Two-dimensional paper networks for high performance multi-step assays at the point-of-care

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2011.10.01

Outline

- Need for higher performance point-of-care tests for low-resource settings
- Two-dimensional paper networks for automated multi-step processing
- Paper fluidics tools for fluid manipulation
- Signal amplification for improved limit of detection

Laboratory-based vs. lateral flow tests

High performance in high-resource setting (e.g. ELISA)

- X Not rapid + transit times
- X Trained personnel
- X Instrumentation
- X Electricity
- ✓ Cost varies, but can be low
- ✓ High sensitivity
- ✓ Quantitative

Appropriate for low-resource settings, but are lacking in performance

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- ✓ Rapid (< 20 min)
- ✓ Easy to use
- ✓ No instrumentation
- ✓ No electricity/refrigeration
- ✓ *Very low* cost
- X Lacks sensitivity
- X Not quantitative

Performance vs. simplicity to run

Multiple sequential sample processing steps for high performance

Example of ELISA

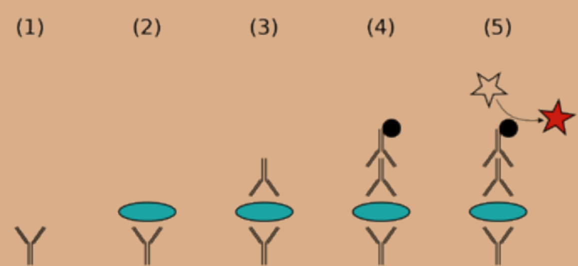
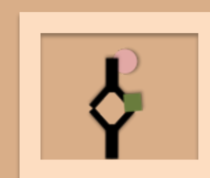


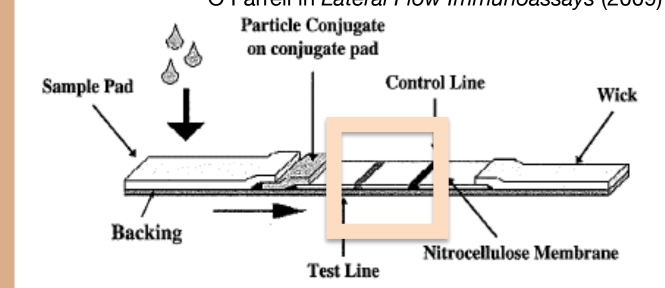
Image credit: wikipedia

- Add sample
- Add label
- Add signal amplification reagent
- Perform multiple washes
- Quantitative instrument readout

Limited to a single chemical delivery step; cannot perform sample pretreatment or signal amplification



O'Farrell in *Lateral Flow Immunoassays* (2009)



- Add sample
- Qualitative readout by eye

Bridge the gap using 2DPNs

High performance

High-resource settings

Need improved performance

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Low-resource settings

Lessons from lateral flow technology

- Capillary flow obviates need for pump-related instrumentation
- Fully disposable so no need for maintenance and repair
- Materials and manufacturing methods are low cost

Engineer paper networks to perform *automated multi-step processes*

Increased performance

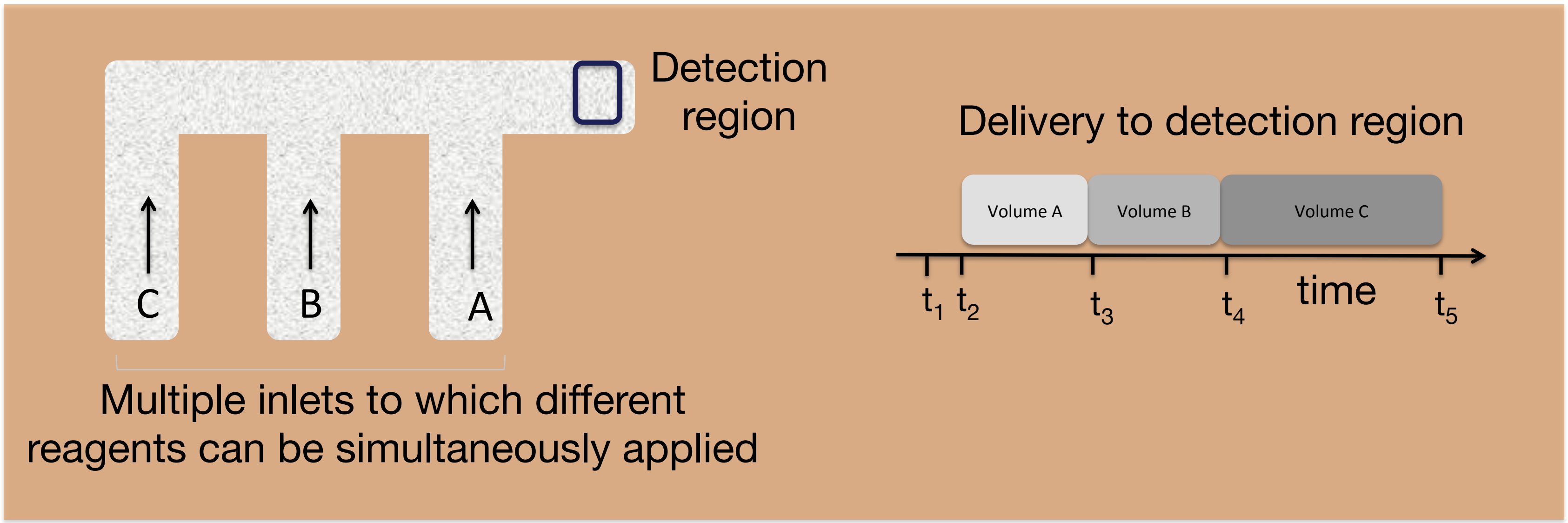
Rapid

Easy to use

Low cost

Two-dimensional paper networks

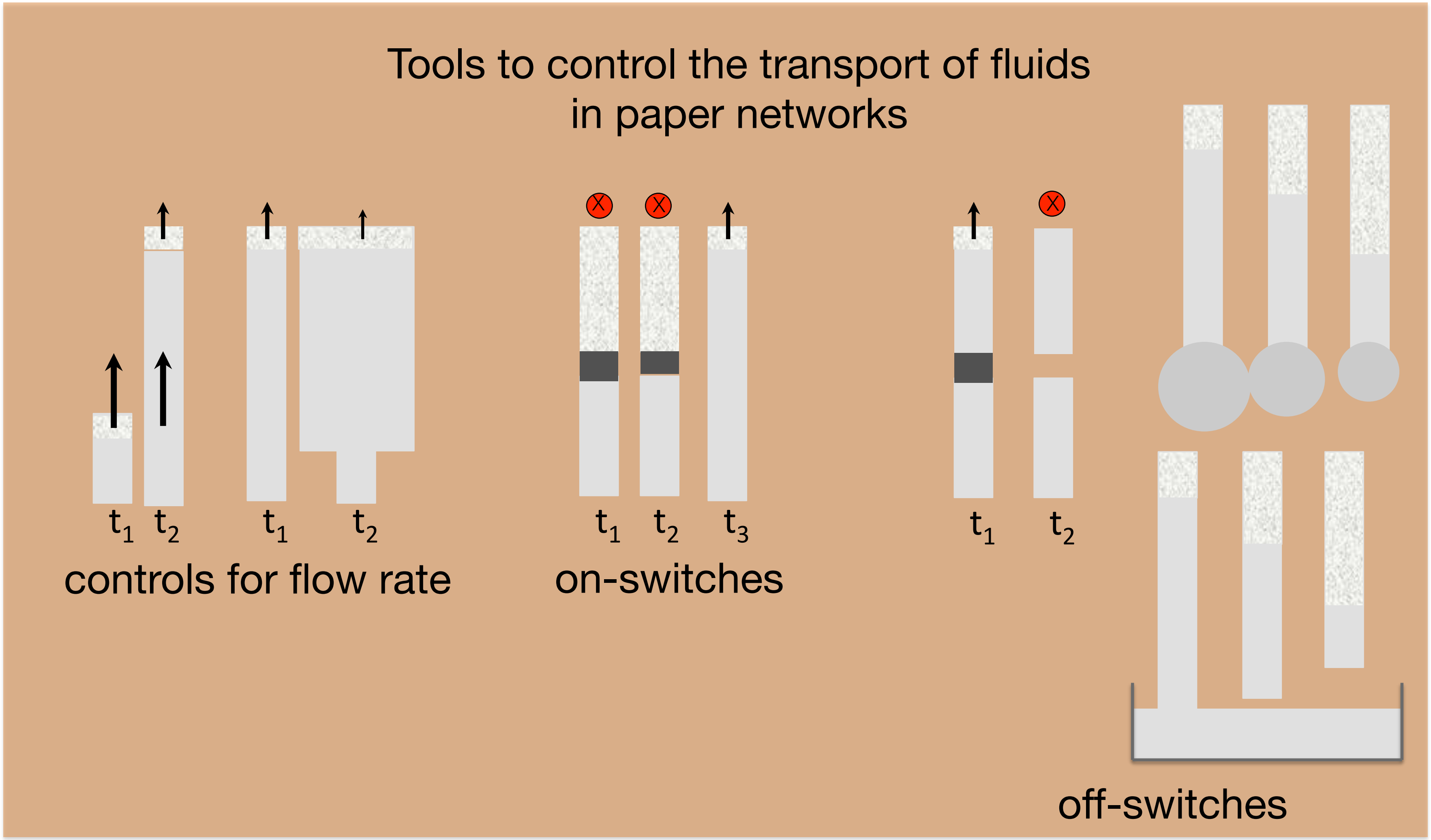
Multiple inlets per detection region



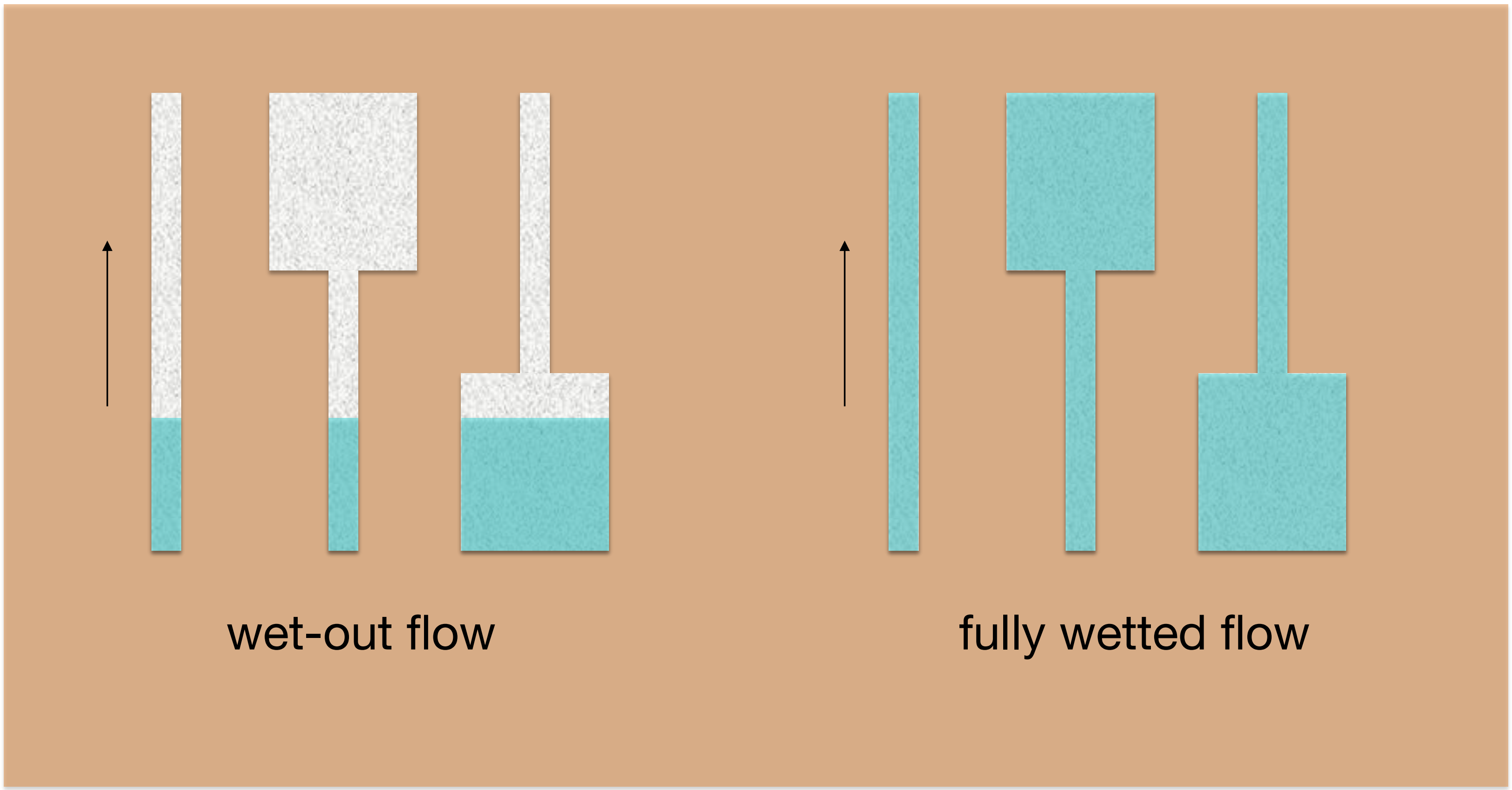
Geometry allows for the sequential delivery of the multiple reagents to the detection region

Key to implementing this is the ability to control well-defined fluid volumes in paper networks

For enabling multi-step processes in paper networks- need paper fluidics tools



Use simple geometries to control flow rates of fluids in paper networks

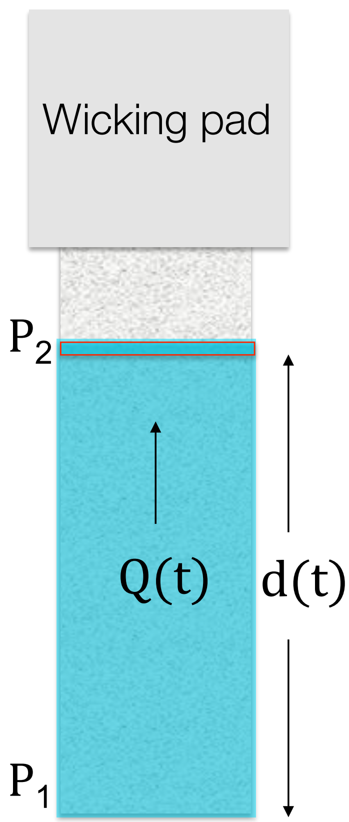


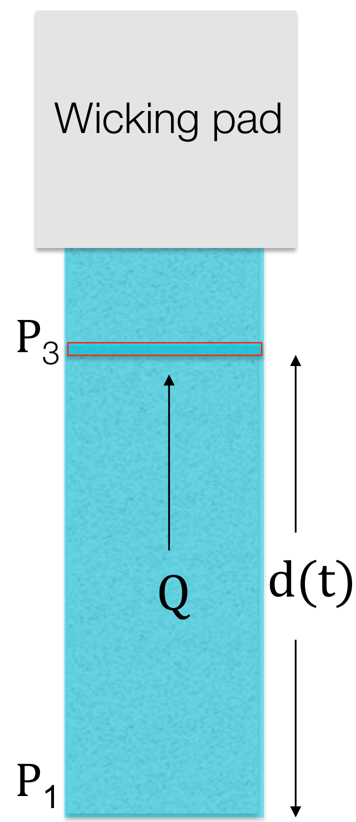
Transport in lateral flow strips

Darcy's Law for 1D flow in porous media: $Q \propto \Delta P / d$

For wet-out flow : $d^2 \propto t$
Washburn relation

For fully wetted flow : $d \sim t$



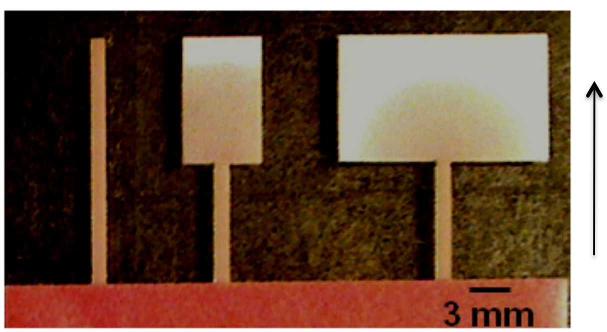


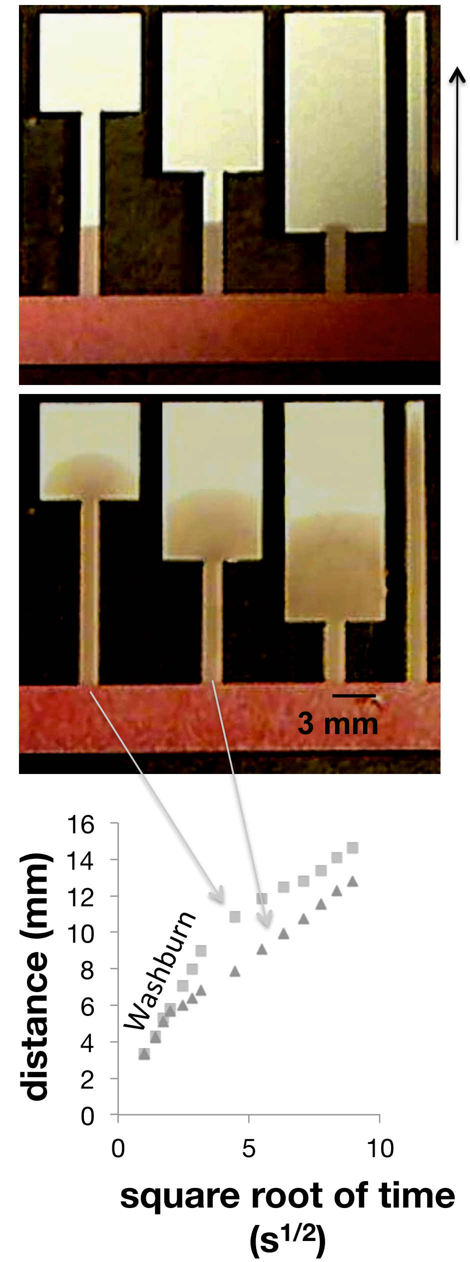
Expansion geometry under wet-out flow

Transition to a greater width results in a greater degree of slowing of the fluid front

Control parameters for slowing fluid front

- Downstream location of the expansion
- Width of the expansion

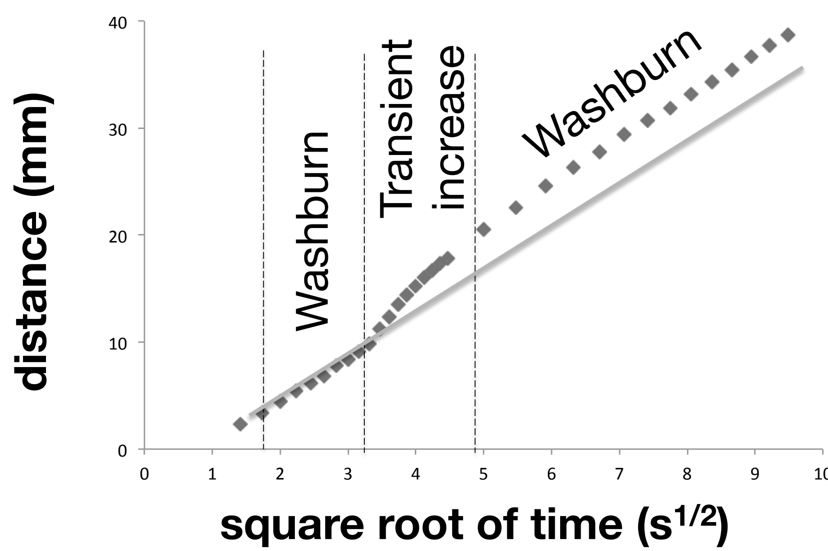


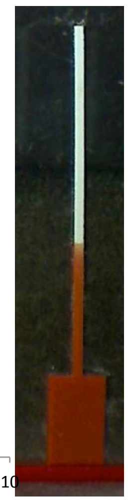


Fu et al., Microfluidics and Nanofluidics (2010)

Contraction geometry under wet-out flow

Flow is initially Washburn, transiently increases at the constriction, and then resumes Washburn flow





Total transport time for the fluid front to move a given distance is decreased relative to that in a constant width strip

Control parameters

- Downstream location of the constriction
- Transport time is minimized for equal length sections

Fu et al., Microfluidics and Nanofluidics (2010)

Electrical circuit analogy for fully-wetted network

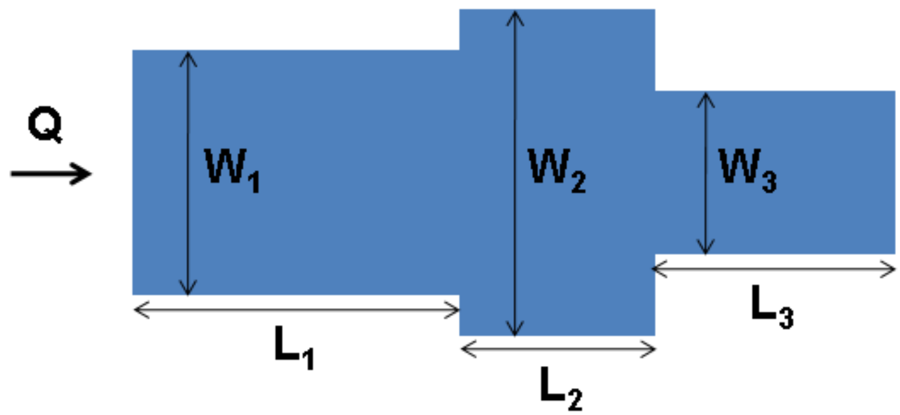
Fluidic circuit:

$$\Delta P = QR_{efffective}$$

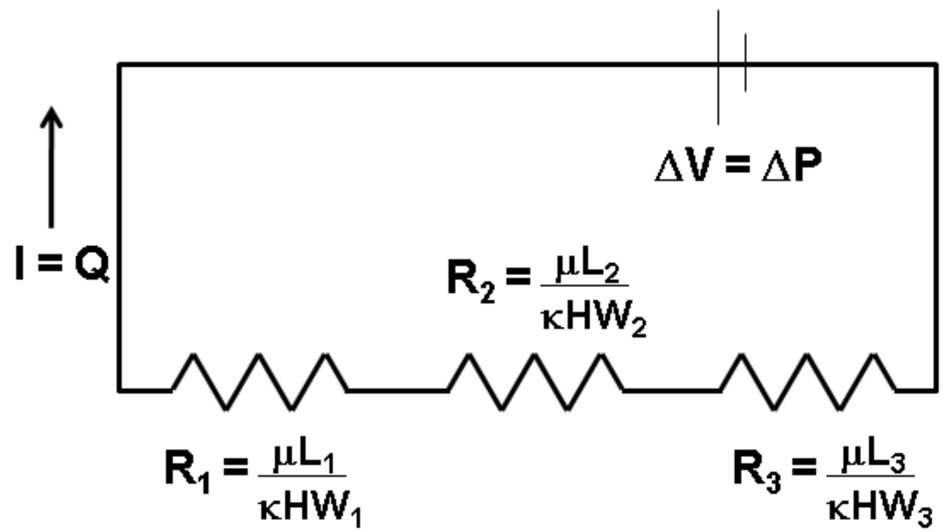
ΔP - total pressure difference across circuit

Q - volumetric flow rate in circuit

$R_{effective}$- effective fluidic resistance



Fluidic resistances in series are summed and those in parallel are added in reciprocals

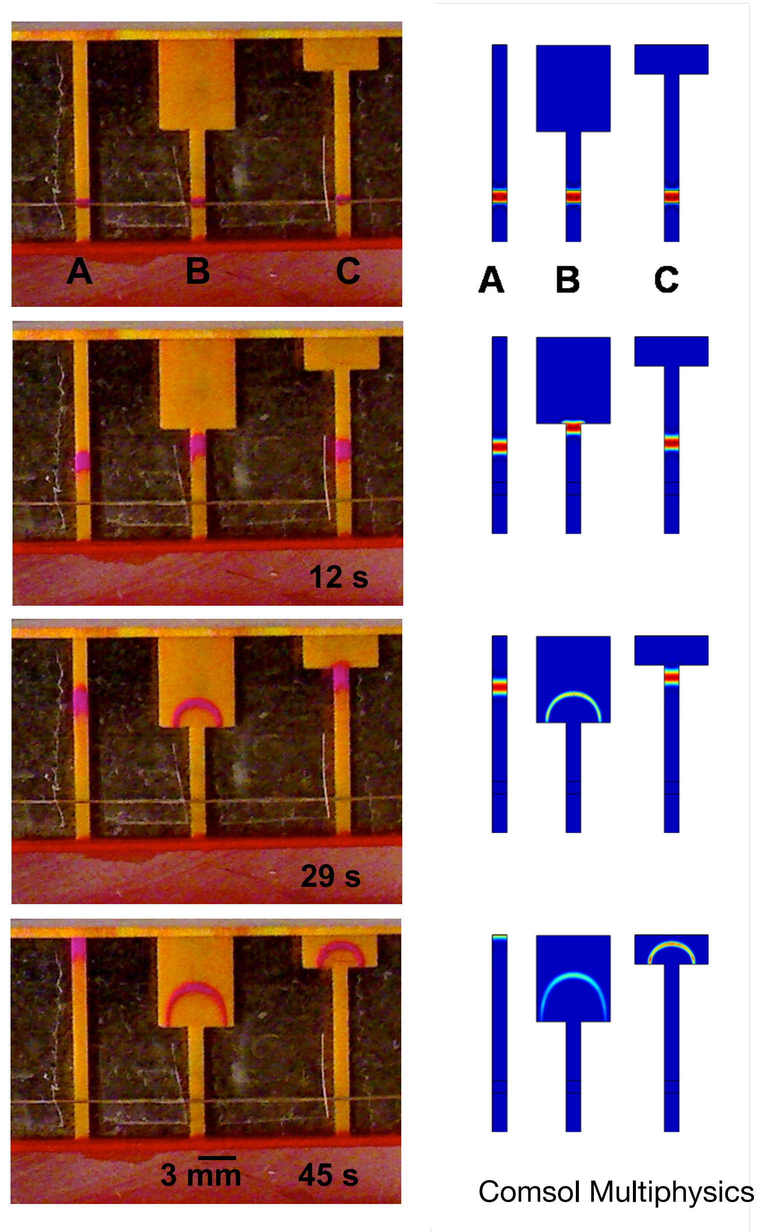


Fully wetted flow in simple geometries

Tracer species to visualize flow in simple geometries (e.g. pH marking method)

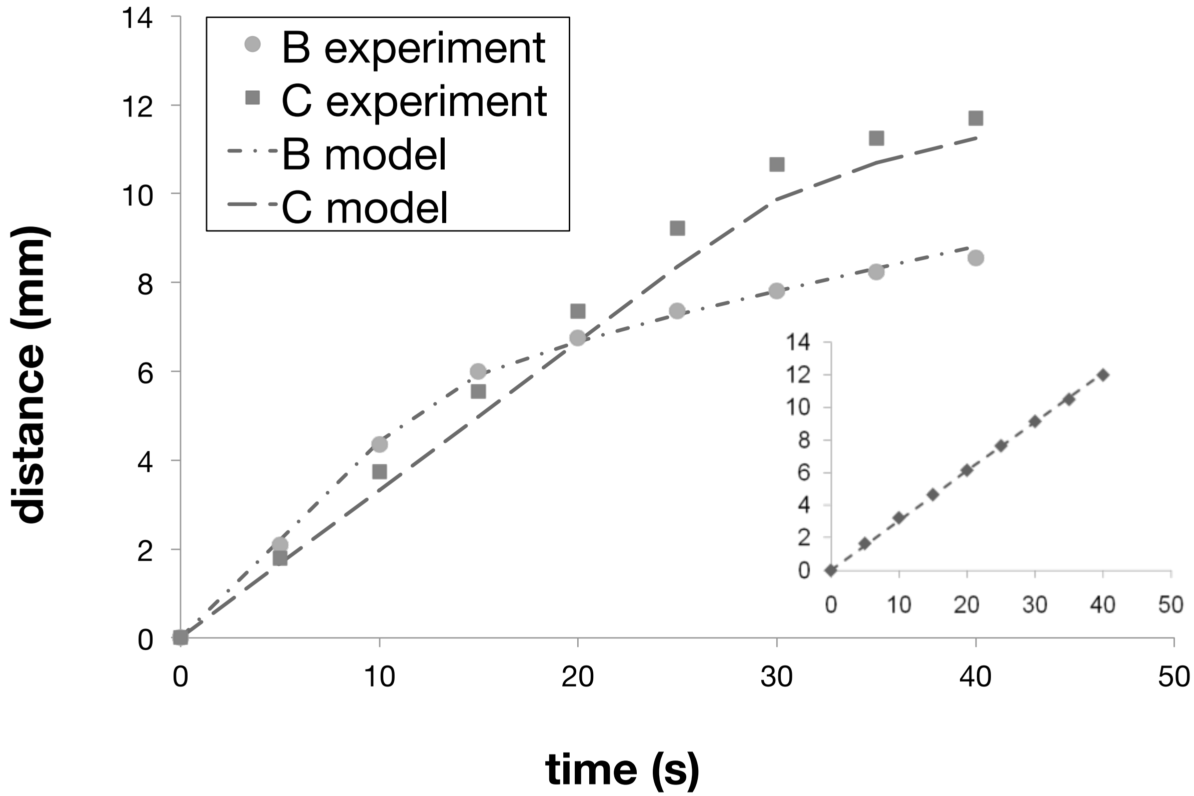
Kauffman et al., Lab on a Chip (2010)

Experimental and model results of the transport of a tracer species show good agreement



Fu et al., Microfluidics and Nanofluidics (2011)

Fully wetted flow in simple geometries

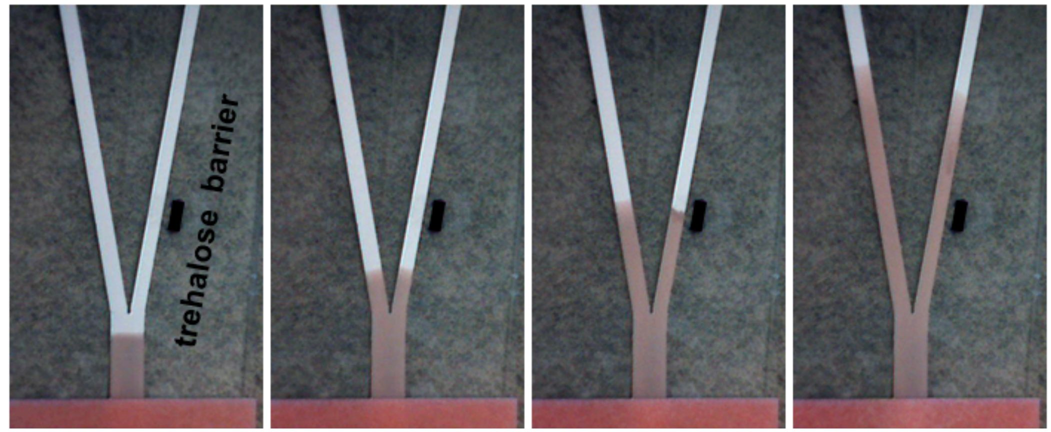


Ability to predict and control flow rates for simple changes in geometry

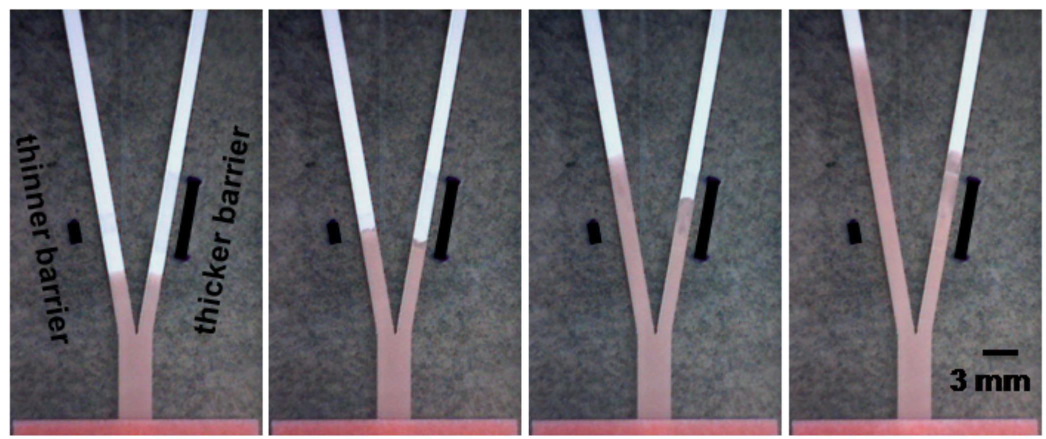
Fu et al., Microfluidics and Nanofluidics (2011)

On-switch for flow – dissolvable barriers

Sugar barriers create delays in transport of a fluid within a network



Extent of barrier can be used to vary delay time

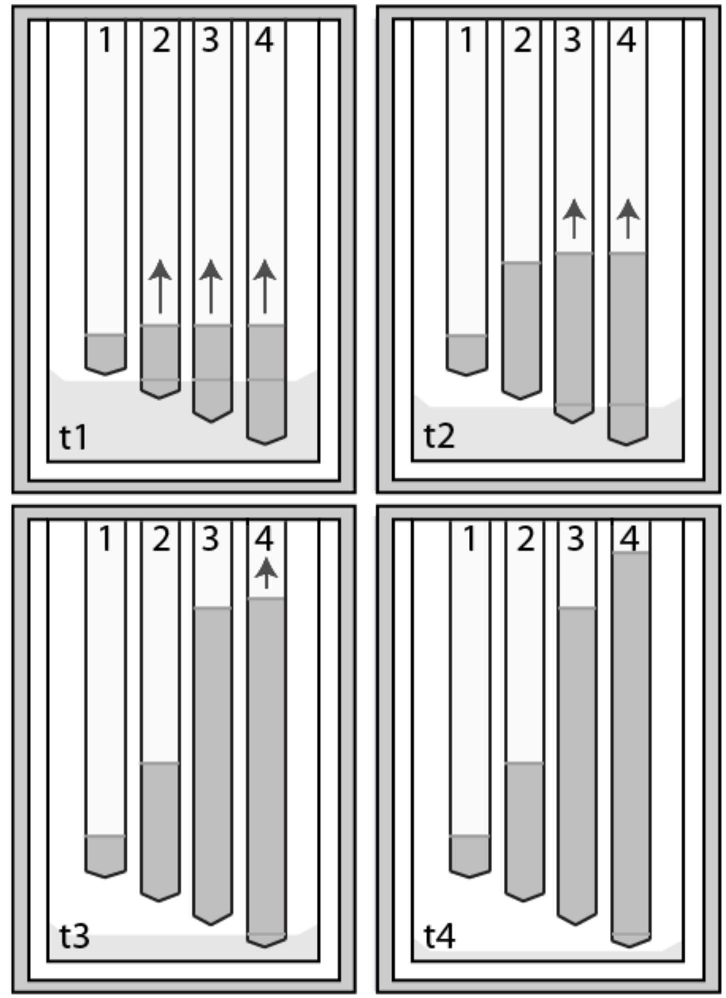


Fu et al., Lab on a Chip (2010)

Metering flows using varying leg lengths

- Inlet legs are submerged by varying distances into a common well to activate flows
- Level of fluid in the well drops as fluid wicks into the paper inlets
- Fluid flow shuts-off from inlets in order of shortest to longest submerged distance

Different volumes of multiple fluids are automatically input

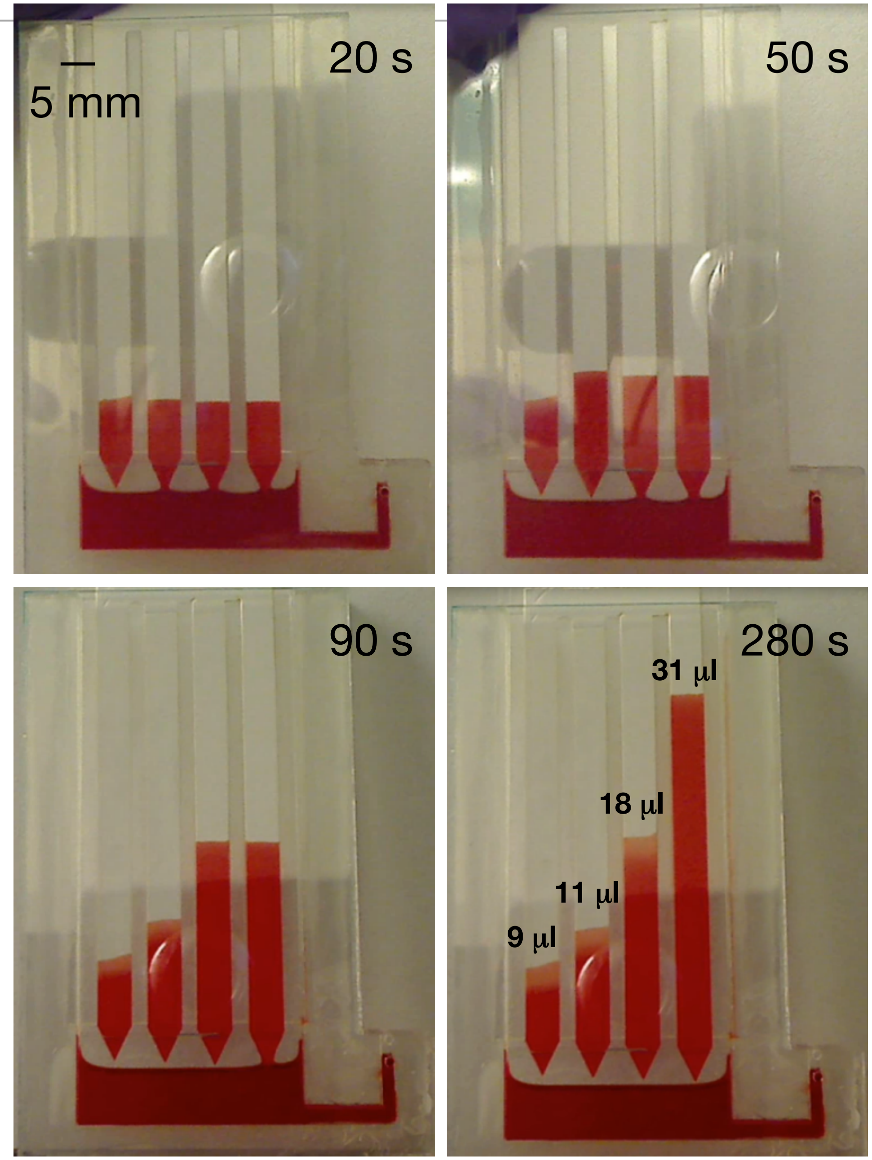


Courtesy of P. Trinh

Metering flows using varying leg lengths

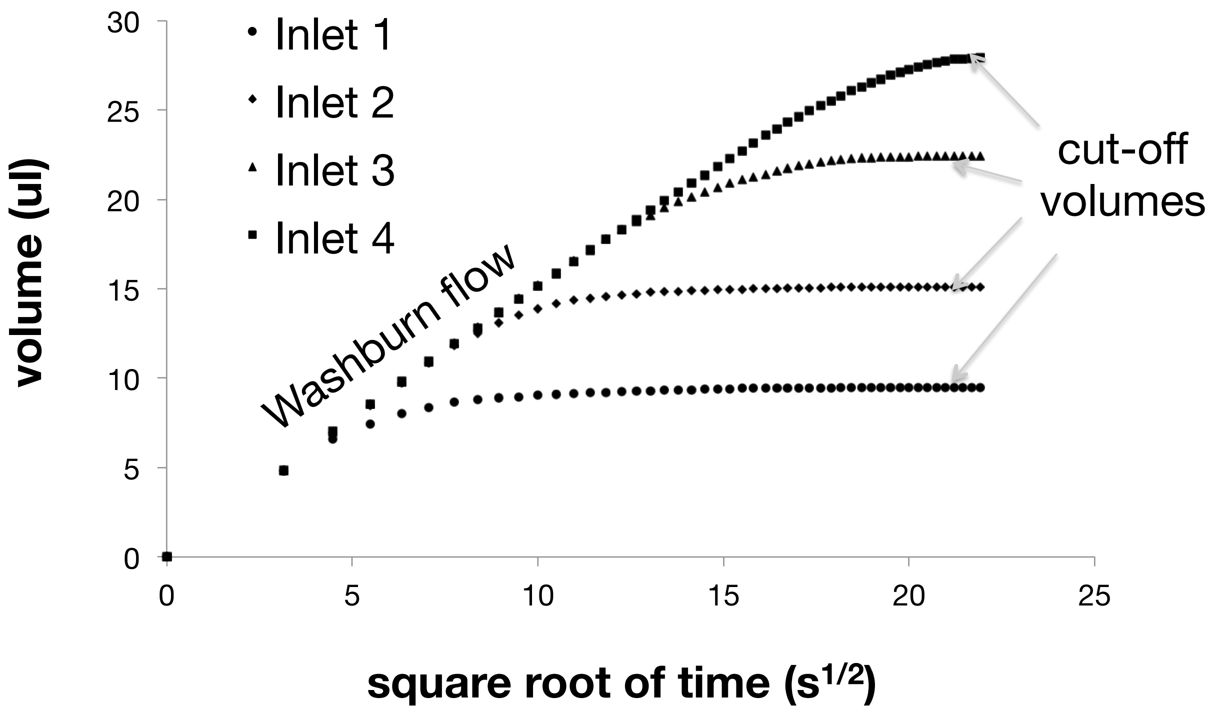
Controls for the volume metering

- Geometry of the fluid well – e.g. cross-sectional area
- Fluid depletion rate from the well - leg geometry and compostion



Data courtesy of P. Trinh

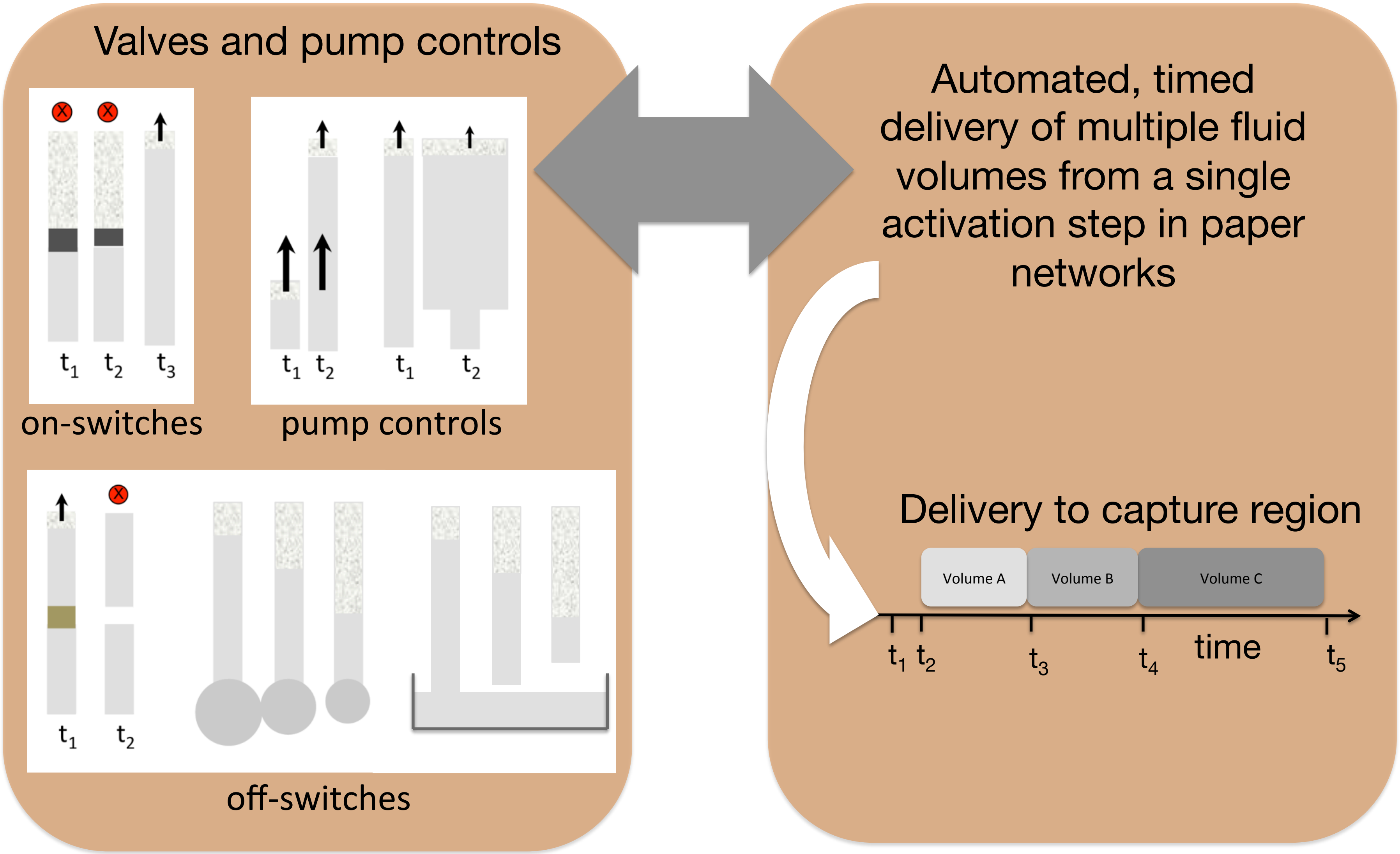
Metering flows using varying leg lengths



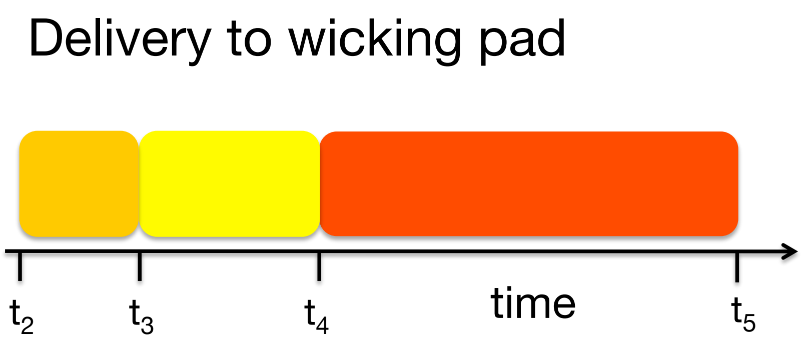
- Flow deviates from Washburn near the programmed cut-off time
- CVs less than 15% in volumes delivered

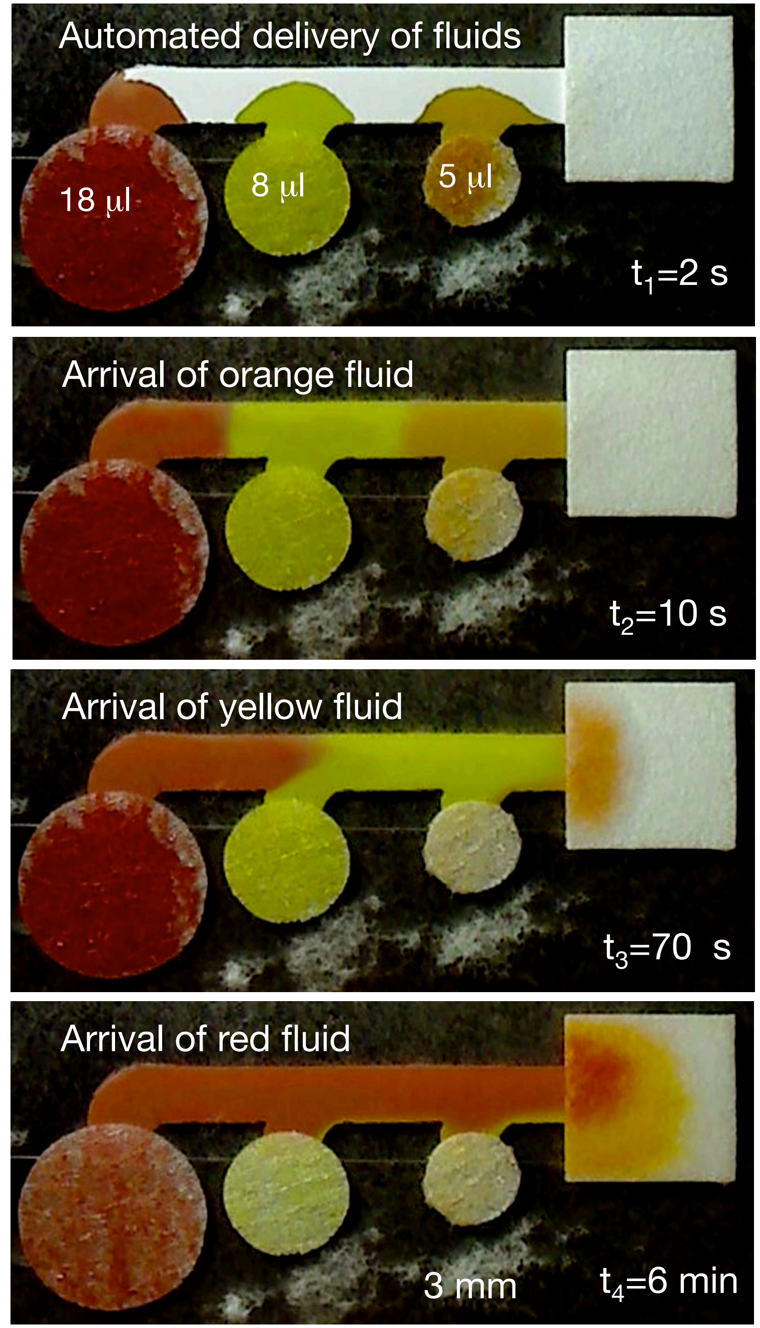
Data courtesy of P. Trinh

Summary of paper fluidics toolbox

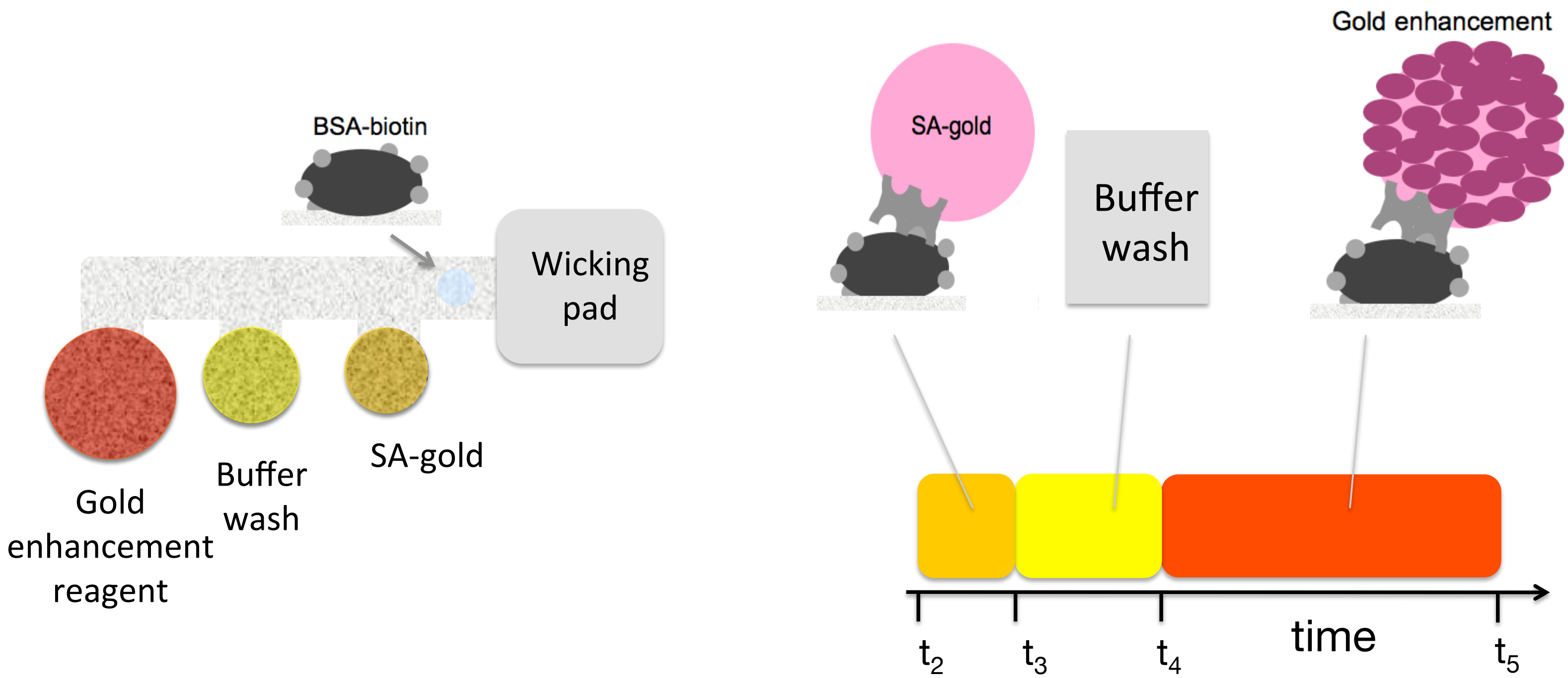


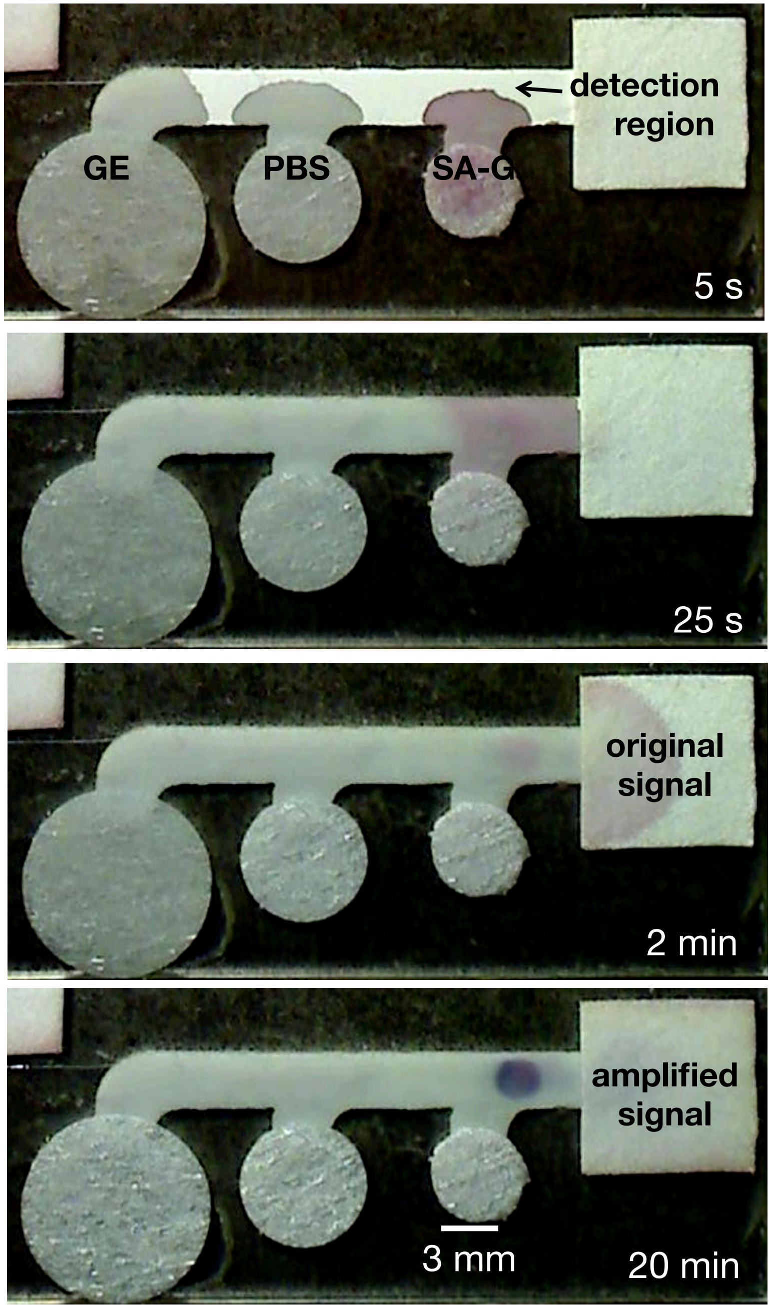
Demonstration of automated delivery of multiple fluid volumes



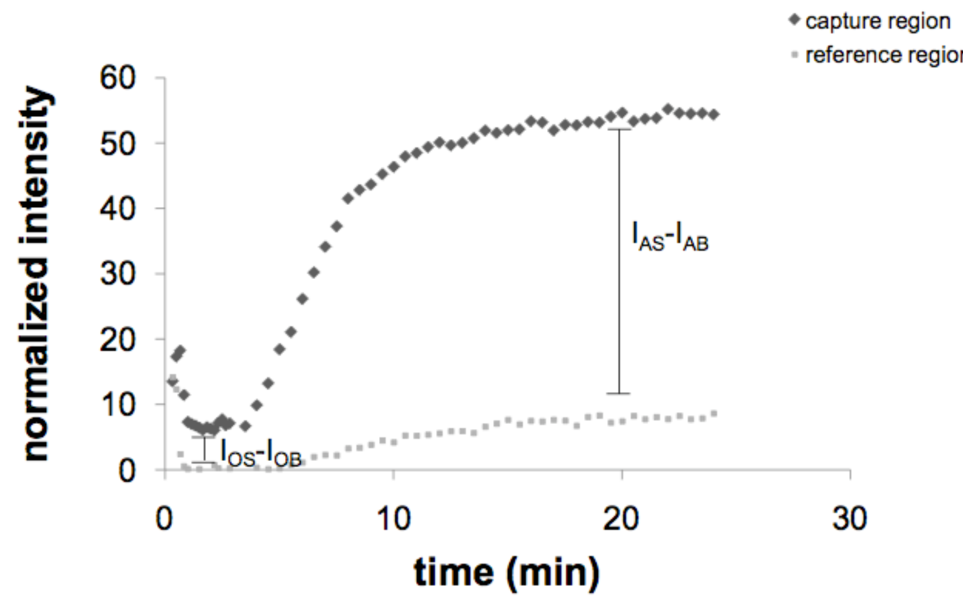


Chemical signal amplification in a paper network





Demonstration of amplified signal



Signal amplification factor (n=3): 6±2

Automated rapid process for improved performance in a paper network

Fu et al., Sensors and Actuators B (2010)

Signal amplification card for PfHRP2

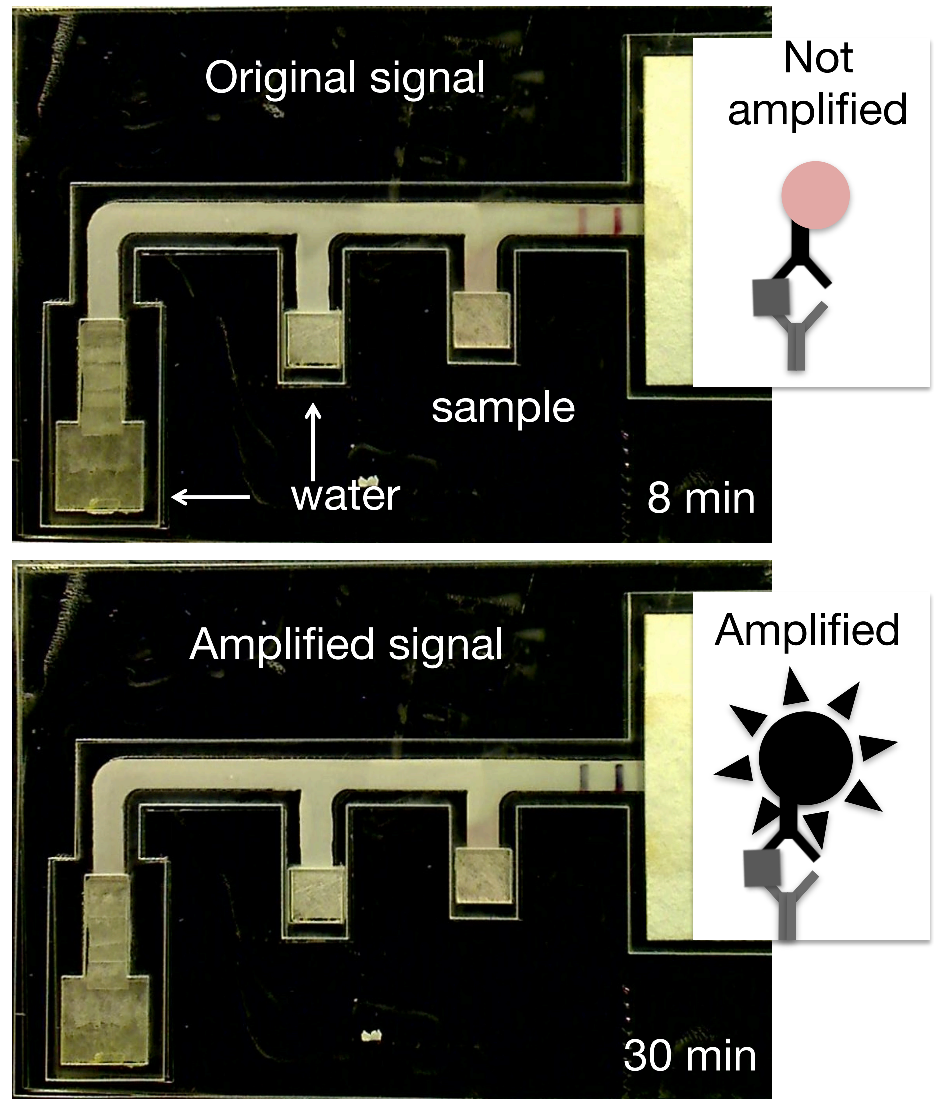
All reagents stored dry on card!

User steps for 2DPN card

1) Add sample and water to
   appropriate pads

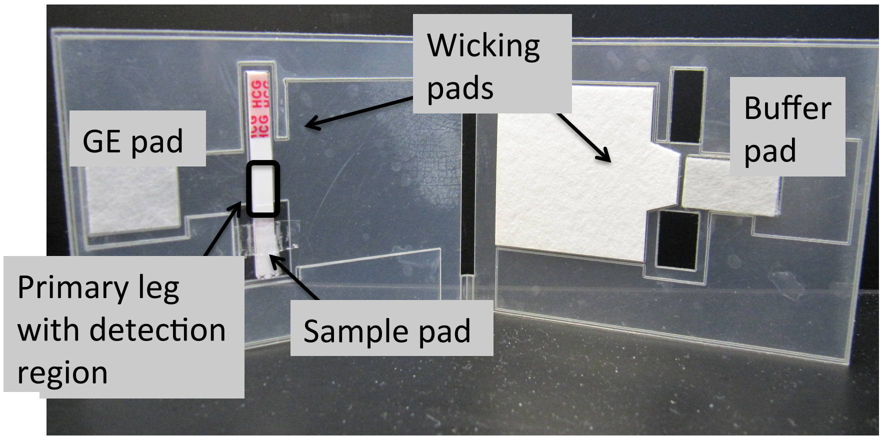
2) Fold card to activate

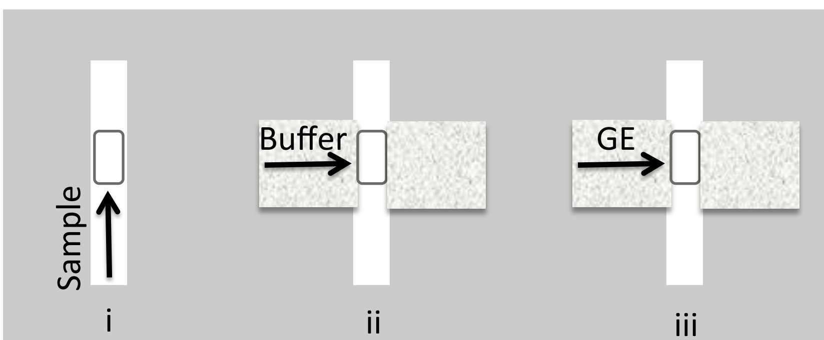
Demonstration is for a mock sample of PfHRP2 in fetal bovine serum



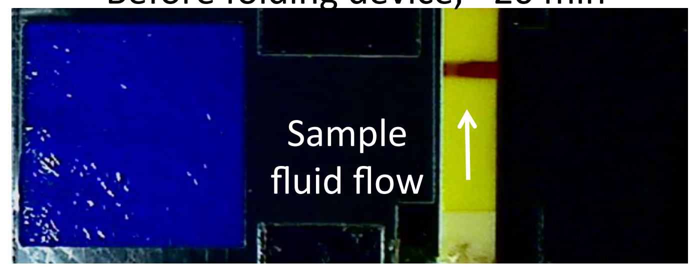
Data courtesy of T. Liang

Signal amplification card for hCG

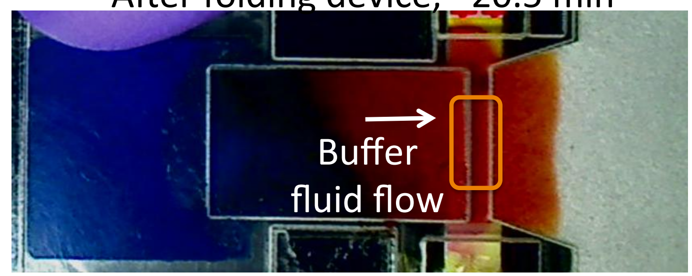




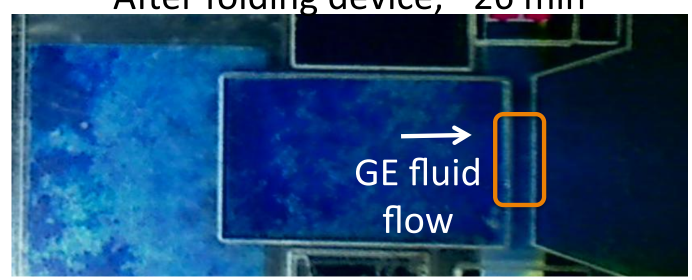
Before folding device, ~20 min



After folding device, ~20.5 min

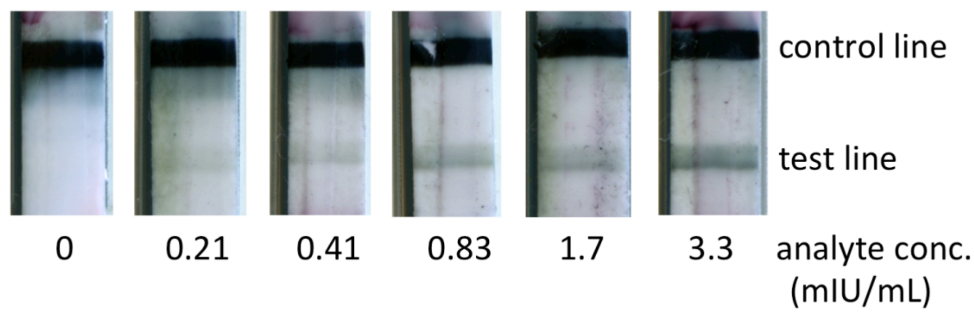


After folding device, ~26 min

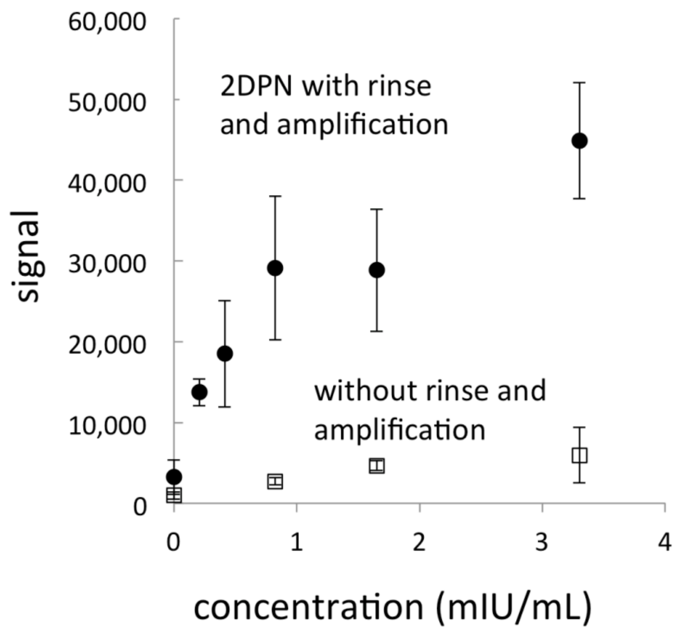


Fu et al., Analytical Chemistry (in press 2011)

Improved limit of detection



Detection region at 35 min



Limit of detection is improved ~4-fold

2DPNs can implement other signal amplification methods with higher performance

Fu et al., Analytical Chemistry (in press 2011)

Summary

Two-dimensional paper networks enable high performance *multi-step* assays while retaining the positive aspects of conventional lateral flow tests

Increased performance

Rapid

Easy to use

Low cost