Forensics-on-chip:
How and where to apply microfluidics in the forensic field?

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Process and Energy

Who am I?

Presentation outline

- Forensic Investigations
- What is Microfluidics?
- Microfluidics in Forensics
- Future Perspectives

Overview on Forensic Investigations

Forensic investigations

Identification and Analysis of

- **Body fluids (forensic serology)**
- **Drugs of abuse**
- **Explosive residues**

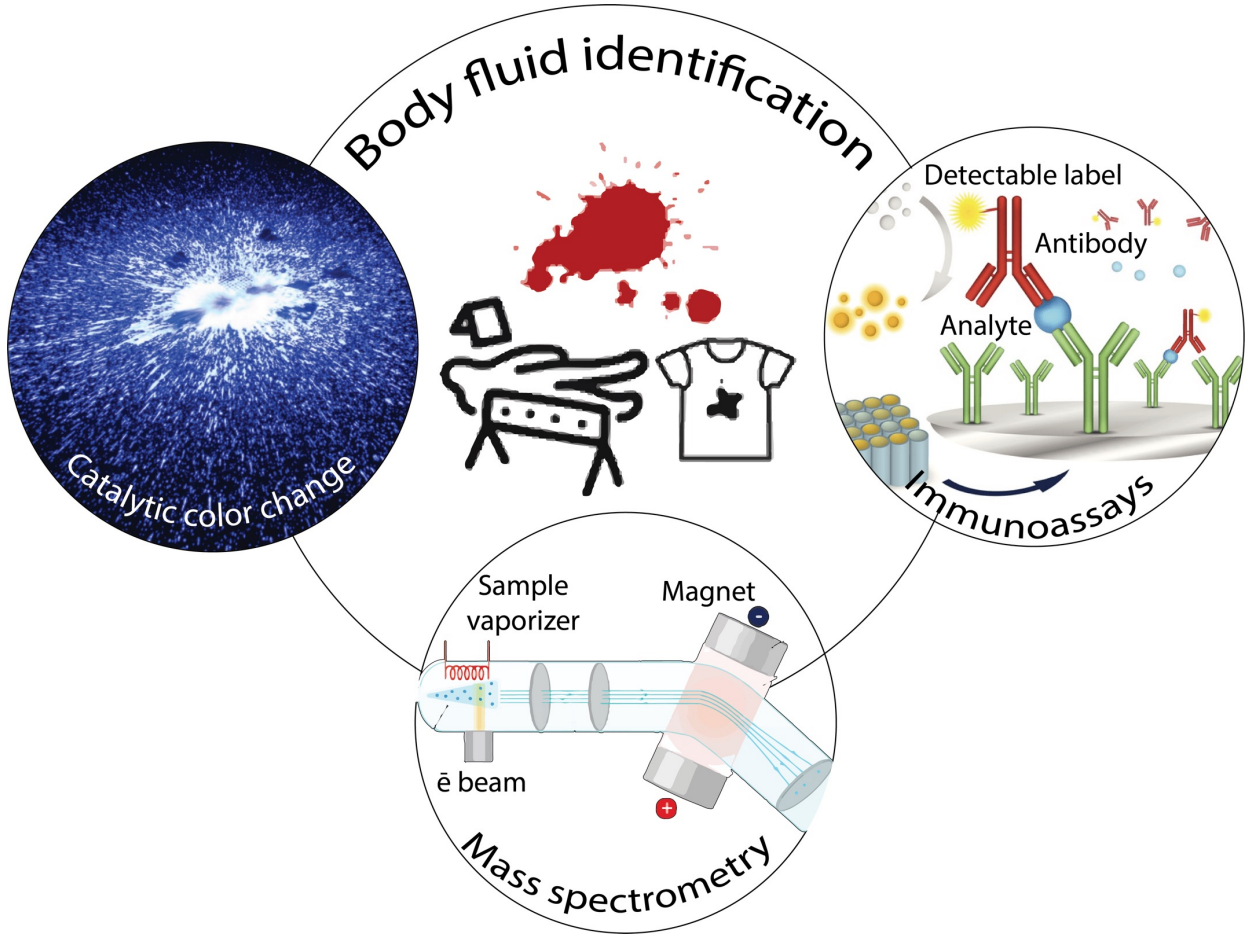
Well-developed presumptive and confirmatory tests are available!

Forensic investigations

Forensic serology

Evidence is sampled and tested for the presence of body fluids (BFs)

Presumptive tests



Confirmatory tests

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Why do we need Microfluidics in Forensics?

Common disadvantages of

Presumptive tests

- Body fluid specific
- Prone to false positive/negative results
- Destructive to genetic (DNA) evidence
- Not label-free
- Susceptible to sample contamination (by chemical reagents)

Confirmatory tests

- Time consuming
- Costly
- Intense sample preparation
- Destructive
- Non-universal

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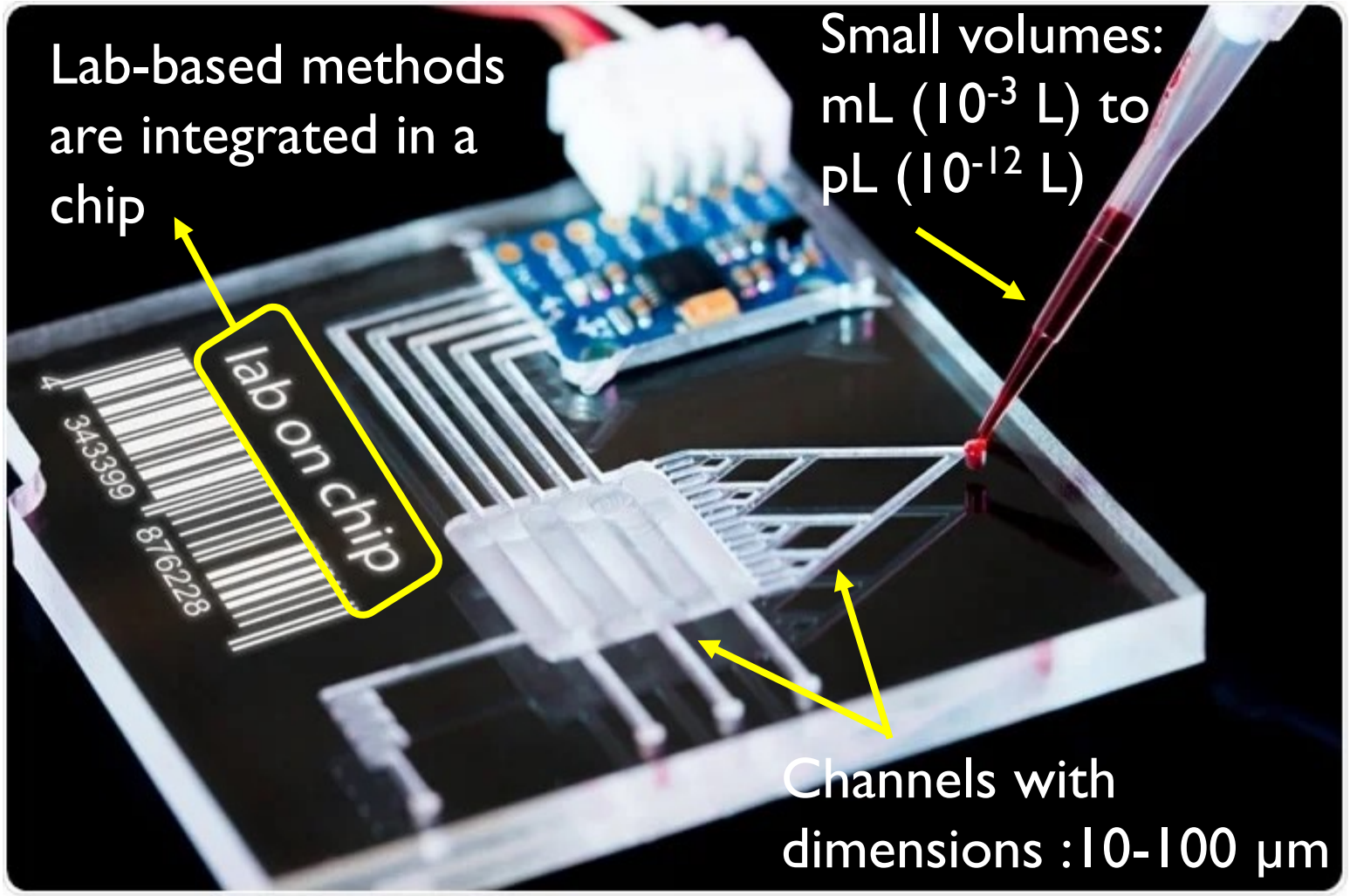
Why do we need Microfluidics in Forensics?

Unique characteristics of Microfluidic devices and lab-on-chip to overcome challenges

+ Rapid analysis
+ Decreased volume of reagents/samples
+ Small footprint
+ Portability
+ Reduced risk of (cross-)contamination
+ Safe sample storage for further analysis
+ Higher surface-to-volume ratio

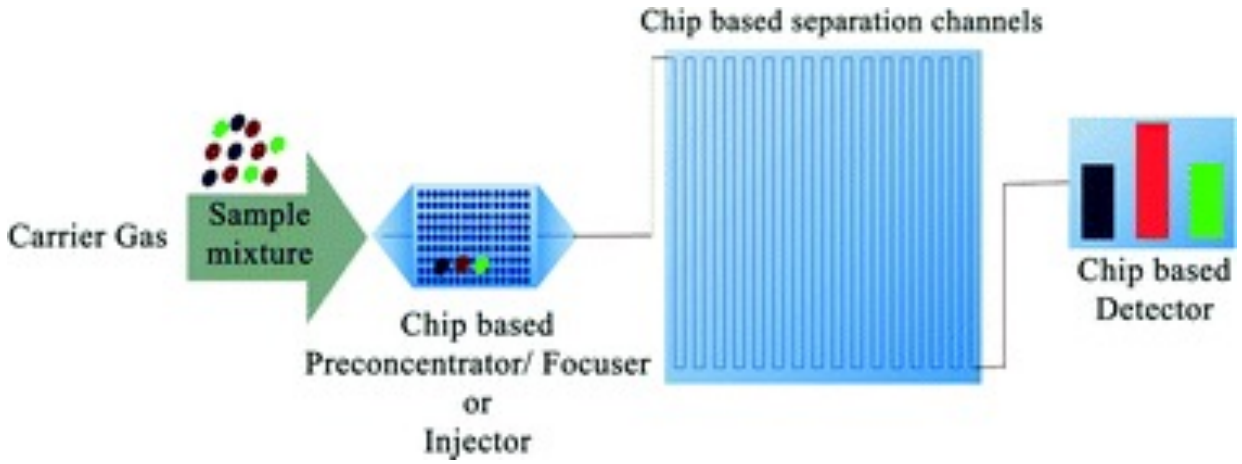
A short summary on Microfluidics

A short summary on Microfluidics

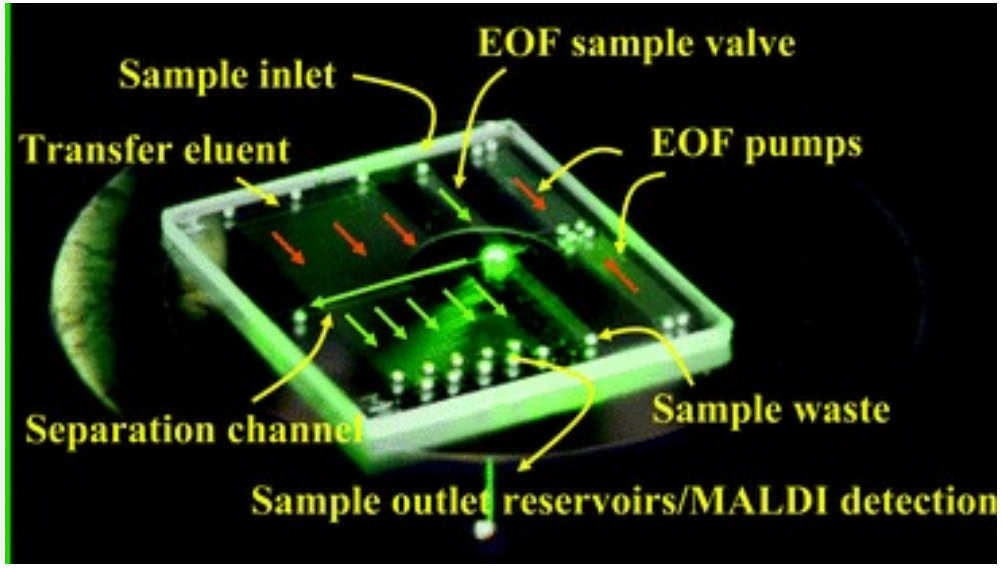


Application of microfluidic devices

Analytical platforms



Gas chromatography on-a-chip [2]

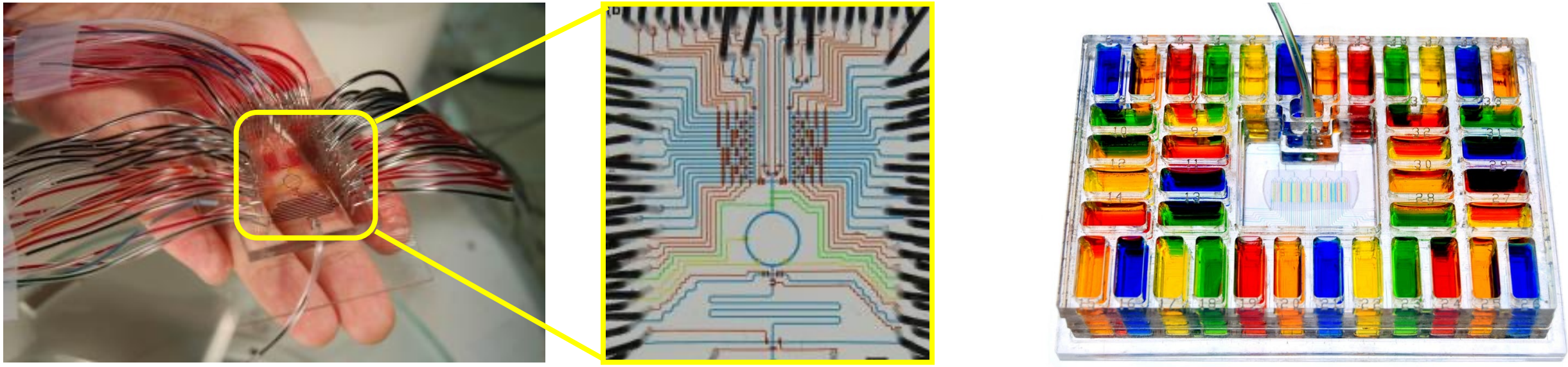


Liquid chromatography interfaced to MALDI-MS detection on-a-chip [3]

[2] F. Haghighi et. al., ***Lab Chip***, 2015,**15**, 2559-2575 .
[3] I. M. Lazar et. al., ***Lab Chip***, 2013,**13**, 2055-2065.

Application of microfluidic devices

Reactions and flow chemistry

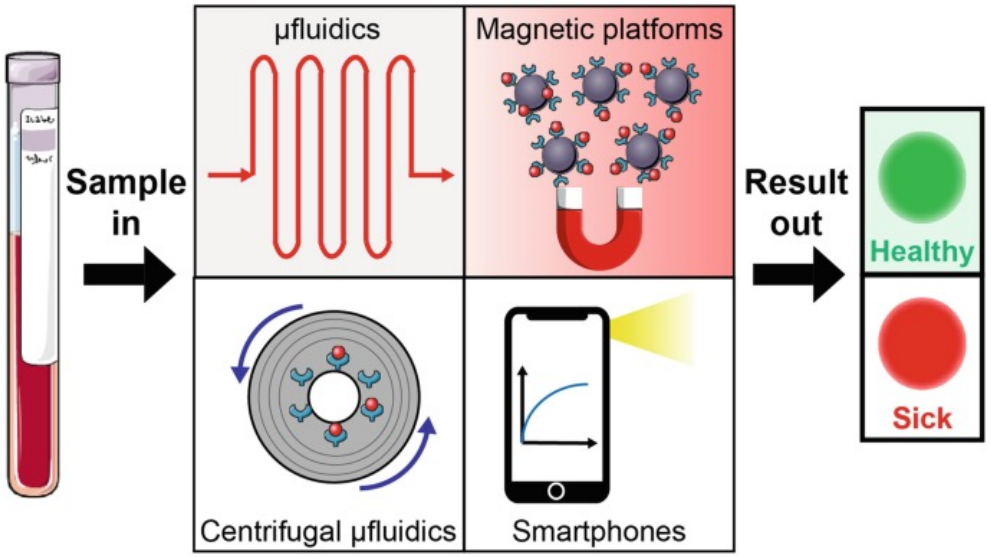


Simultaneous multiple chemical reactions at once!

A short summary on Microfluidics

Application of microfluidic devices

Point-of-care diagnosis [4]



Diagnostic testing at the location of patient

[4] S.A. Graham et. al. (2020), Lab-on-a-Chip Devices for Point-of-Care Medical Diagnostics. In: Bahnemann, J., Grünberger, A. (eds) Microfluidics in Biotechnology. Advances in Biochemical Engineering/Biotechnology, vol 179. Springer.

A short summary on Microfluidics

Materials and fabrication techniques

Inorganic

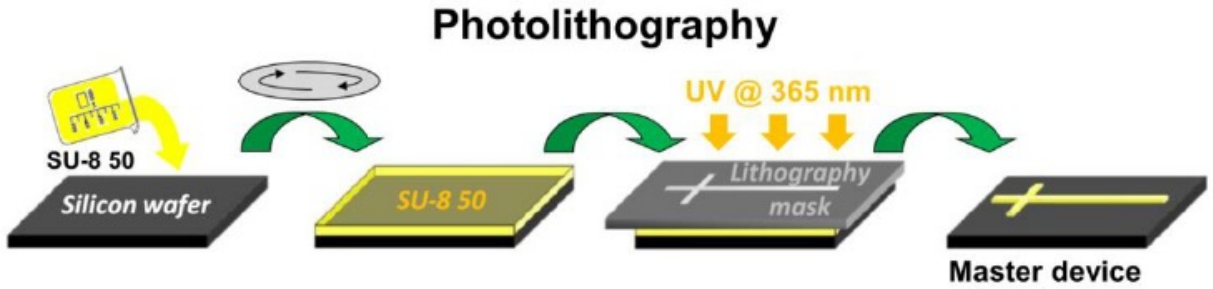
- Silicon
- Glass

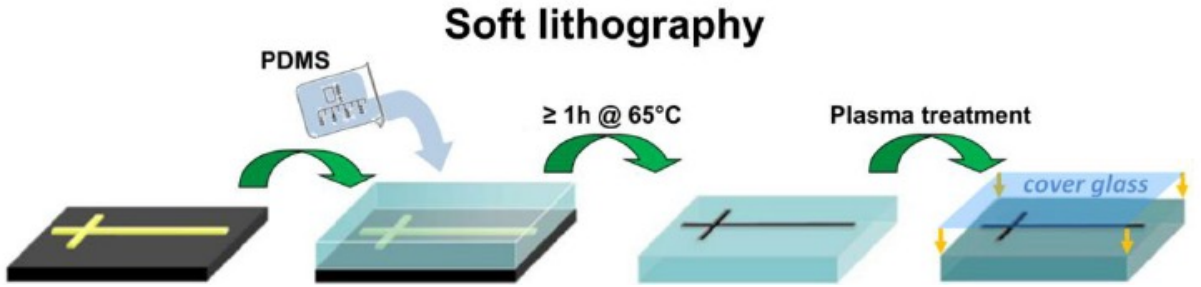
Organic (polymers)

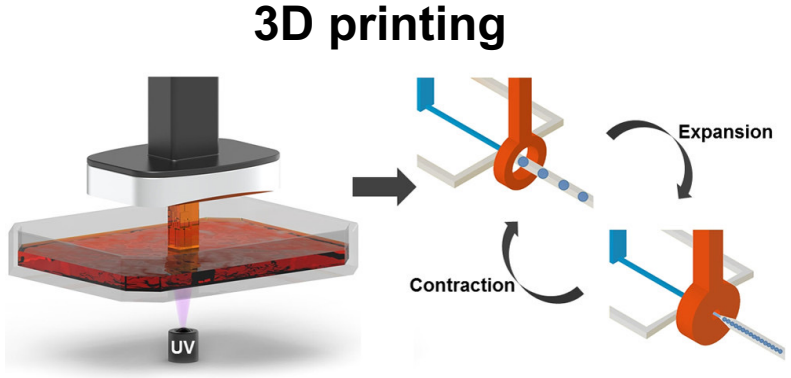
- Elastomers: PDMS
- Thermoplastic: PMMA, PC
- Cyclic Olefin Polymers (COP)

Paper

- Paper cellulosic fibers: pure cotton







[5] Y. Ma et. al., ***Chem. Commun.***, 2014, **50**, 112-114.
[6] N. Weigel et. al, ***ACS Appl. Mater. Interfaces***, 2021, **13**, 31086–31101.

A short summary on Microfluidics

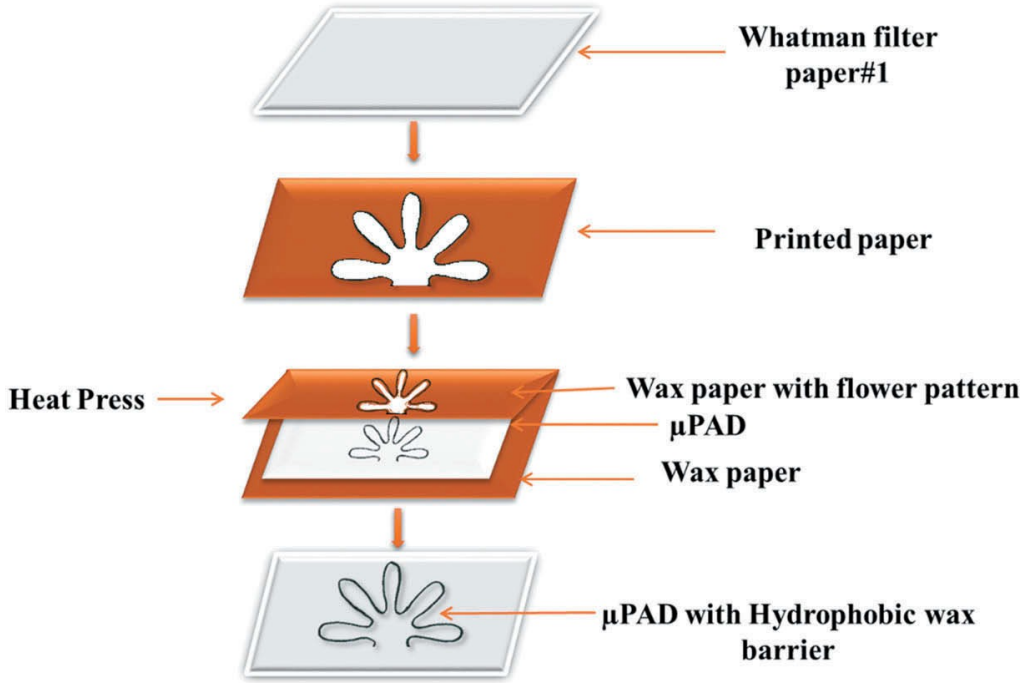
Microfluidic Paper-based Analytical Devices (µPAD)

+ Simple with low-cost material
+ Reduce final cost
+ Limited need of peripherals (due to capillary action (no need for pump)
+ Can be used by non-trained personnel
+ Can be used in remote areas
+ Satisfies ASSURED criteria*

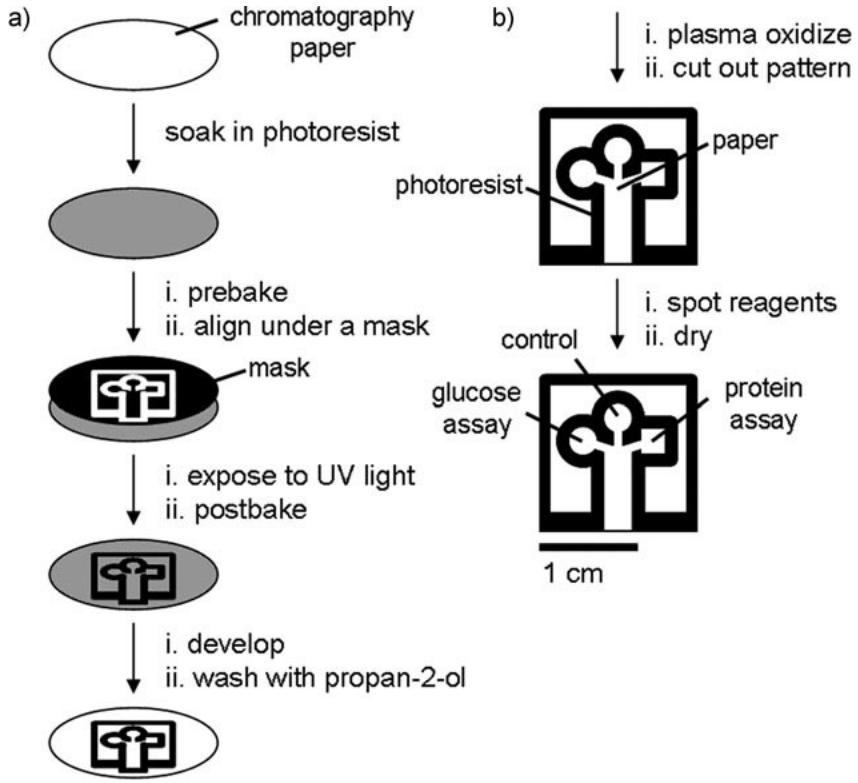
*ASSURED criteria:
Any analytical device must be **A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free, and **D**eliverable to enable analysis outside of well-equipped laboratories.

Microfluidic Paper-based Analytical Devices (µPAD)

Fabrication methodology



Wax printing on Whatman filter paper [7]



Photolithography for patterning paper [8]

[7] N. Ansari et. al., ***Australian Journal of Forensic Sciences***, 2021, **534**, 407-418.

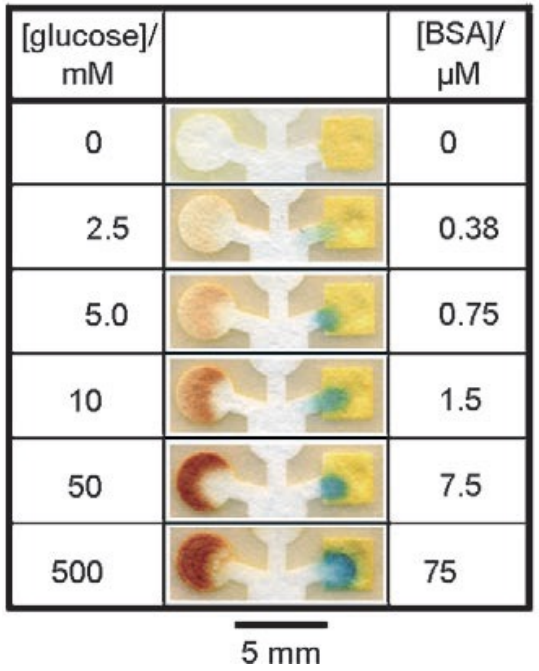
[8] A.W. Martinez et. al., ***Angew. Chem. Int. Ed.*** 2007, **46**, 1318 –1320.

A short summary on Microfluidics

Microfluidic Paper-based Analytical Devices (µPAD)

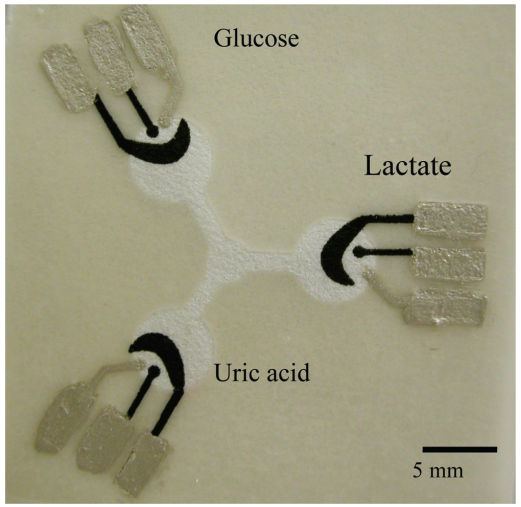
Sensing mechanisms for analyte detection

Colorimetric



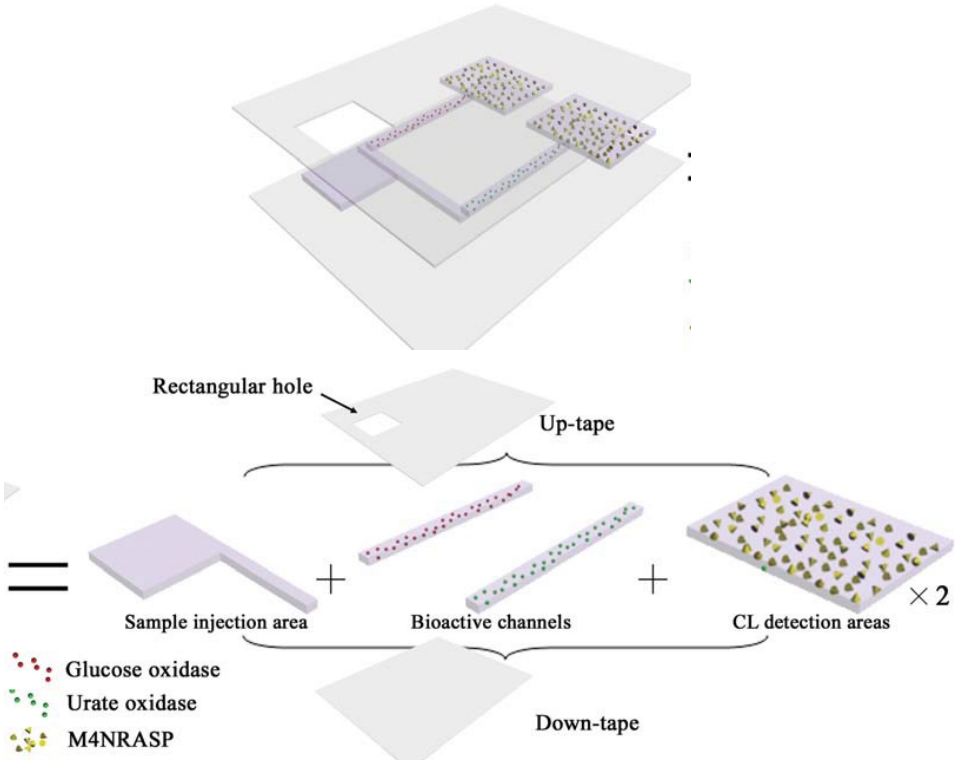
Artificial urine

Electrochemical



Biological sample

Chemiluminescence



Artificial urine

Microfluidics in Forensic Applications

Body fluid screening and identification

Simultaneous identification of BFs [9]

Kastle-Meyer

Phenolphthalein

Sodium perborate tetrahydrate

Starch iodine

Acid phosphatase

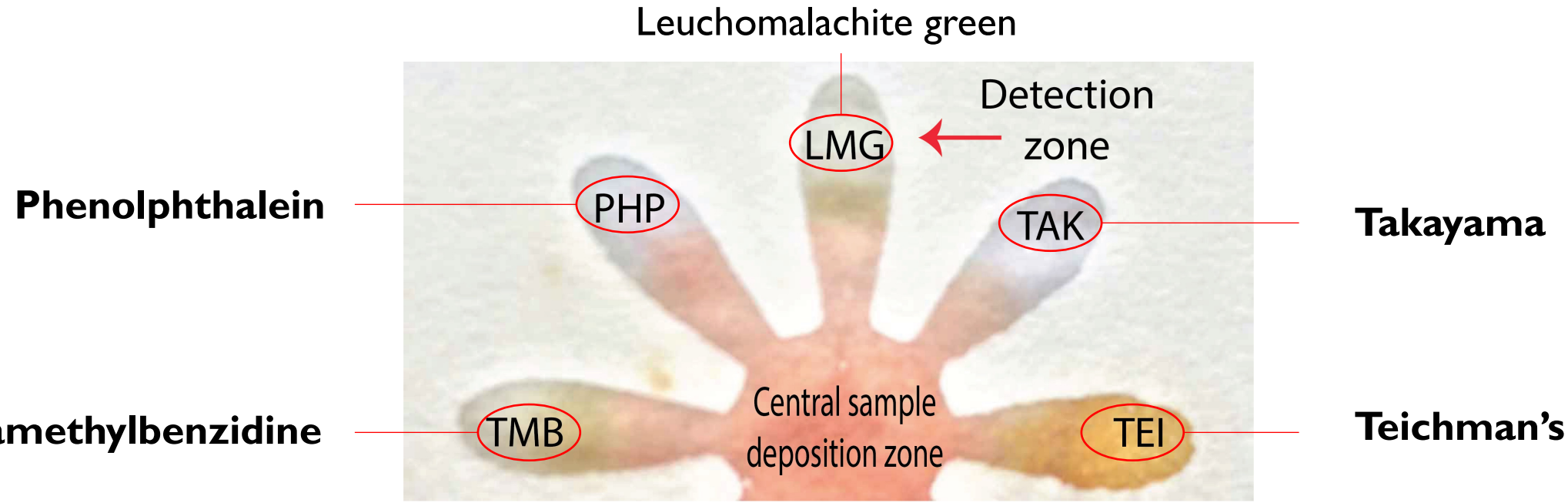
Nesslers reagent

Multiplexed µPAD before and after use in forensic serology for simultaneous colorimetric detection of urine, blood, saliva, and semen in 10-15 min.

[9] R. L. Cromartie, ***Anal. Methods***, 2019,**11**, 587-595.

Body fluid screening and identification

Blood detection and blood typing assays



Rapid blood detection based on presumptive and confirmatory colorimetric reagents

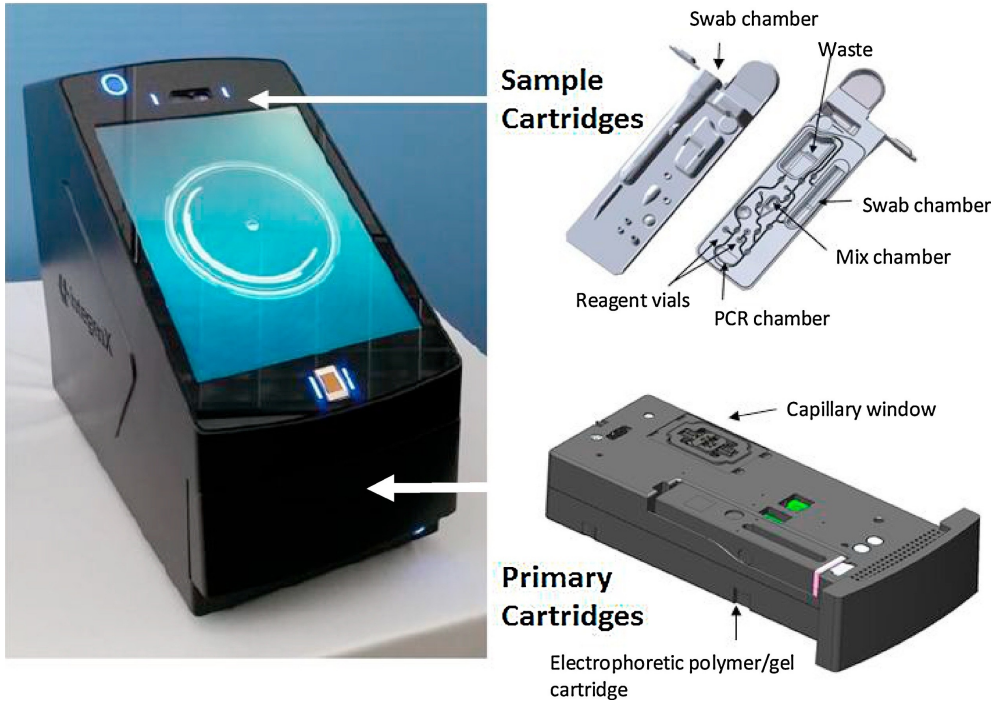
[10] N. Ansari et. al., ***Australian Journal of Forensic Sciences***, 2021, **534**, 407-418.

Microfluidics in Forensic applications

Genetic profiling and human identification

Forensic DNA analysis in microfluidics

Rapid DNA initiative proposed by FBI in 2010!



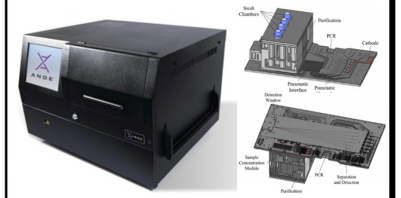
Rapid HIT® ID system for DNA analysis from IntegenX [11]

[11] R. Wiley, et. al., ***Forensic Science International: Genetics***, 2017, **31**, 180-188.

Genetic profiling and human identification

Rapid DNA systems

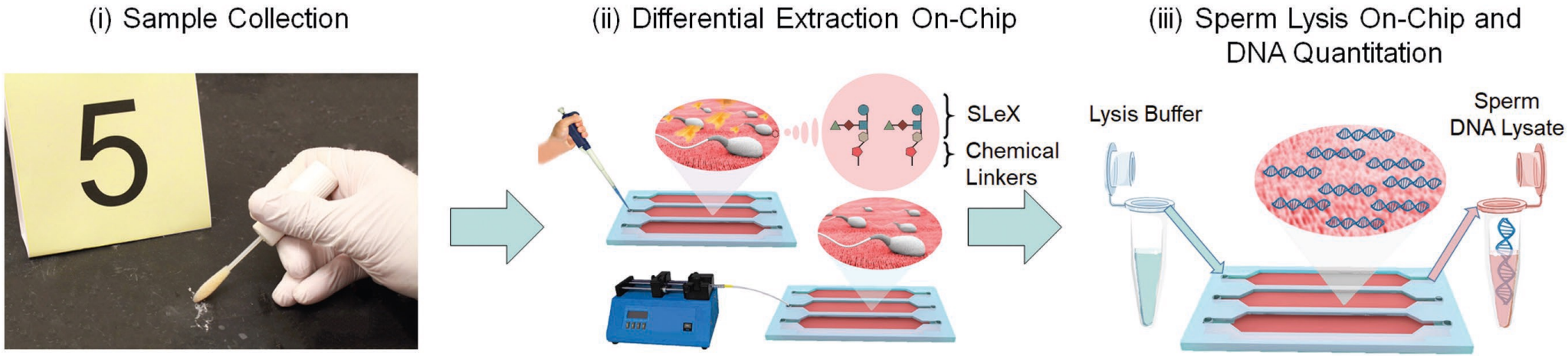
Automated **swab-in-profile-out**: only *few* are commercially available

Rapid DNA System Comparison		RapidHIT ID System	ANDE 6C Rapid DNA System
			
Cost	System Cost	~150 – 200 k	~250 k
	Cost per Sample	~$150/sample	~$250/sample
Investigative Time	Analysis time	90 mins	90 mins
	Hands-On Time	~1 minute	~1 minute
Practical Implementation	System Size (L x W x H)	19 x 10.5 x 21 inches 62.6 lbs	17.7 x 29.5 x 23.6 inches 117 lbs
	Cold Storage	Required	Not Required
	Throughput	1 sample per run	4 – 5 samples per run
	NDIS Approval	✓	✓

[1] R. Turiello, ***Forensic Science International: Genetics***, 2023, **63**, 102824

Genetic profiling and human identification

DNA extraction and purification on a chip

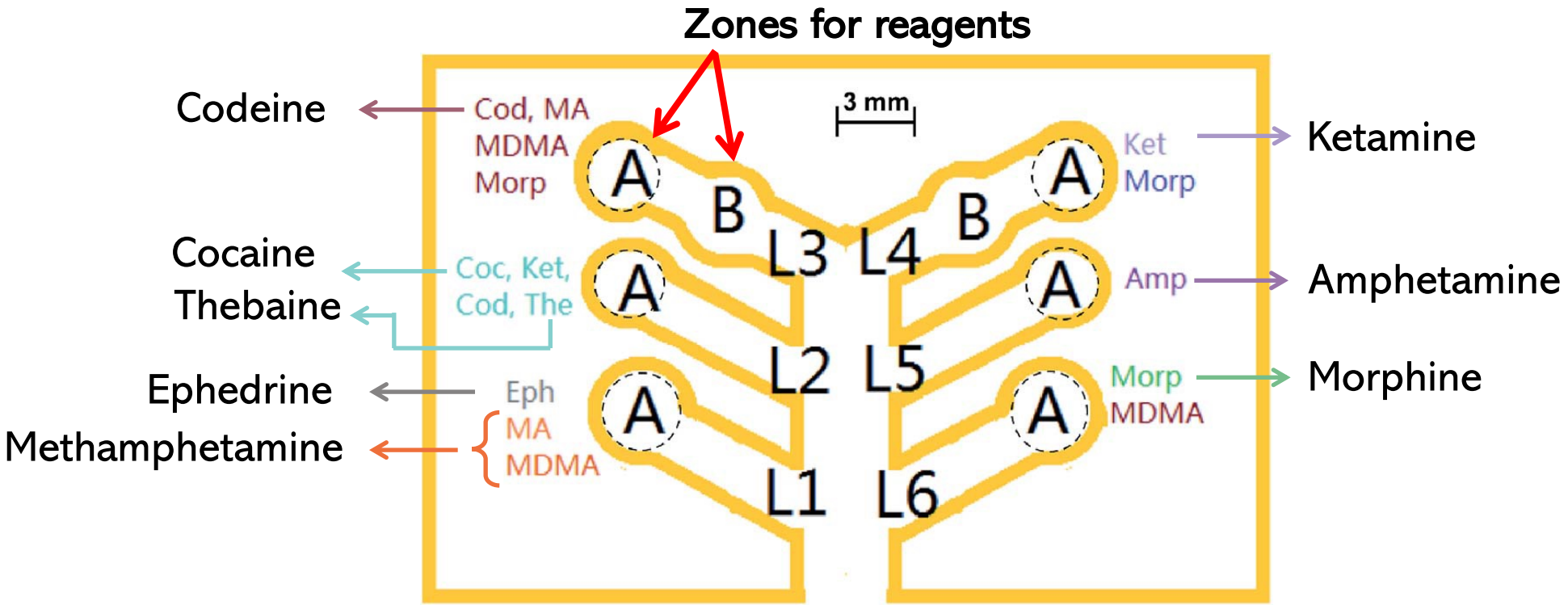


On-chip selective capturing of sperm cells followed by sperm lysis in forensic assault cases [12]

[12] F. Inci, et. al., ***Adv. Sci.*** 2018, **5**, 1800121.

Forensic Drug analysis (FDA)

Detection of seized drugs using μPADs



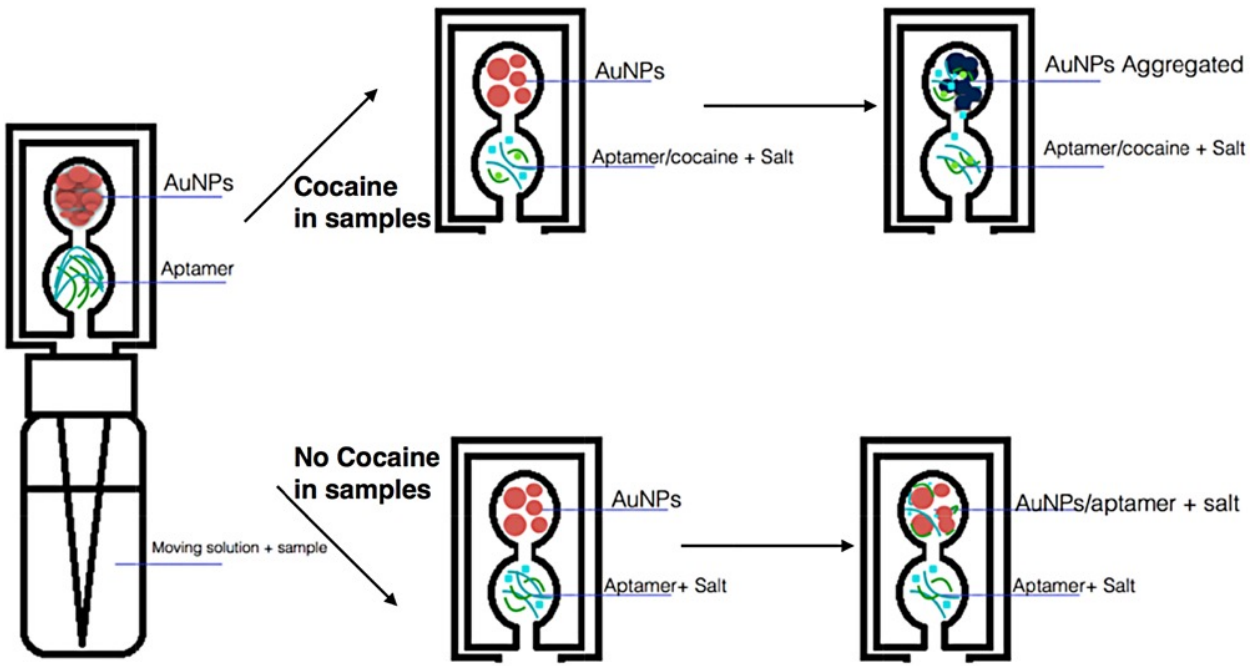
Multiplexed colorimetric detection of various drug compounds. [15]

[15] G. Musile et. al., ***Anal. Methods***, 2015, **7**, 8025.

Illicit drugs and drugs of abuse

Aptamer/antibody recognition for more selective detection

Aptamers: Engineered nucleic acids with specific recognition characteristics for small molecules.

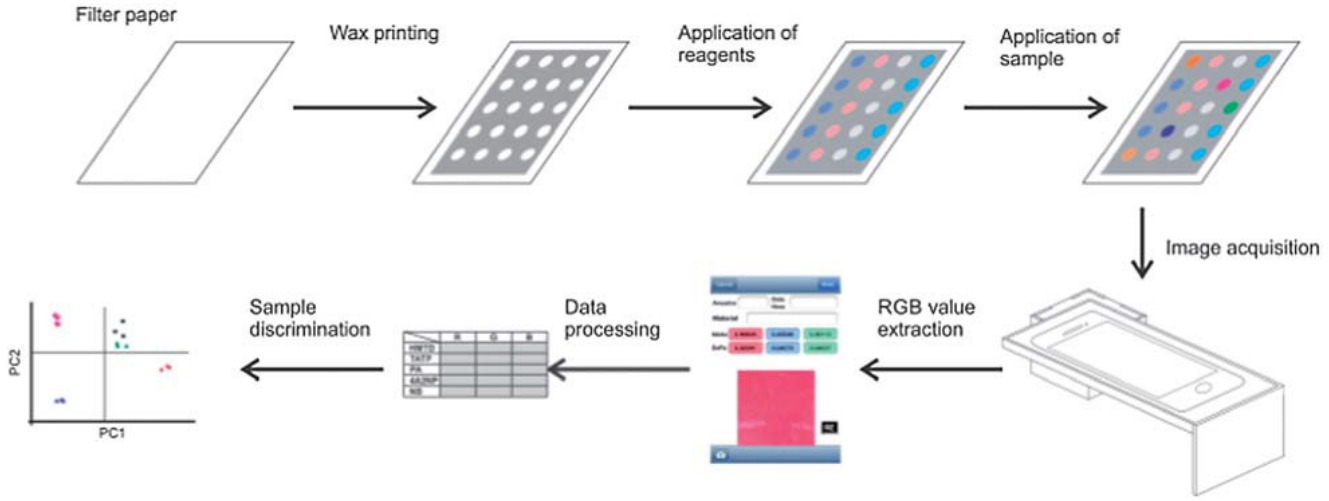


μPAD with gold nano particles and anti-cocaine aptamers. [16]

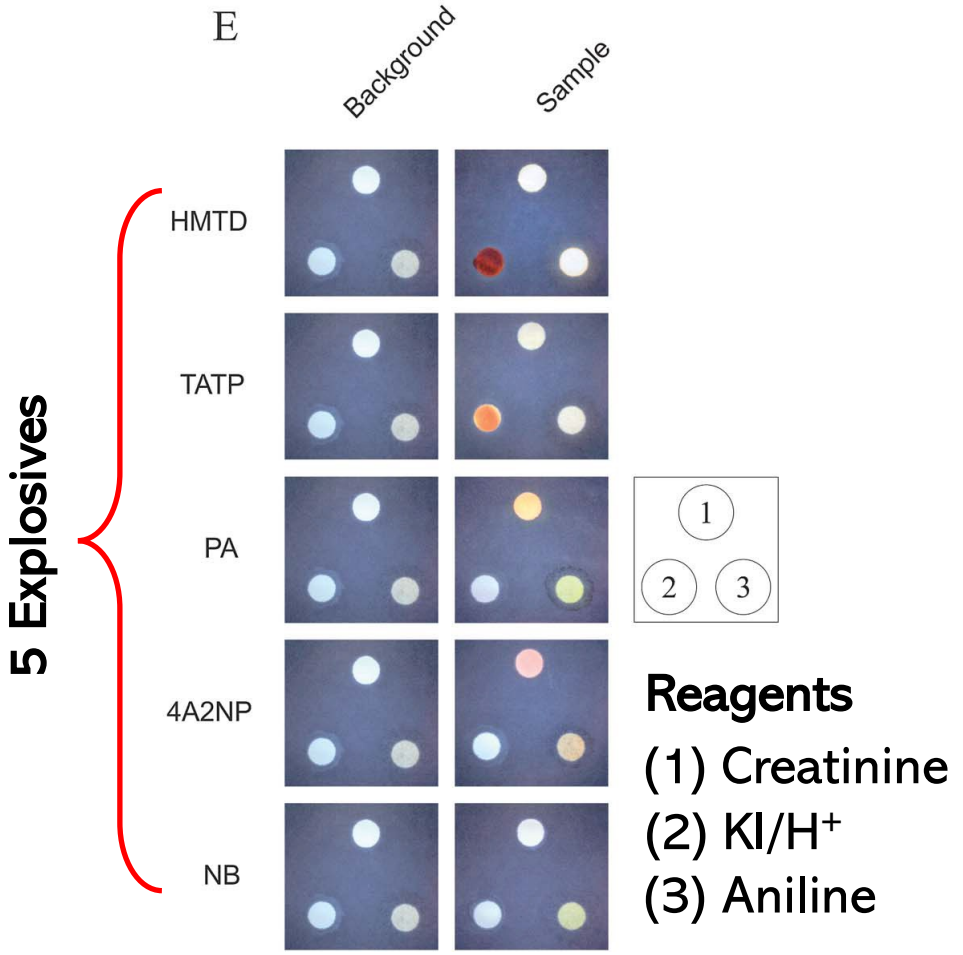
[16] L. Wang, et. al., ***Electrophoresis*** 2018, **39**, 470–475.

Explosive residues

Detection of high explosives using μPADs



Fabrication and measurement principles. [17]

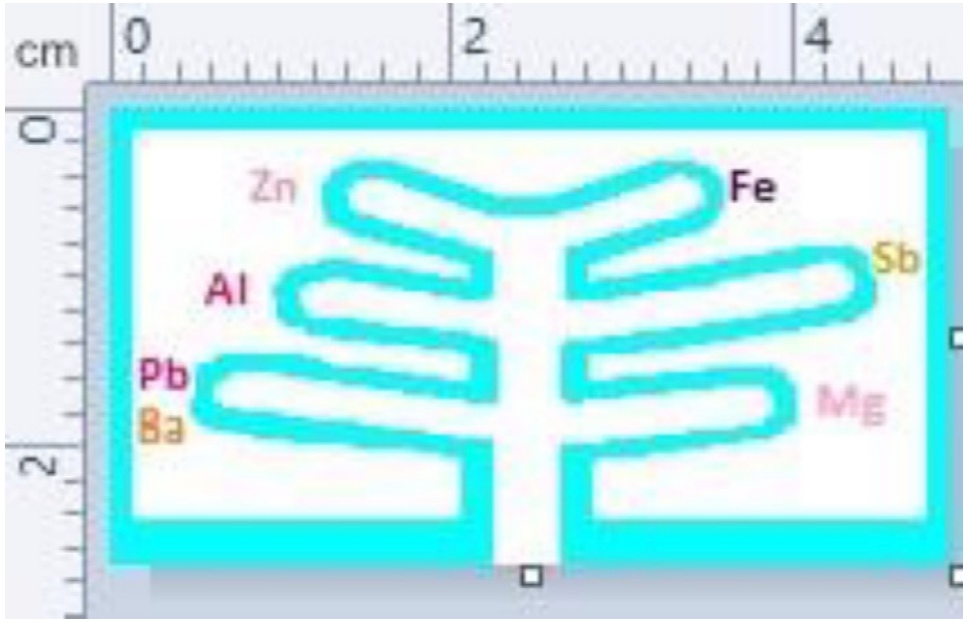


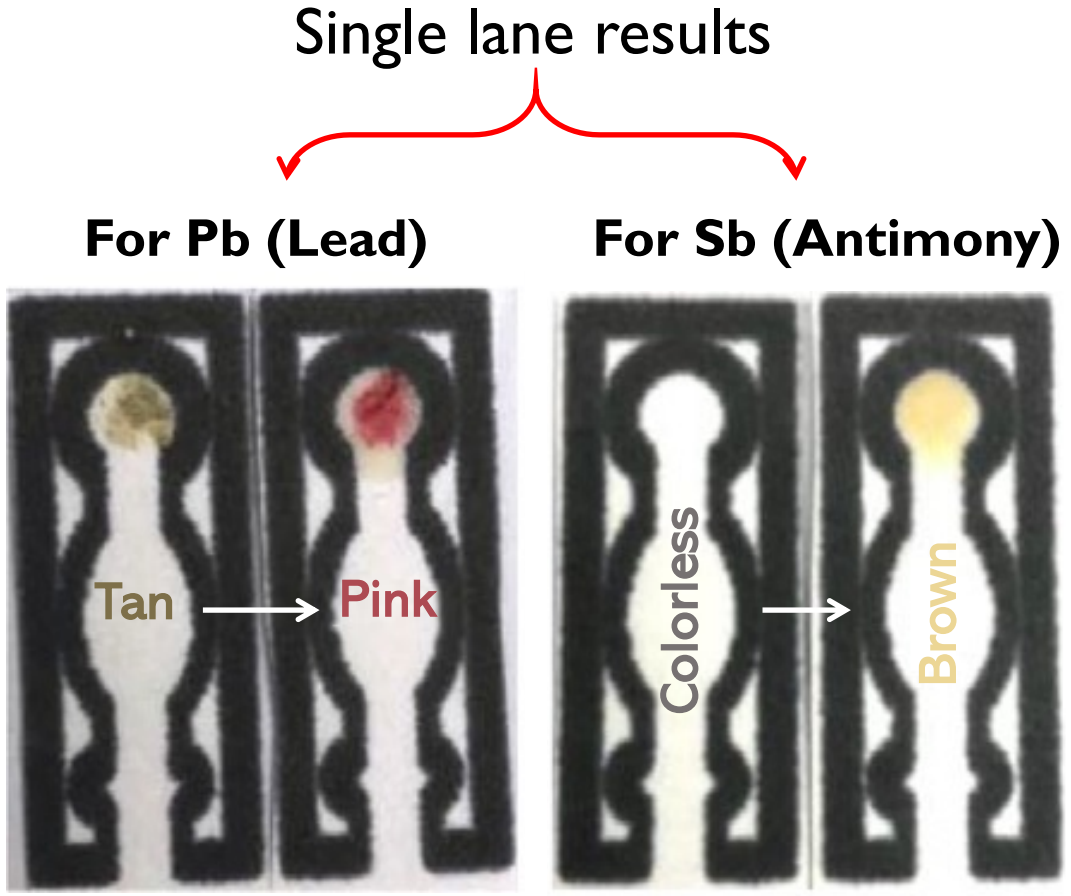
[17] M. O. Salles, ***Anal. Methods***, 2014, **6**, 2047.

Microfluidics in Forensic applications

Explosive residues

Detection of low explosives using μPADs





Multiplexed μPAD for detection of metal salts (inorganic residues of low explosives). [18]

[18] K. R. Chabaud, ***Forensic Chemistry***, 2018, **9**, 35–41.

The road ahead

Shortcomings of the microfluidic technology

Microfluidics for forensics are not yet universally implemented due to:

(1) Lack of standardization (specially for µPADs)

- Cannot withstand harsh conditions
- Sensitive to temperature and/or humidity
- Limited stability of chemical reagents
- Variations from batch to batch

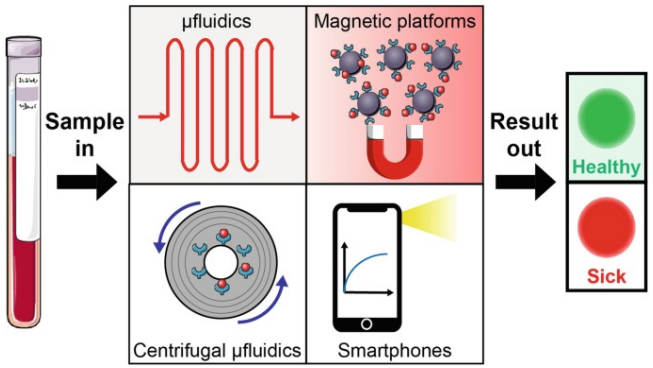
[19] H. Bazyar, On the application of microfluidic-based technologies in forensics: A review, ***Sensors: Special issue: Advanced Analysis and Sensing at the (Crime) Scene or Location of Interest***, 2023, **Submitted** (under review).

Shortcomings of the microfluidic technology

Microfluidics for forensics are not yet universally implemented due to:

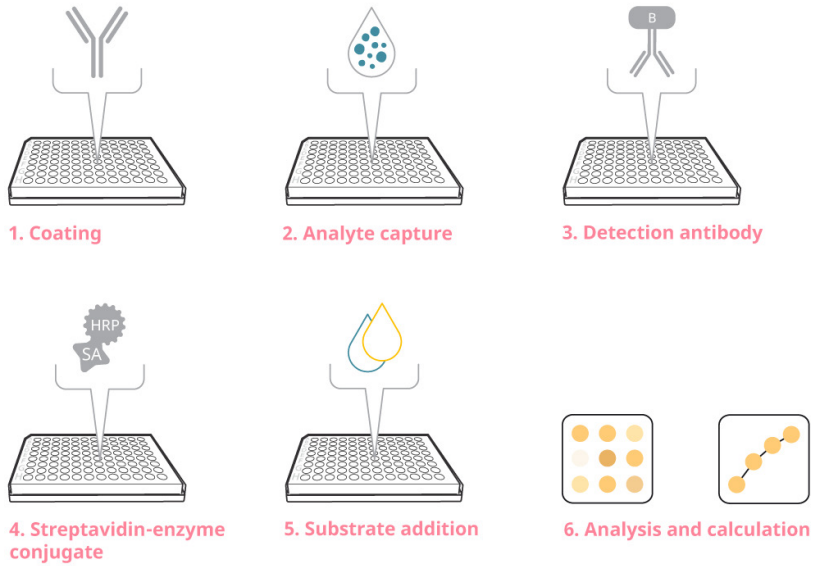
(2) Challenges in integration

Ideal microfluidic for on-scene application



Sample-to-answer platform

Lab-based assays involve multistep procedure



Proof of concept for individual steps have been developed

To realize end product, all discrete steps must be integrated!

Shortcomings of the microfluidic technology

Microfluidics for forensics are not yet universally implemented due to:

(3) Product cost

Cleanroom or lithography methods are needed

For Silicon, glass, or PDMS chips

Plastic and paper-based chips are affordable for mass production

Universal applicability of these chips?

Choice of materials

Depends on application and compatibility with sample

Shortcomings of the microfluidic technology

Microfluidics for forensics are not yet universally implemented due to:

(4) Associated trade-offs (with rapid DNA systems)

- **Reduced sensitivity**
- **Higher costs**
- **Reduced speed and throughput**

These trade-offs along with cultural forensic landscape, limits application of sample-to-answer platforms!

Future perspectives

(1) Enhancing the existing capabilities

Focus change towards *standardization* and *integration* within paper and plastic-based microfluidics.

(2) Innovative and court-proof platforms to empower existing technology

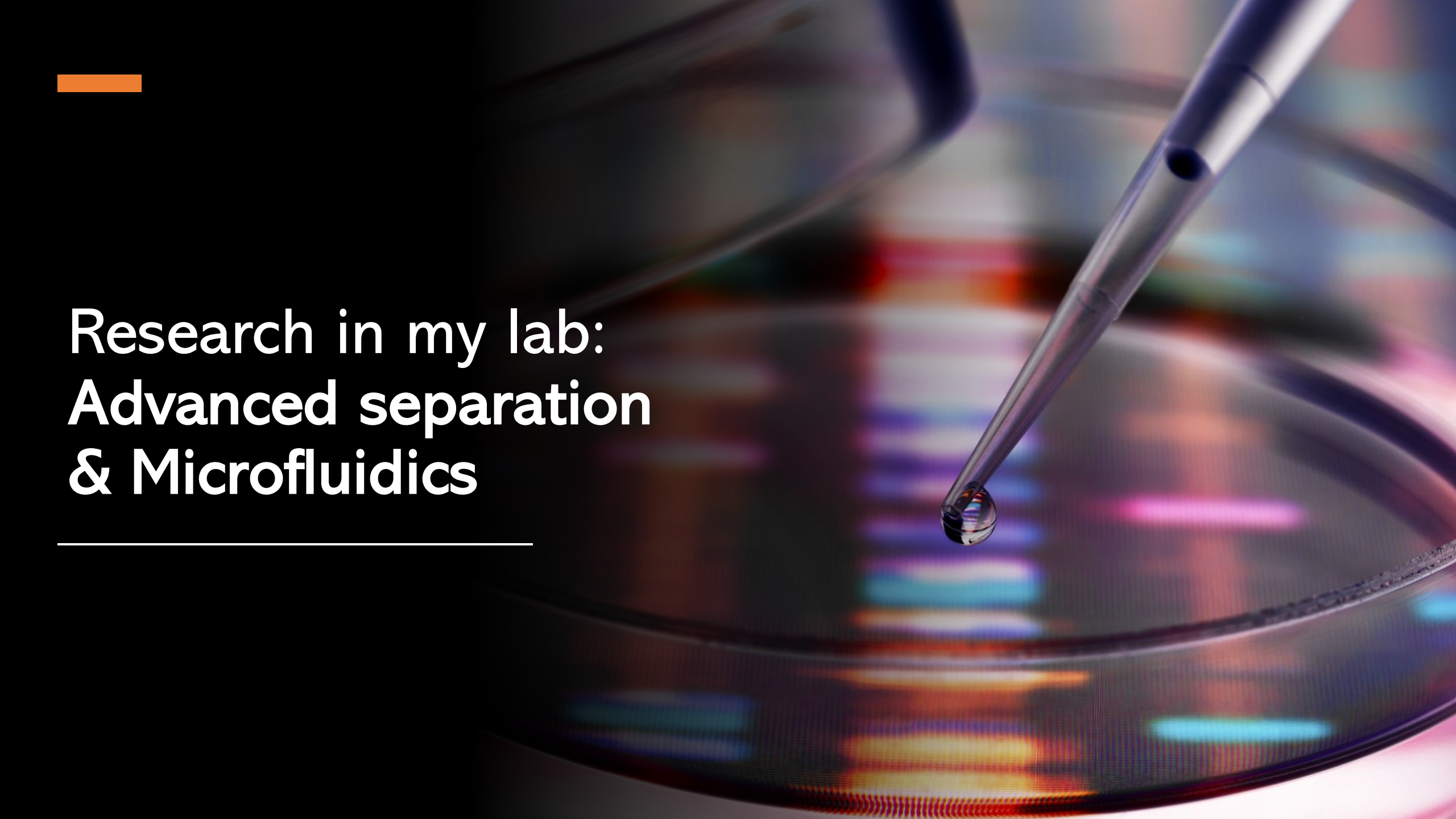
Instead of developing competing technology with the current state-of-the-art methods

(3) Miniaturization of bulky peripherals (e.g., pumps, detectors)

All components should be miniaturized to achieve fully portable platforms

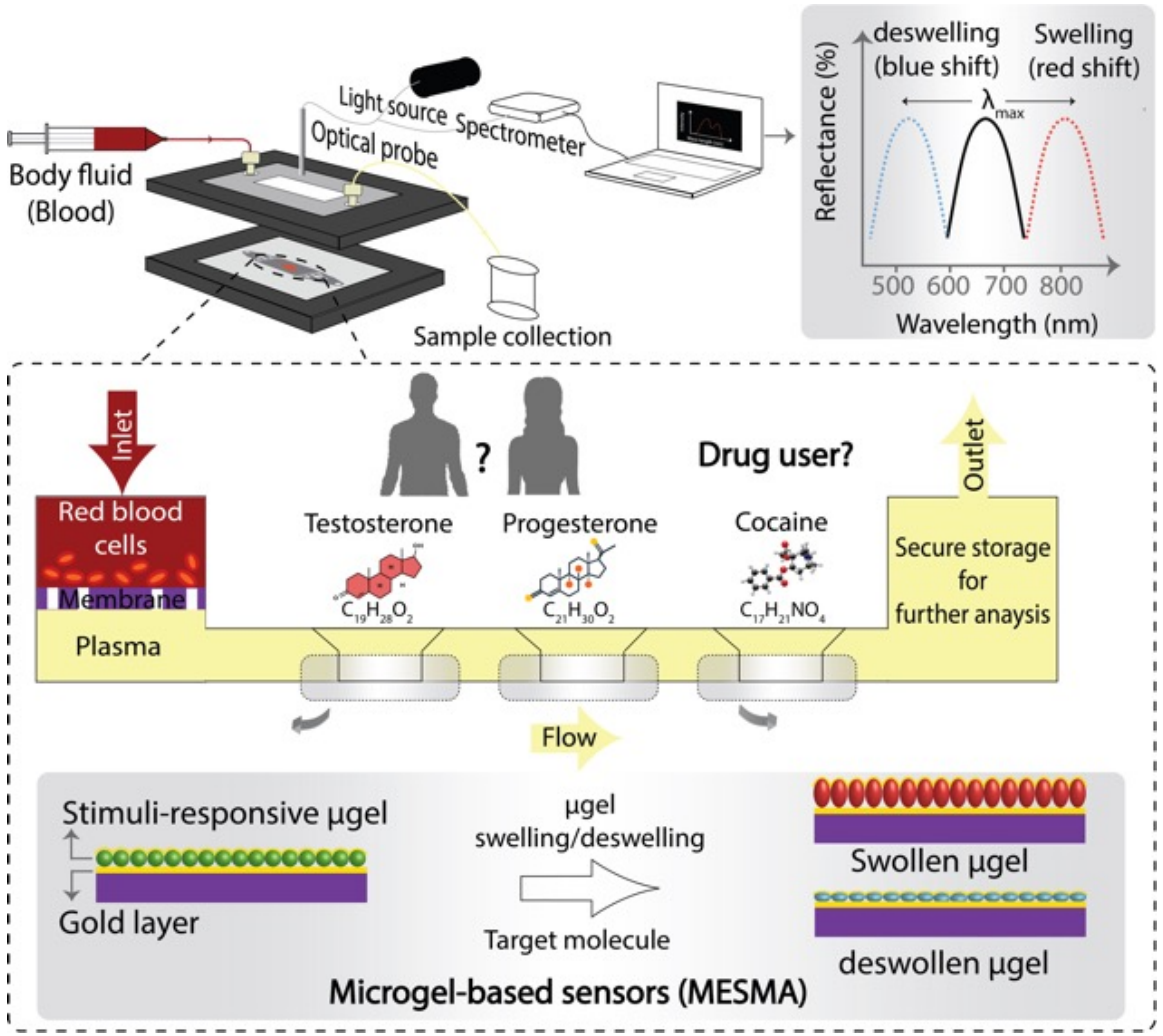
[19] H. Bazyar, On the application of microfluidic-based technologies in forensics: A review, ***Sensors: Special issue: Advanced Analysis and Sensing at the (Crime) Scene or Location of Interest***, 2023, **Submitted** (under review).

Research in my lab: **Advanced separation & Microfluidics**



Forensics-on-chip

Recently granted postdoc project (1.5 year)



(2) Innovative platforms to empower existing technology!

Thank you

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