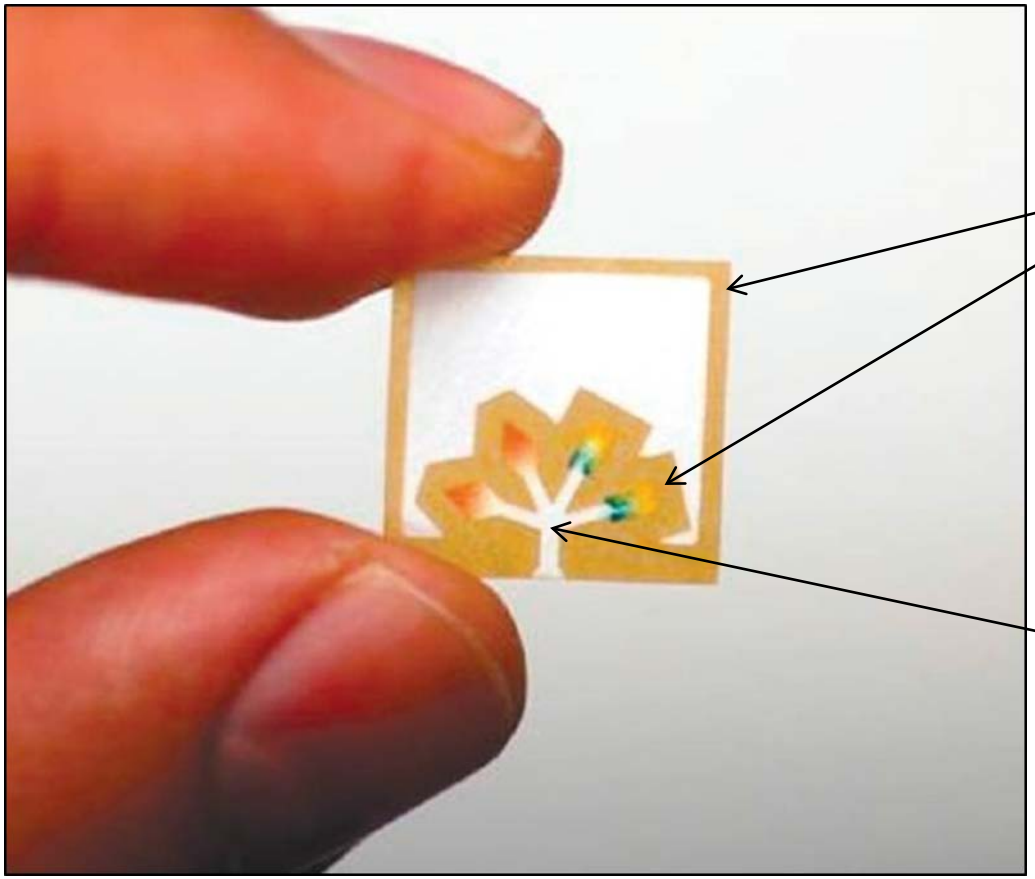

Hydrophobic barrier walls that extend throughout the paper

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Interconnected hydrophilic fluid-flow channels

Requirements for creating such devices on paper

- Pattern paper to form fluidic patterns
- Deposit reagents for implementing the assay

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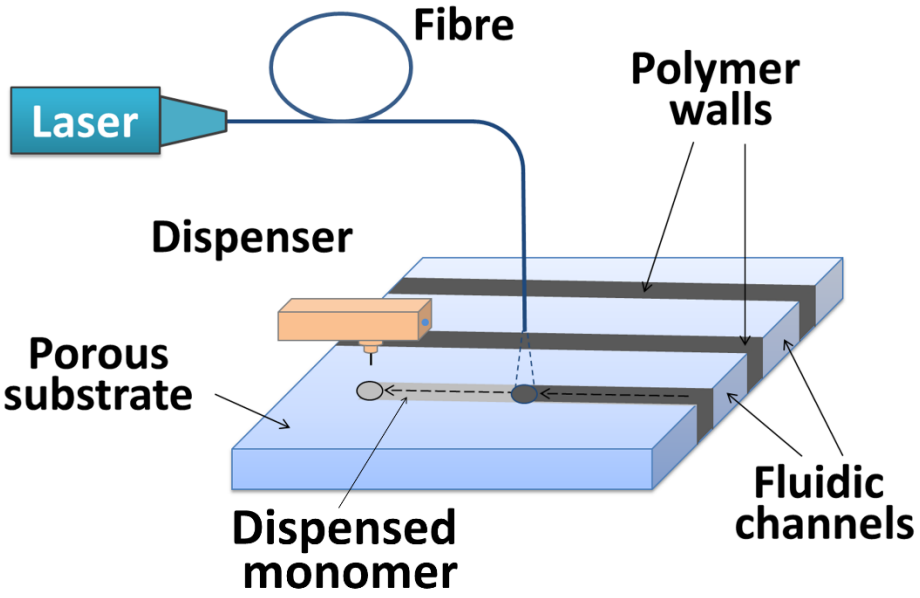
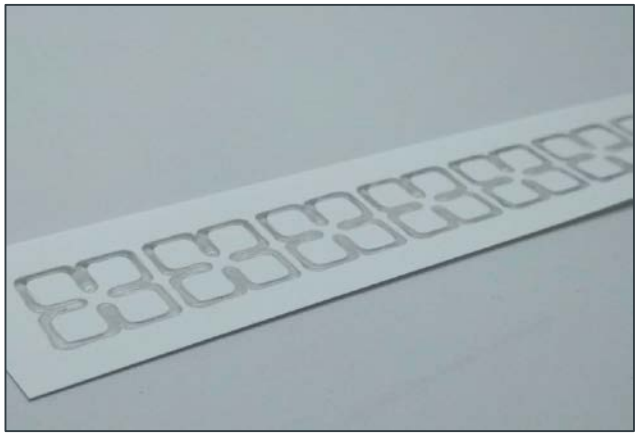
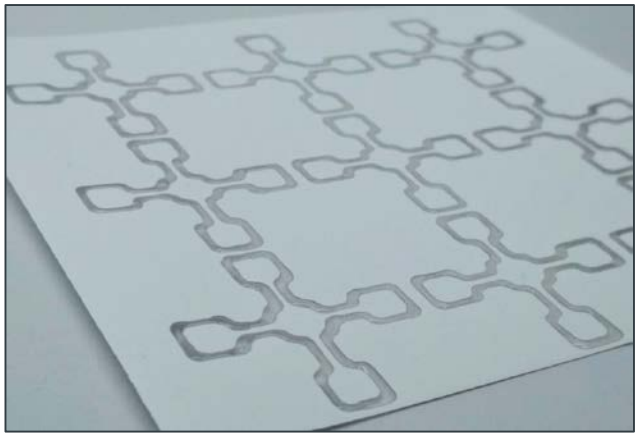
Our LDW patterning approach

Technique that allows *creation of fluid-flow channels/patterns in paper*

1. A local-deposition assisted laser-direct write procedure
2. Relies on the concept of light-induced polymerisation







Lasers used
Few mW of 405 nm c. w. lasers

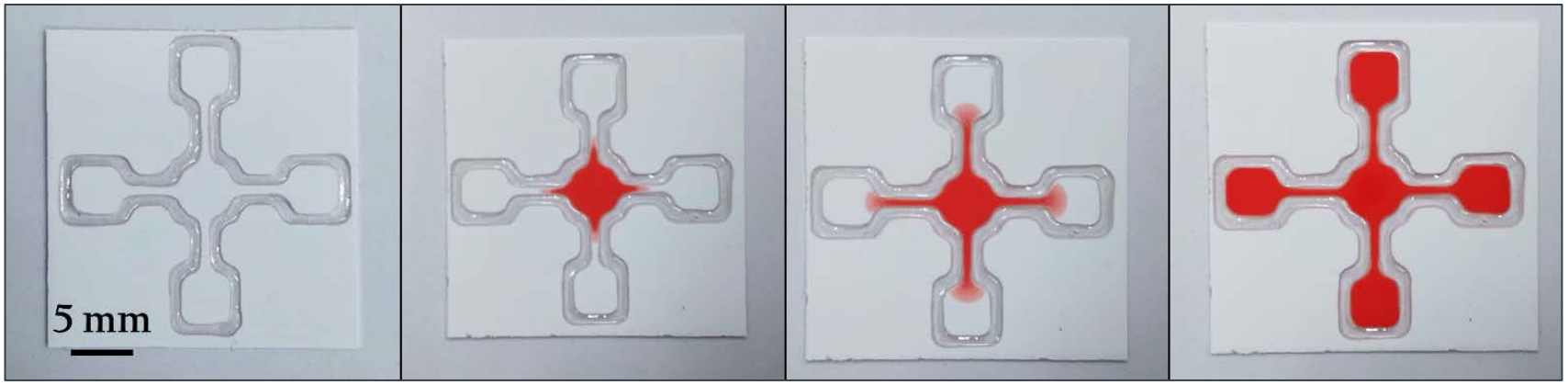
Polymers used
Desolite 3471-3-14

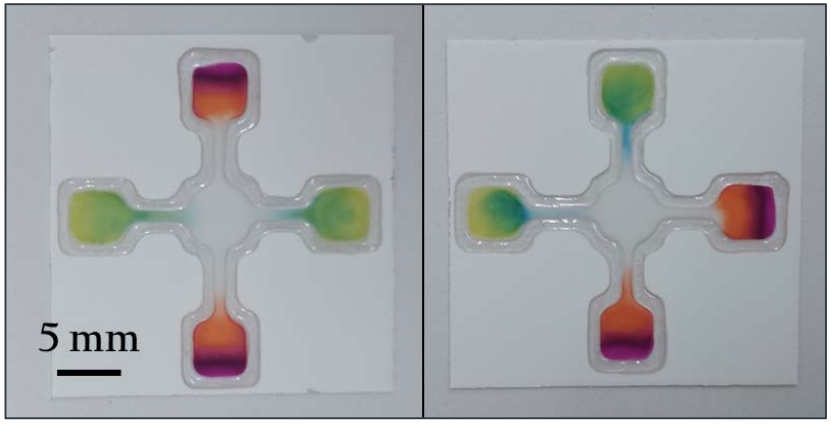
Porous materials patterned
Cellulose, Nitrocellulose membranes, glass-fibre filters, and fabrics

Patterning speeds
> Metre per second

Patterning of porous materials

Results – patterning of user-defined devices





Device allows simultaneous detection of four different bio-markers from within a common fluidic 'sample'.

- Yellow-green indicates the presence of BSA in the sample
- Purple-red indicated the presence of nitrite in the sample

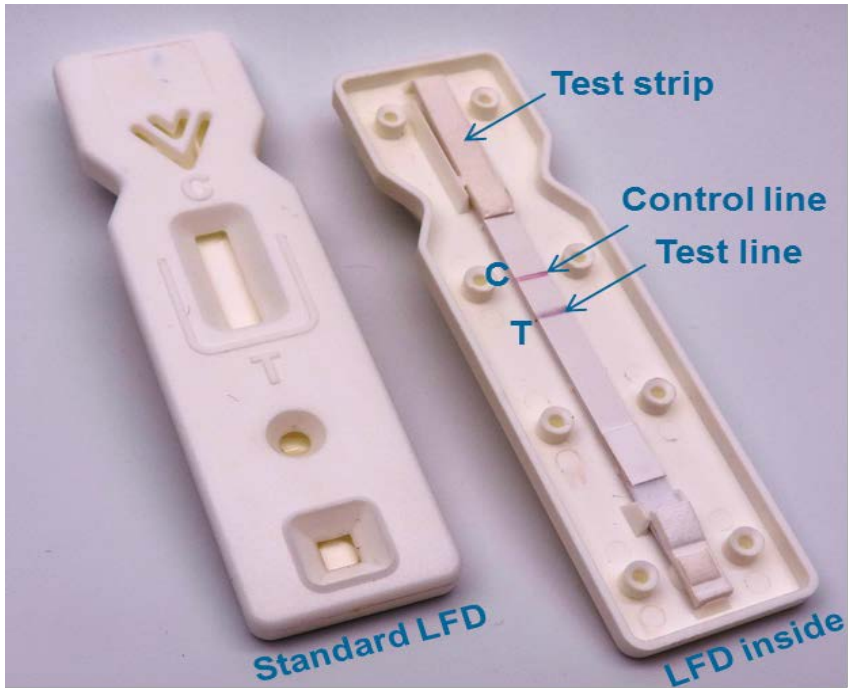
- Example of a device which will enable multiplexed detection or semi-quantitative detection
- Can be a compact device, small footprint, but can be scaled-up, very easy

Patterning of Lateral-flow Devices (LFDs)

Lateral Flow Devices (LFDs) or Dip-Sticks

Their advantages -

- ✓ Used at point-of-care
- ✓ Provide rapid results (~few min.)
- ✓ Are easy-to-use
- ✓ Are affordable (~ few £s)



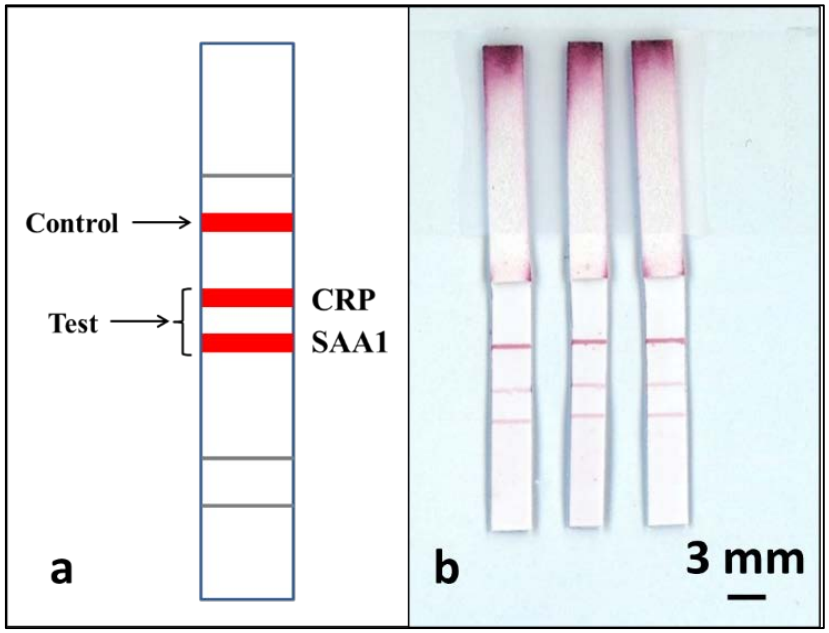
Their disadvantages -

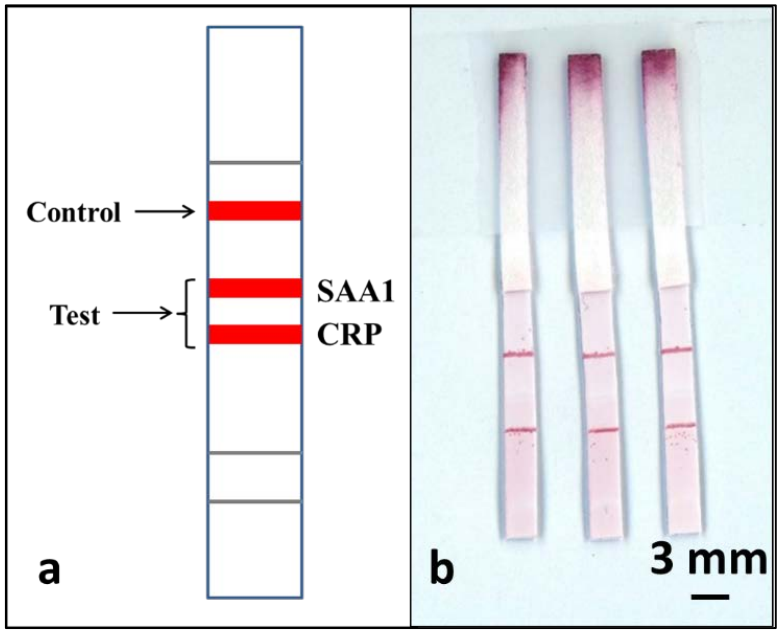
- X Detect a single condition or disease only
- X Give a yes/no answer only
- X Have low sensitivities

Hence have minimal clinical use

Introduction to multiplexed detection on LFDs

Current method – multiplexing in a single flow path





Results for multiplexed detection of CRP and SAA1 in a single LFD with multiple detection sites in the same flow path.

Drawbacks - undesired interference between different detection.

Detection of multiple inflammation markers on a single LFD

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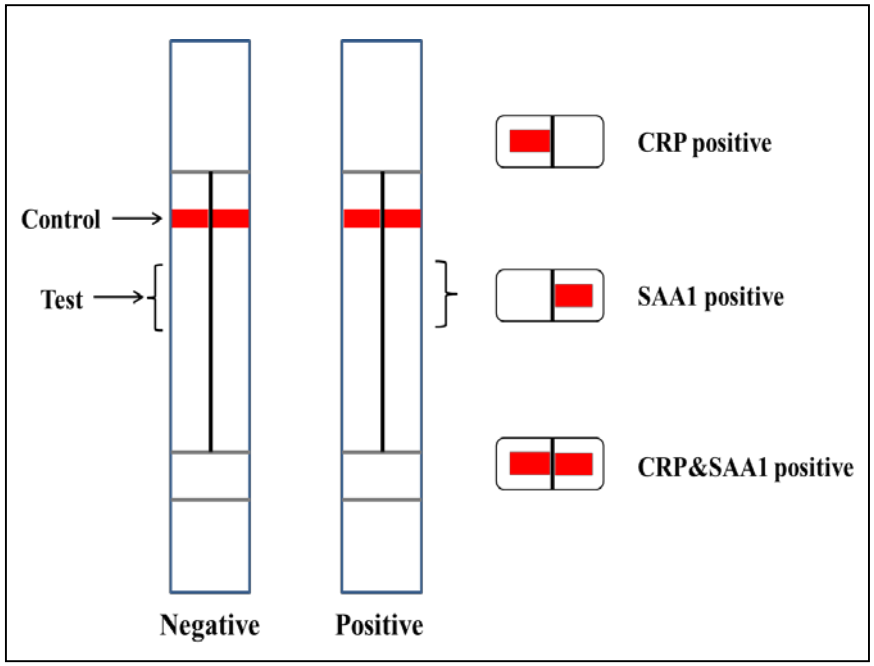
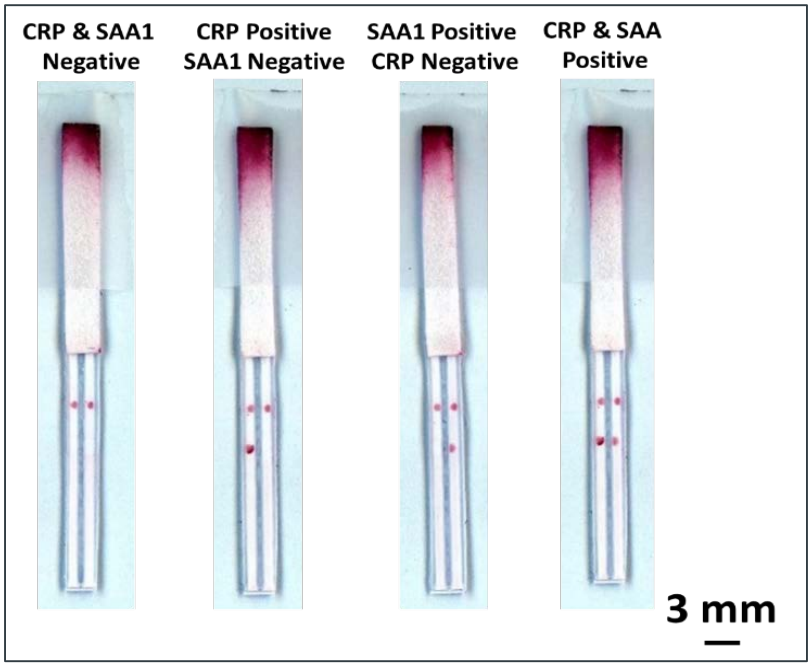
Our solution – multiplexing in multiple isolated flow-paths





Results for detection of CRP and SAA1 using LFDs with multiple flow paths.

Advantages:

- No interference of multiple test sites positioned in the same flow path
- No need for increased device dimension
- No need for addition sample volume

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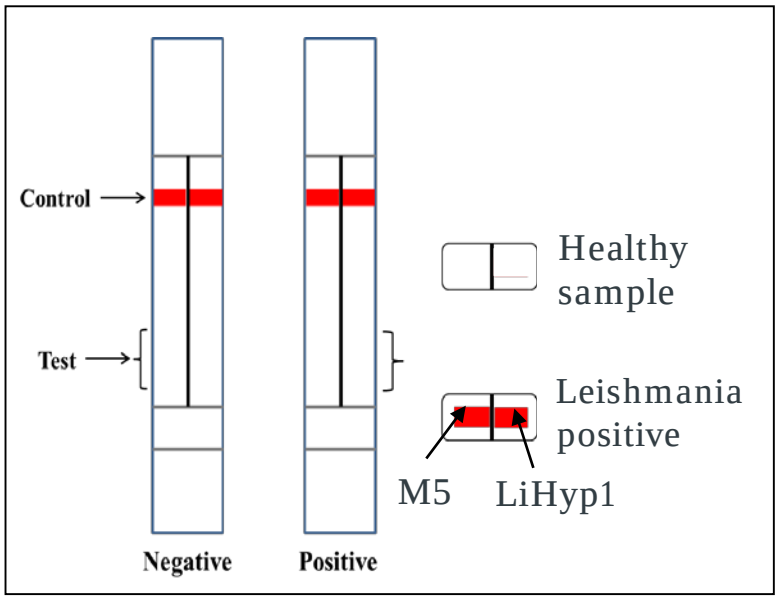
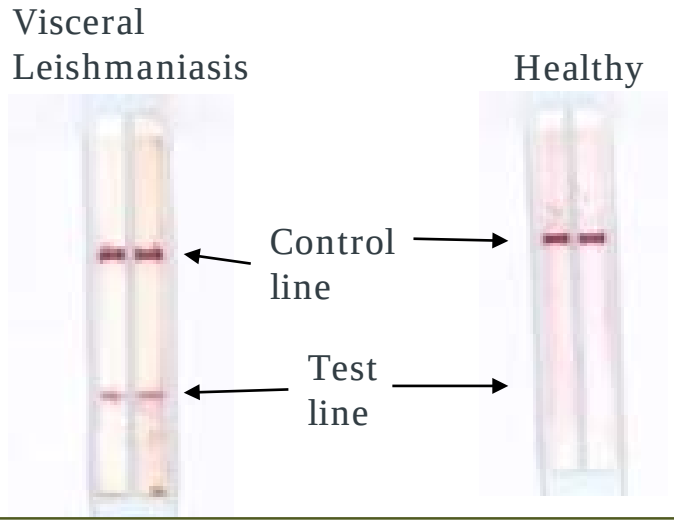
Multiplexed detection of Leishmaniasis



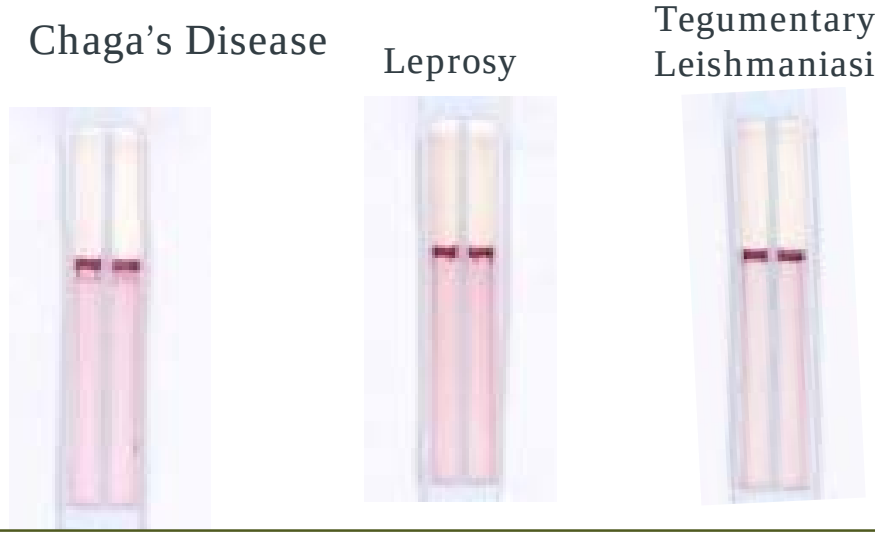
Samples used – Serum and Whole blood

Healthy negative controls, Visceral Leishmaniasis, Chaga's disease, Leprosis, Tegumentary Leishmaniasis

Testing for positive and negative samples:

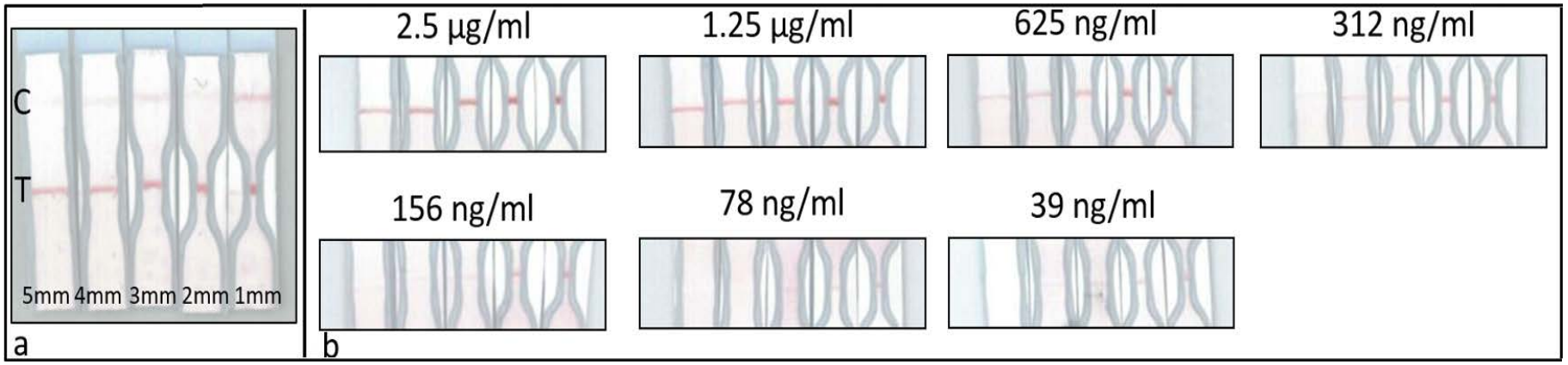


Testing for cross-reaction:



Introduction to improved sensitivity and limit-of-detection on LFDs

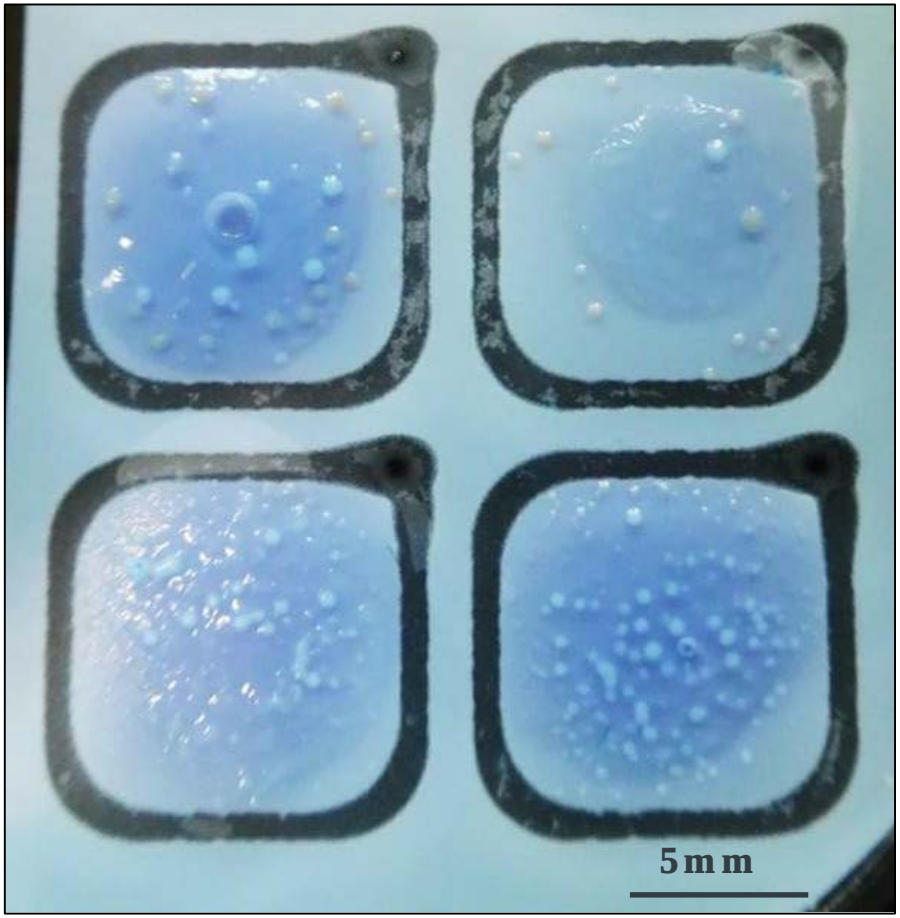
Example– sensitivity and limit-of-detection increase for a CRP assay



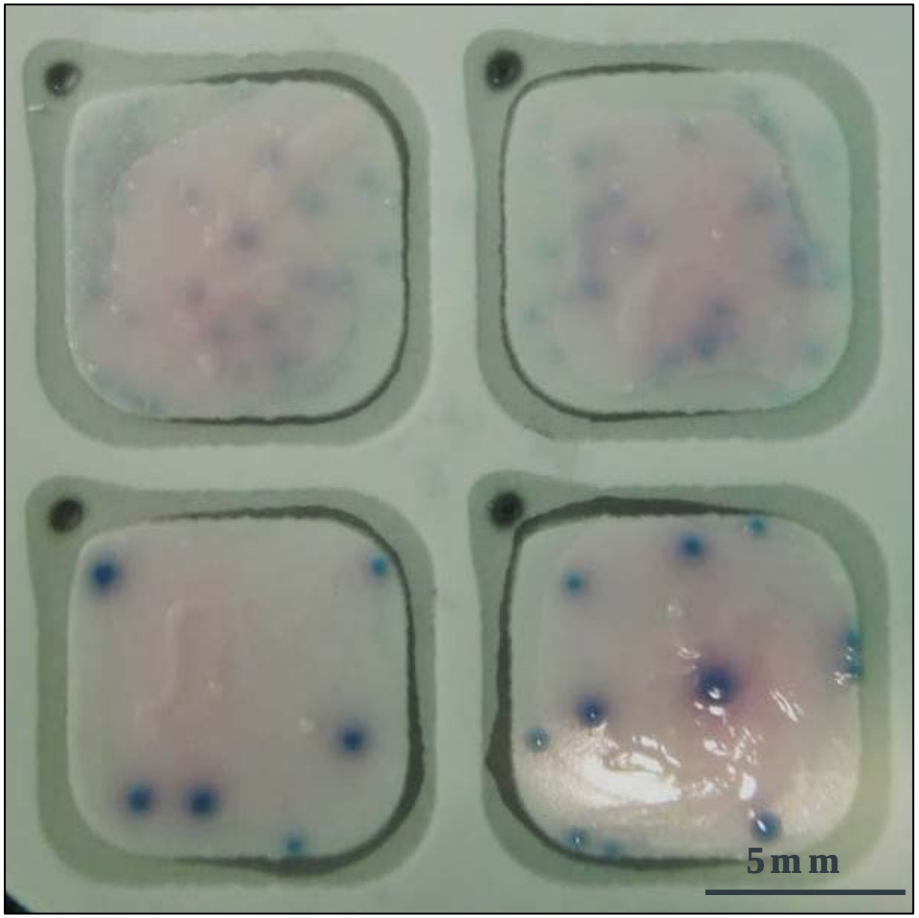
A clear signal enhancement can be observed with the decrease of the constriction widths for each concentrations.

- Improve the sensitivity of the assay by 62x;
- Improve the limit of detection by 32x.

Confinement of bacteria cultures using paper-based wells



Detection of MRSA
Grown on Oxacillin Resistance Screening Agar



Detection of E. Coli
Grown on Brilliance Coliform Selective Agar

Antibiotic resistance testing

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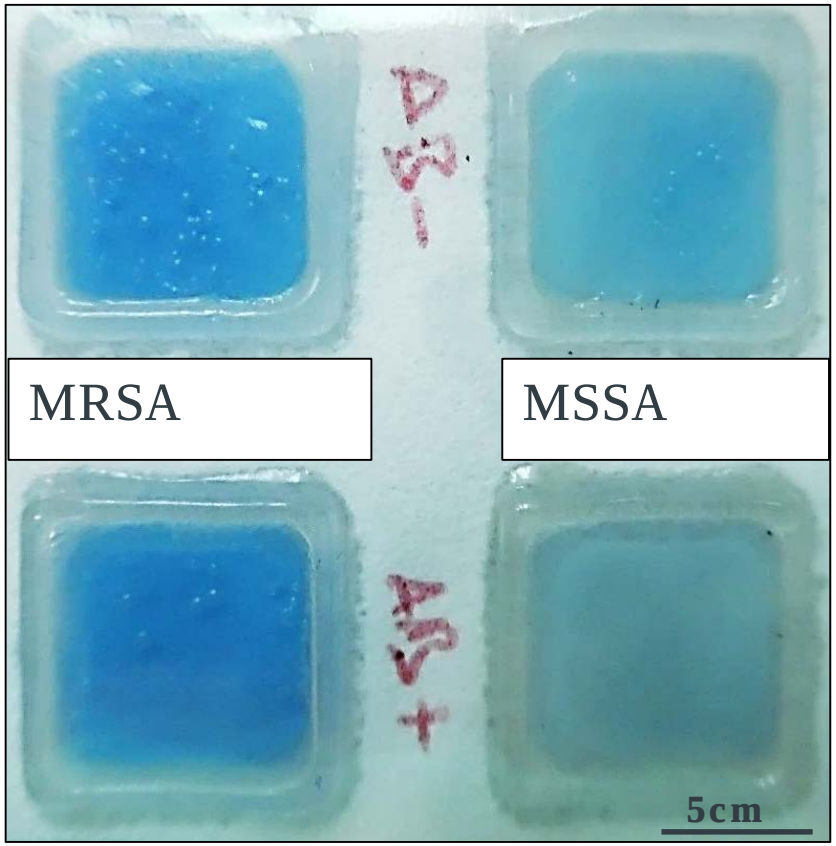

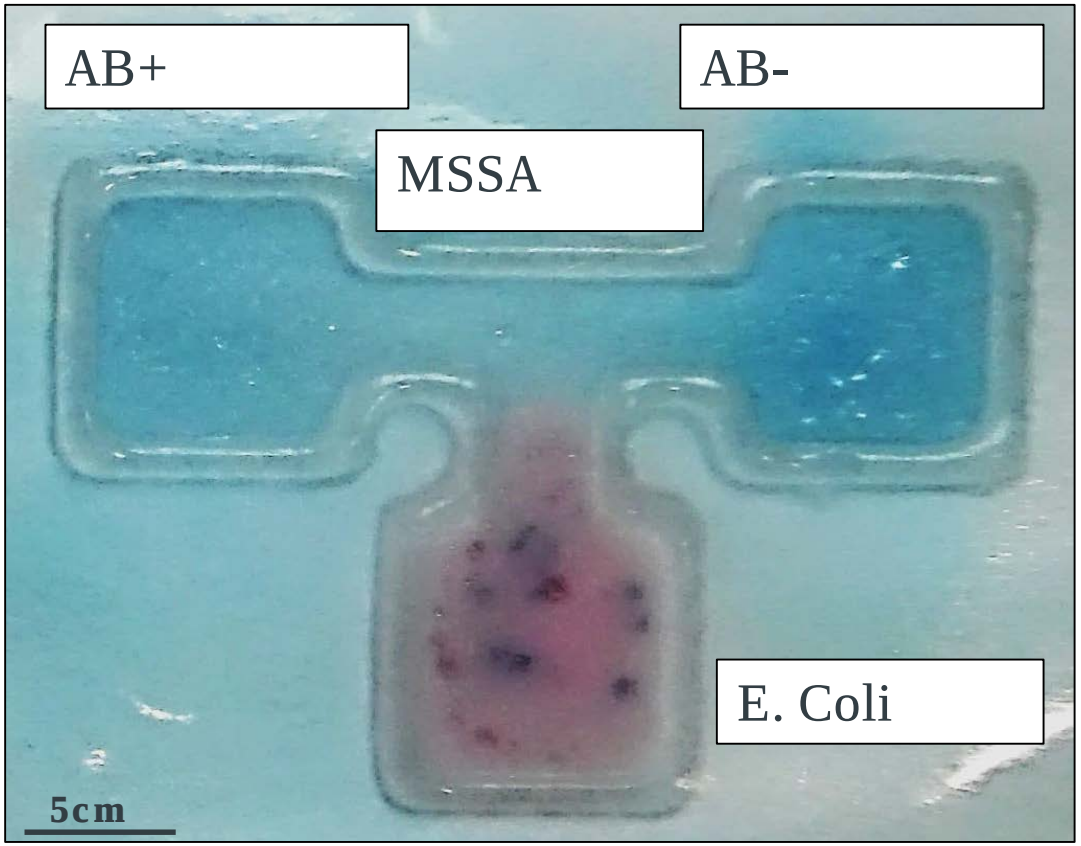

- Testing resistance of MRSA and MSSA to Oxacillin on paper-based mini petri dishes

Conclusion and Collaborations

Fabrication & preparation of paper-based devices by Laser-based direct-write (LDW) methodologies

- No need for specialist environment
- Flexible – changes to laser parameters
- Reducing of feature dimensions
- Rapid prototyping or even large-scale manufacture

✓ Offers a mature solution for multiplexing and enhanced sensitivity and limit of detection on LFDs.

Platform which can be adapted according to the needs

(our) platform + (your) assay = Device as per the user's needs

Highfield Diagnostics

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Enabling point-of-care diagnostics of the future

LaserWrite™ - patented technology

Business Statement:

A spin-out company from The University of Southampton to commercialise novel technology that enables the research, development and manufacture of multiplexed and quantitative point-of-care diagnostic devices by creating fluidic structures within materials used in lateral flow devices.

Visit us on: http://highfielddiagnostics.co.uk/

Thank you!

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Public Health England

Yoperon immuno & molecular diagnostics

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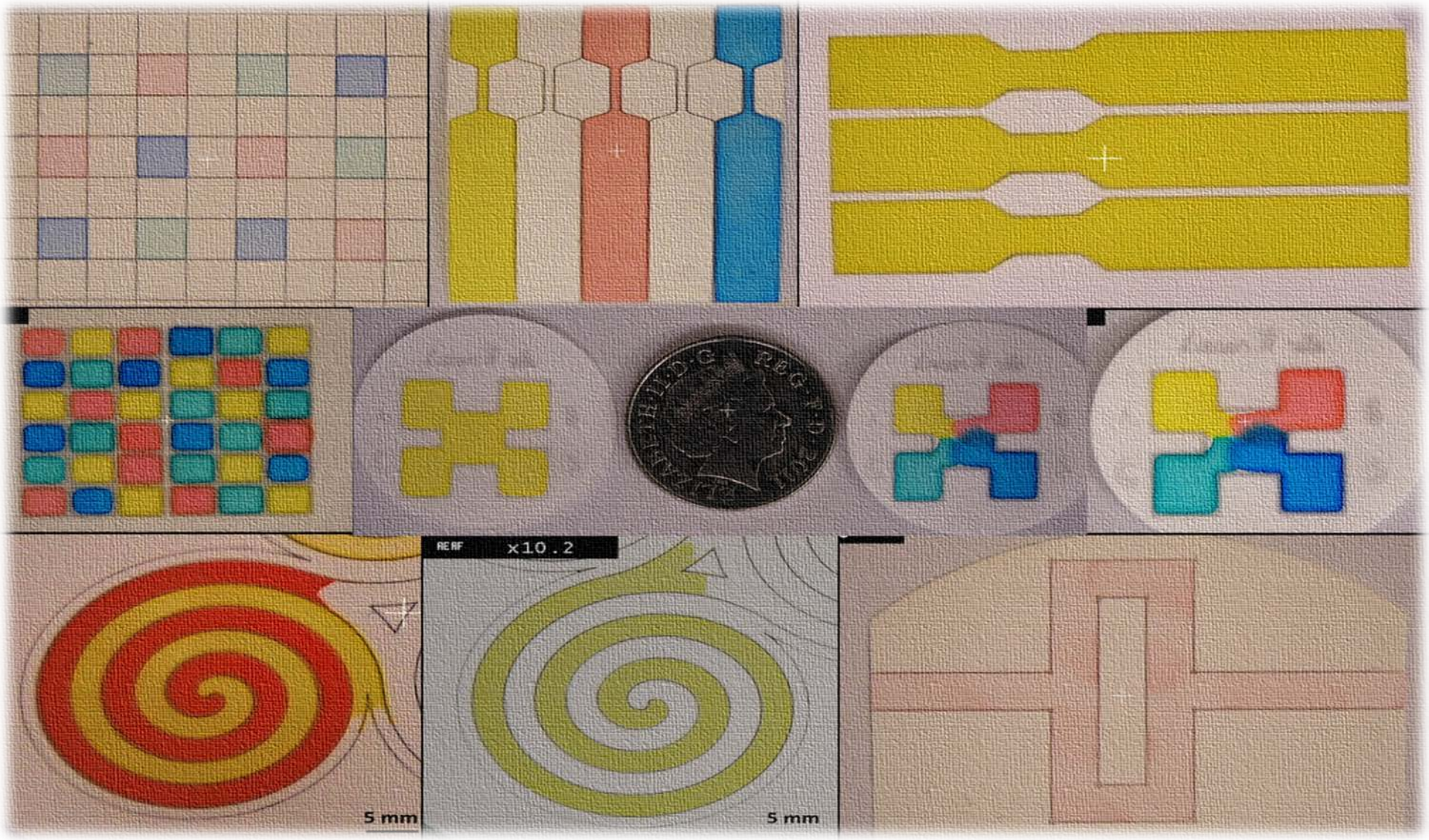
THANKS FOR YOUR ATTENTION



LDW for fabrication of paper-based devices

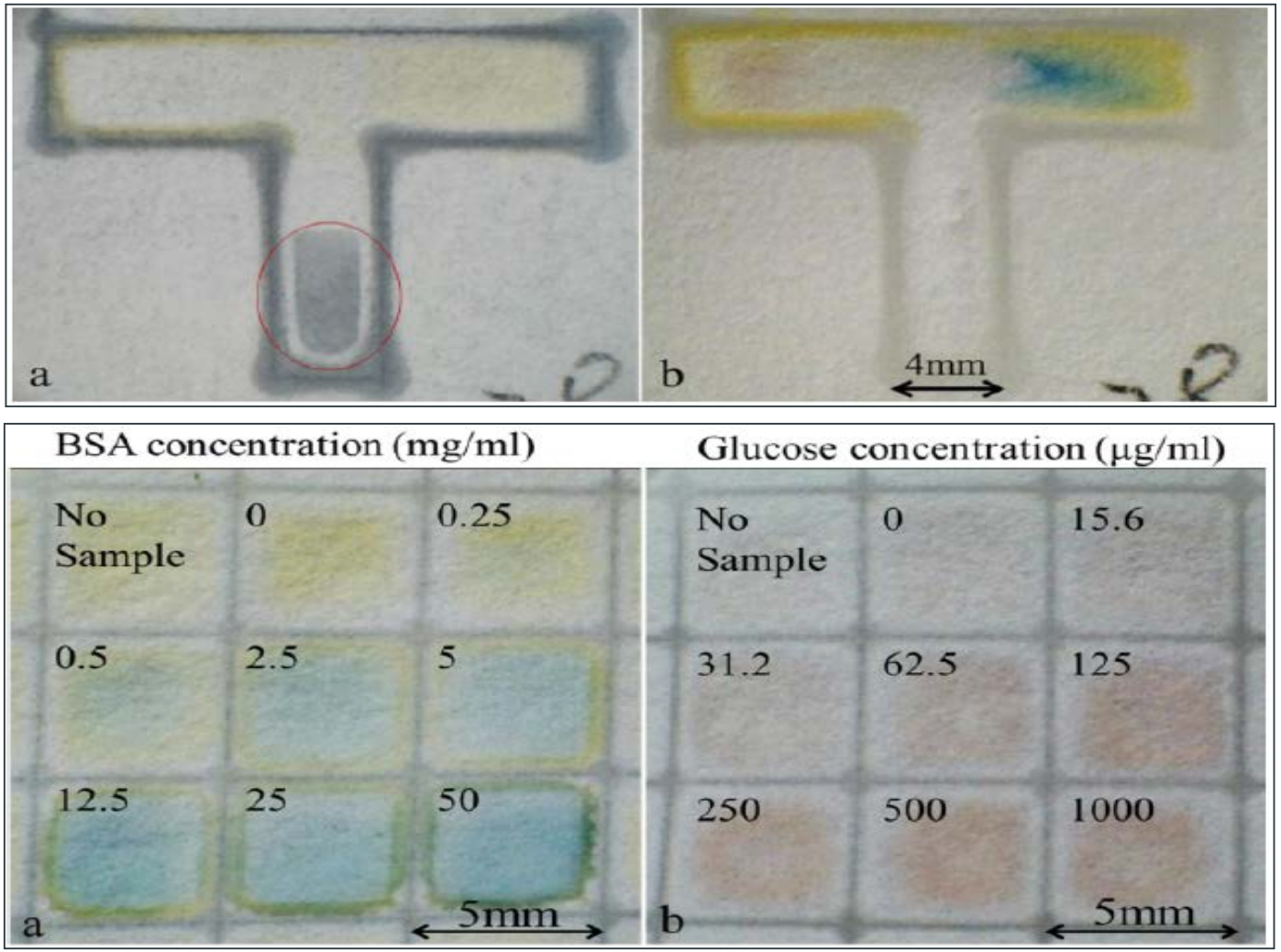
Polymer barriers compatibility

1. The following table shows the compatibility of various solvents and reagents with the photopolymer barriers;

Aqueous Solvent	Compatibility	Polar Organic Solvent	Compatibility	Non Polar Organic Solvent	Compatibility
BSA	Yes	Acetone	Yes	Hexane	Yes
Buffers pH 3 to 10	Yes	Isopropanol	Yes	Toluene	Yes
PBS/TBS	Yes	Ethanol	Yes	Xylene	Yes
Surfactants	Yes	Methanol	Yes		
Water	Yes				

2. No degradation with time (6 months) and temperature (RT – 120 ^{o}C);

Single-step colorimetric assay on cellulose



- Patterned cellulose paper used for detection of BSA and Glucose
- Semi-quantitative detection using a mobile phone camera

Introduction - Paper-based fluidics

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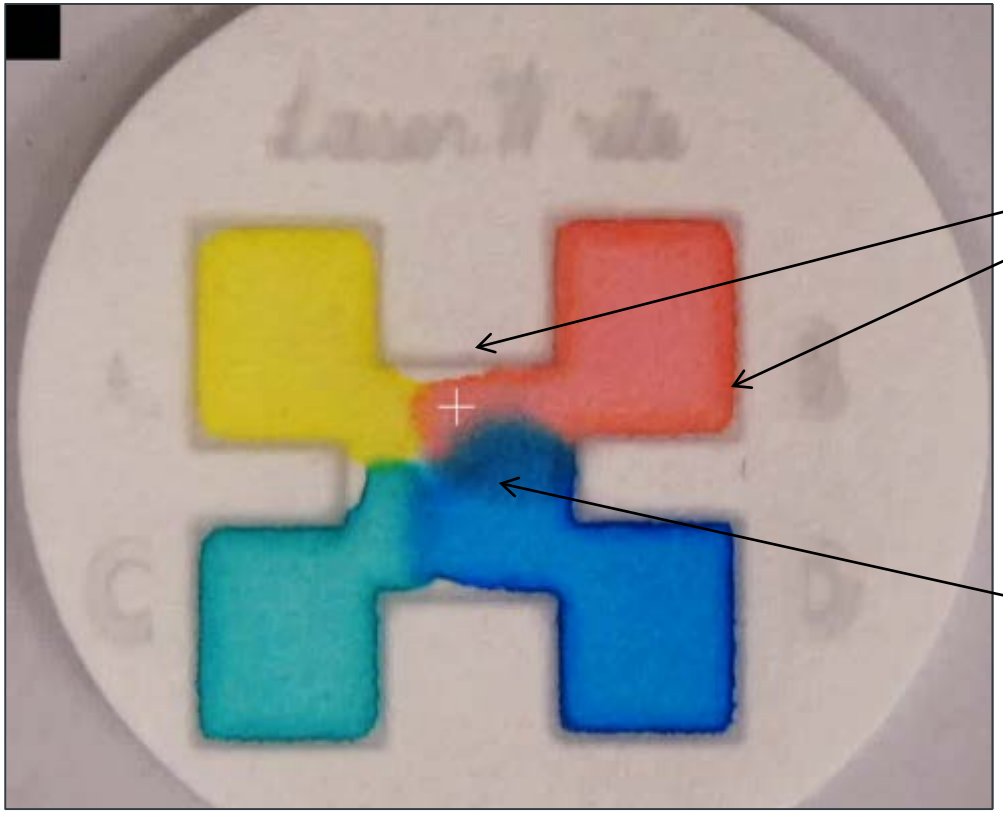

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