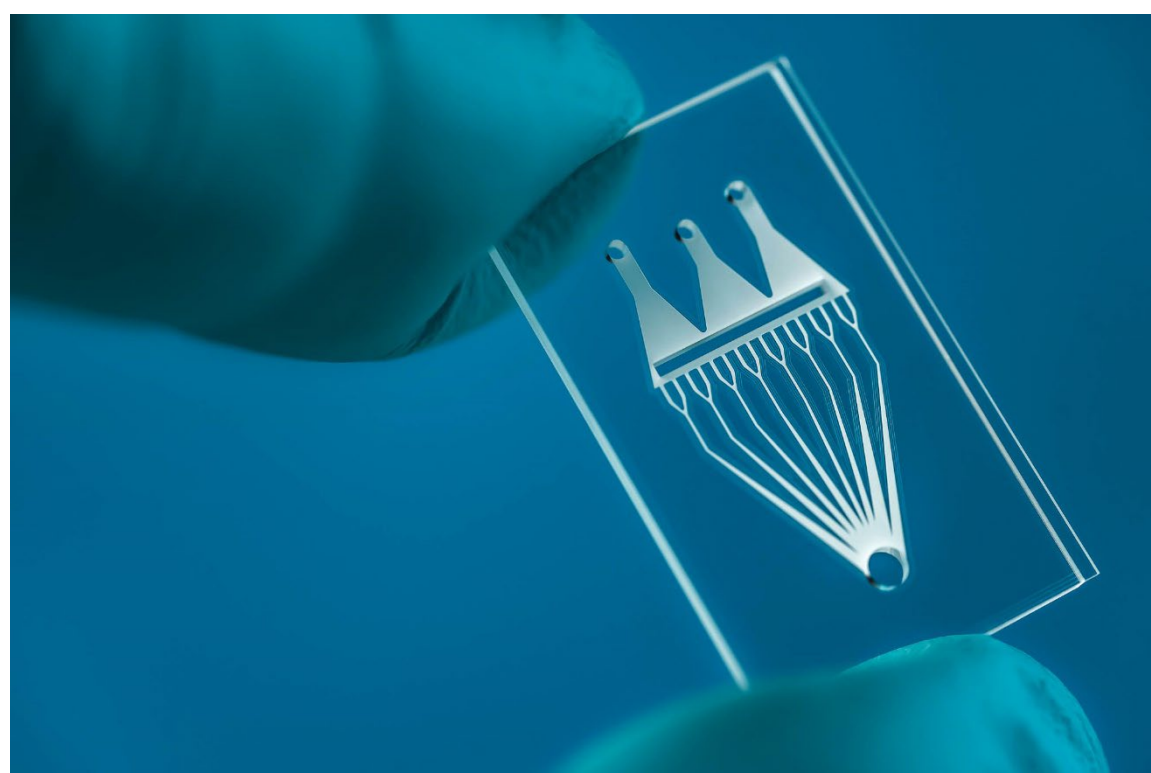


Lecture – Microfluidics

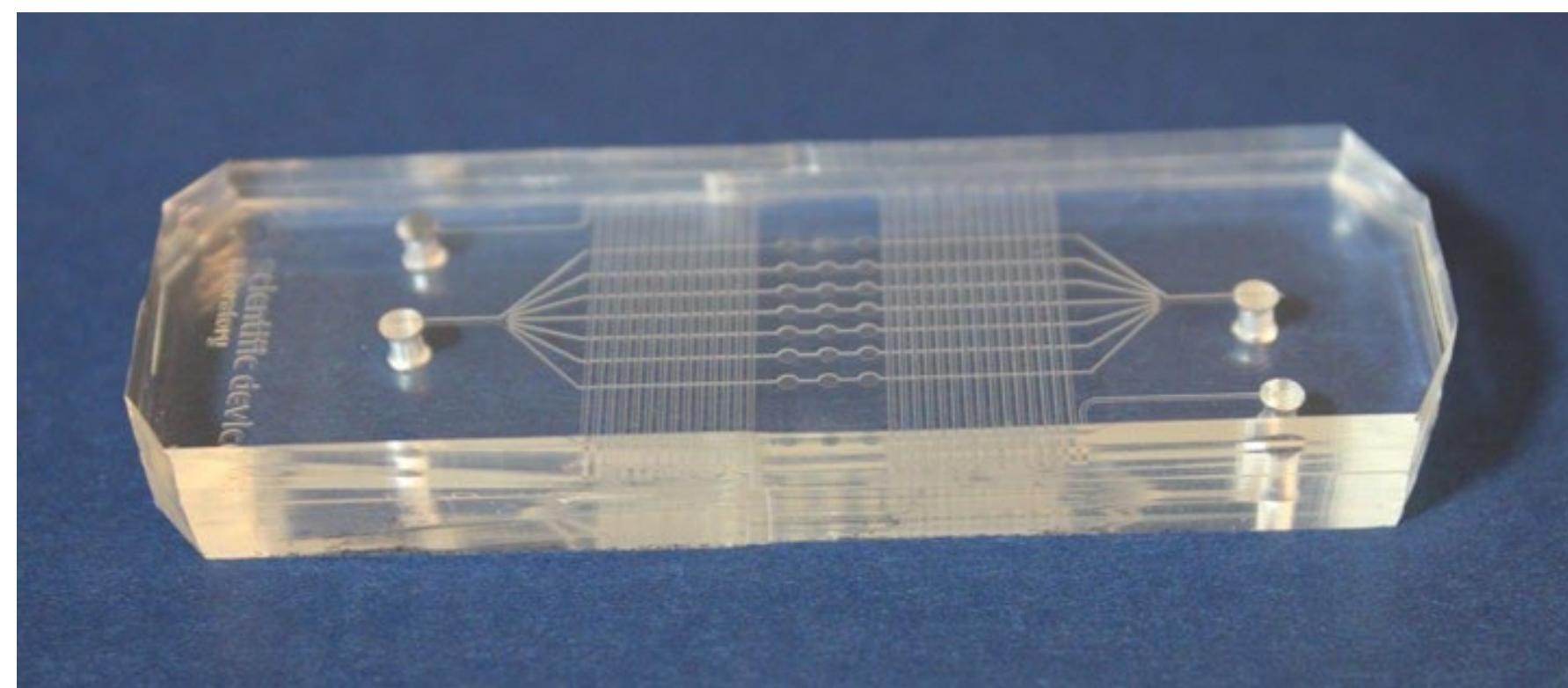


Microfluidic Materials

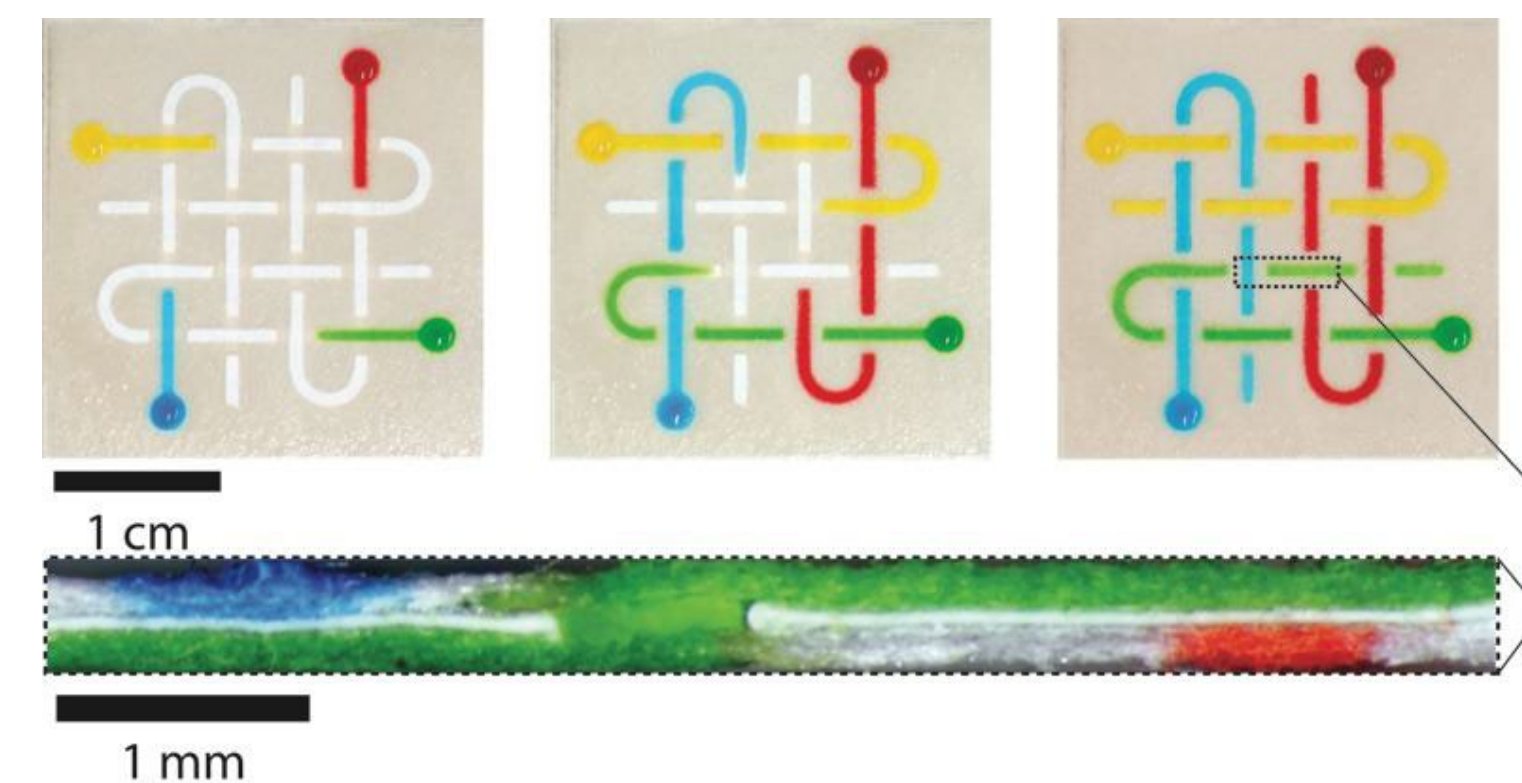
Glass / hard plastic



Elastomers (PDMS)

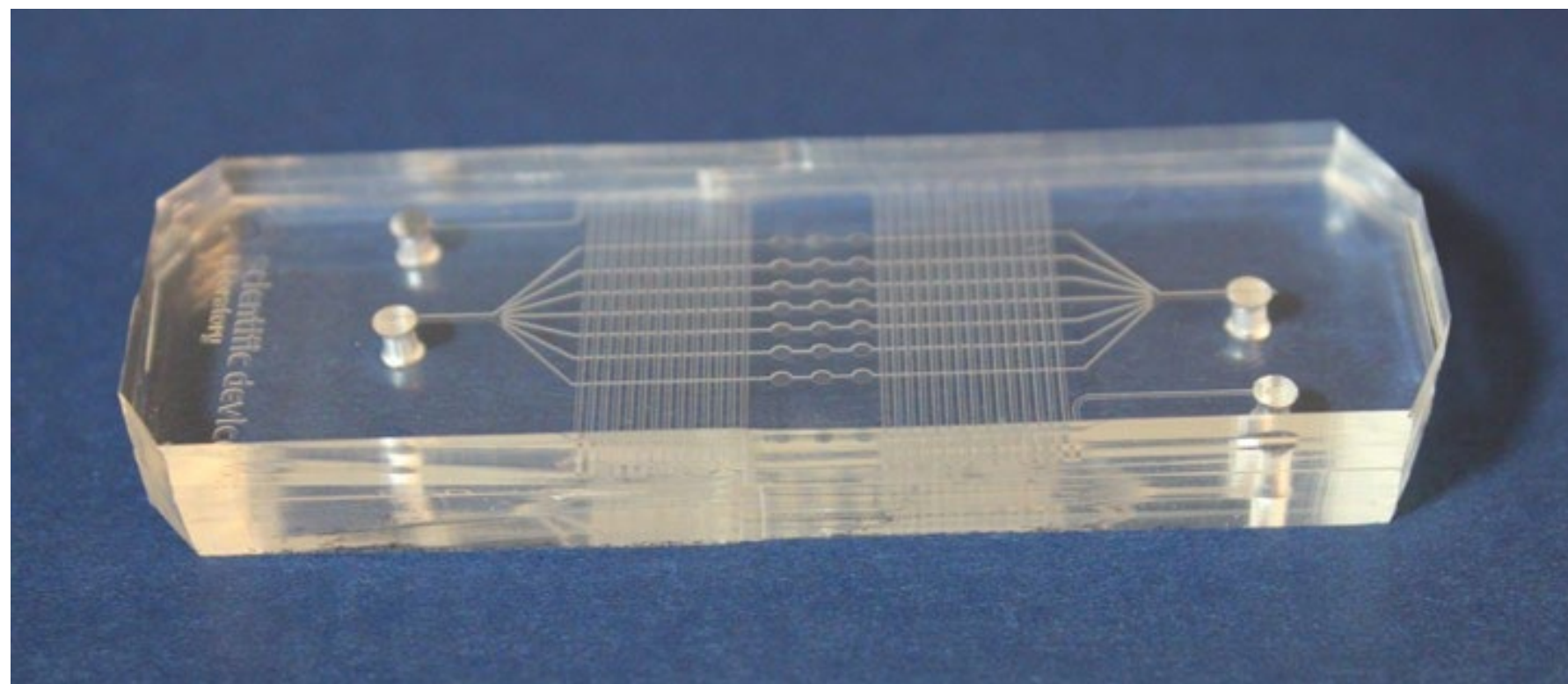


Paper

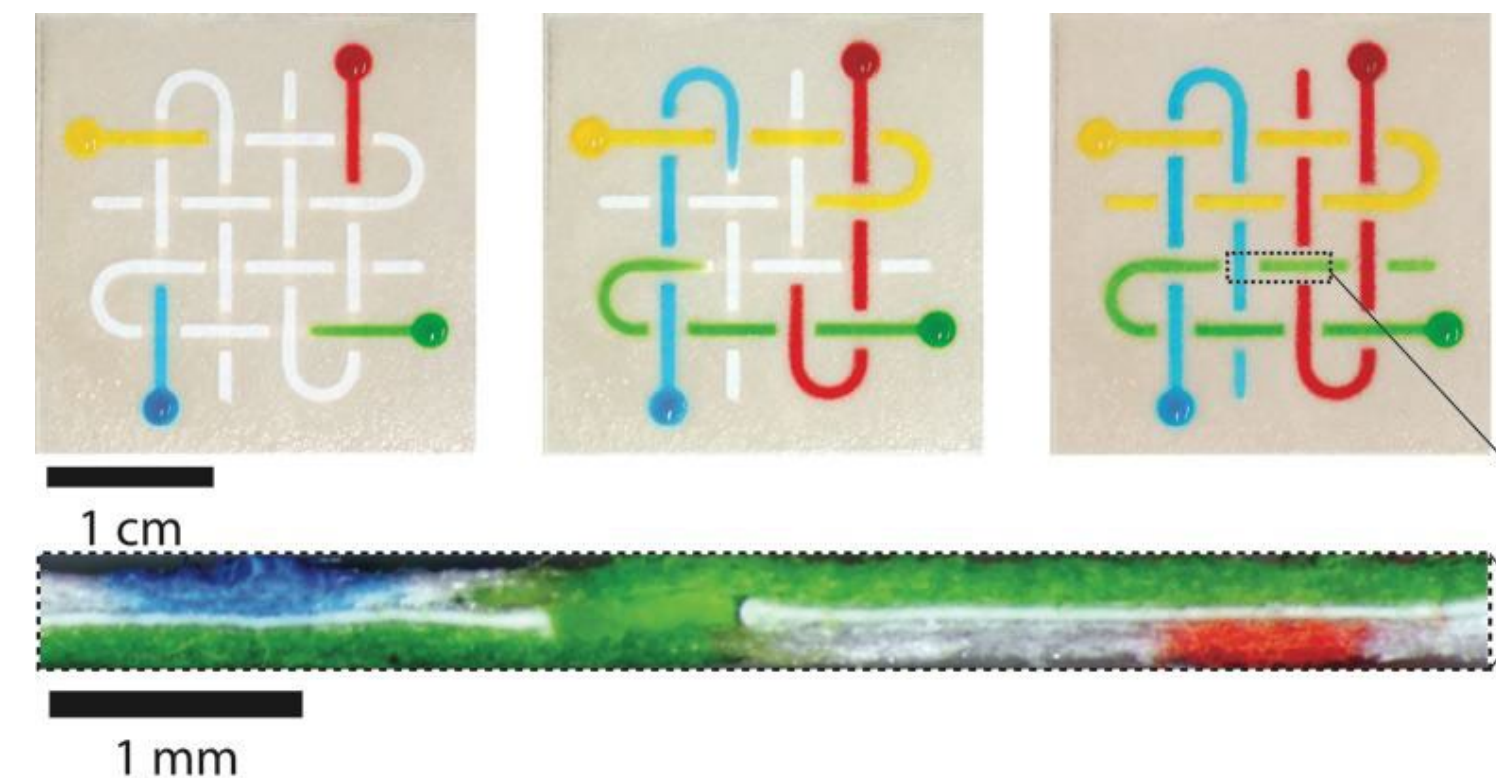


Microfluidic Classification

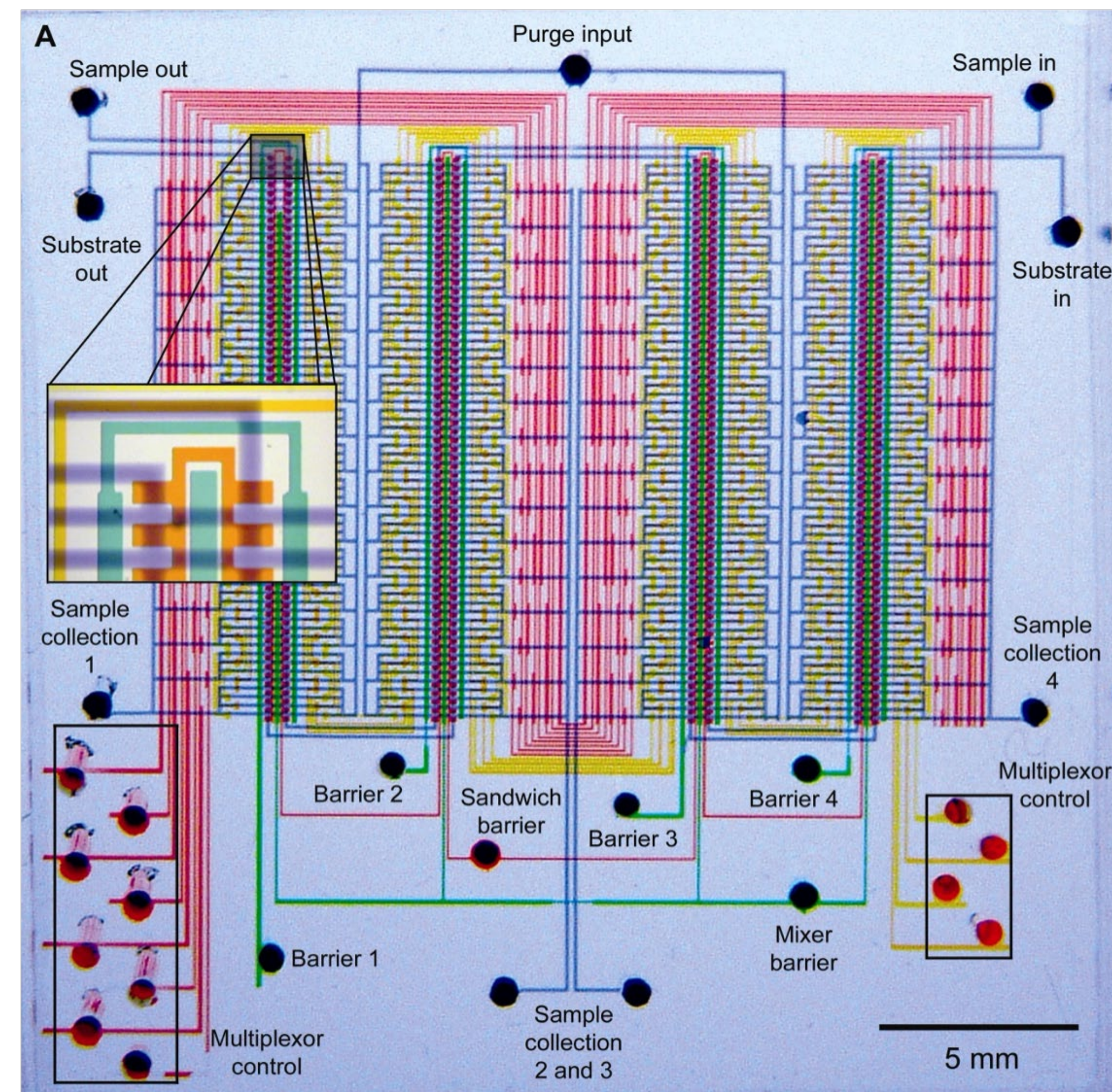
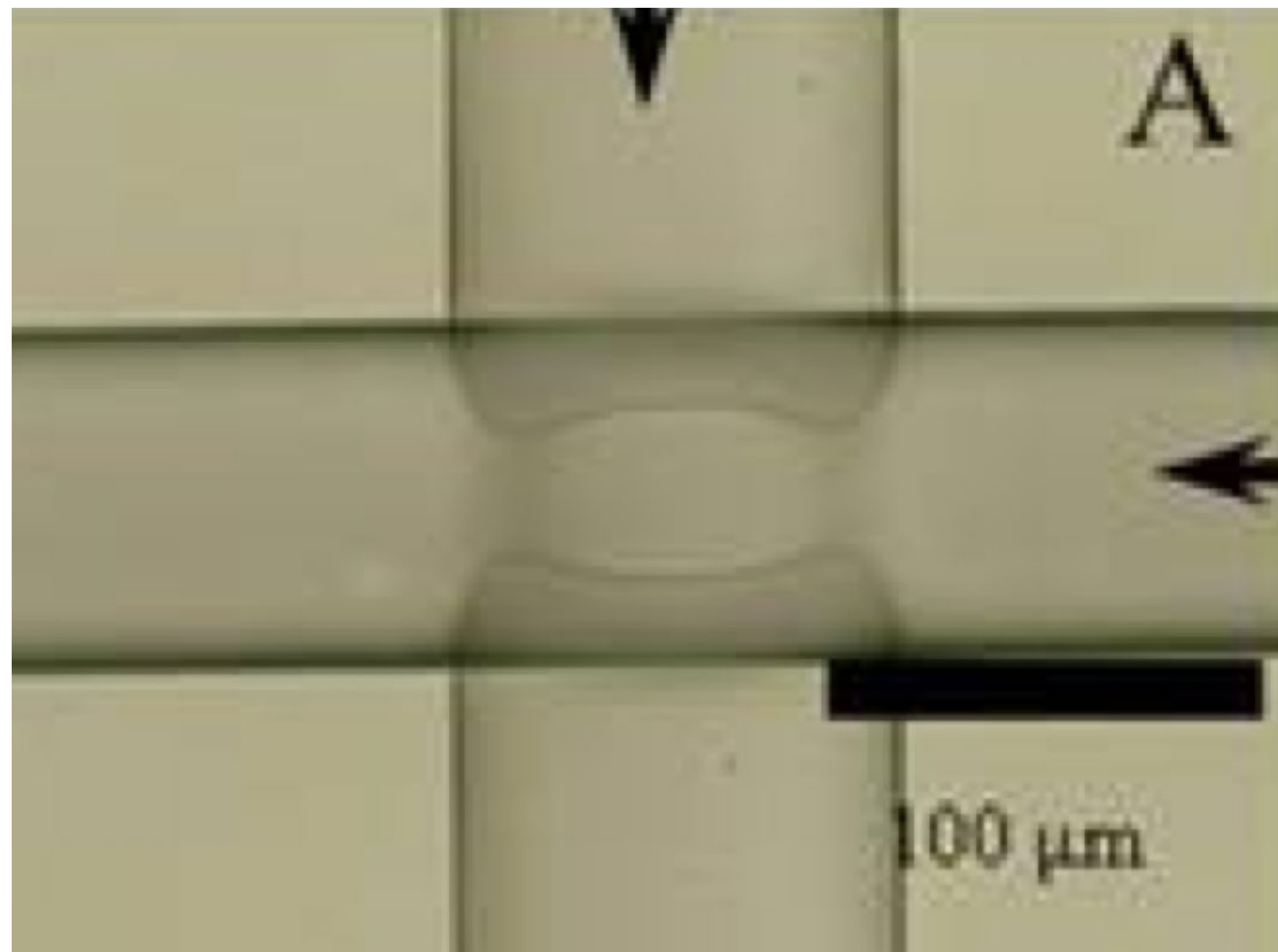
Active Devices



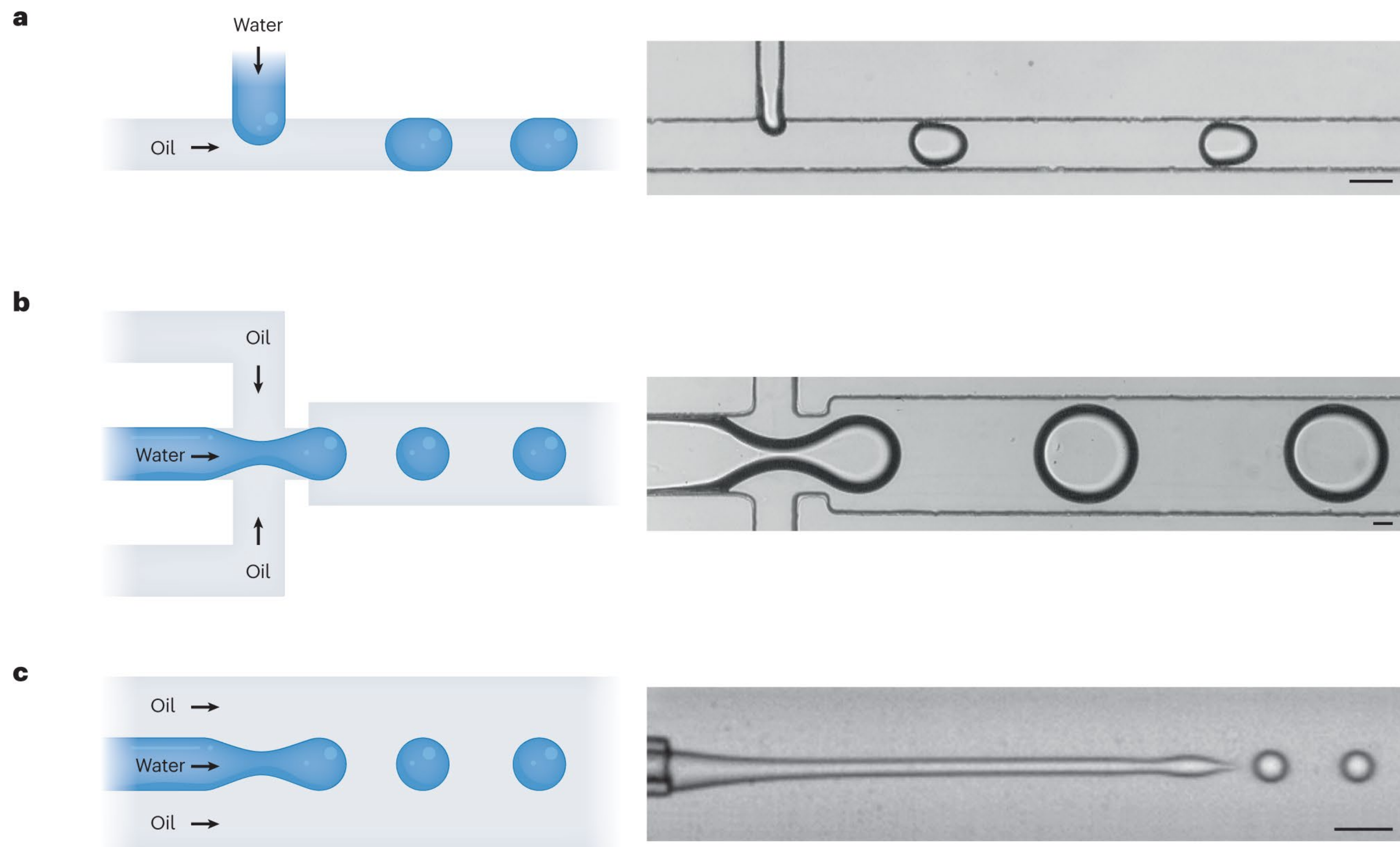
Passive Devices



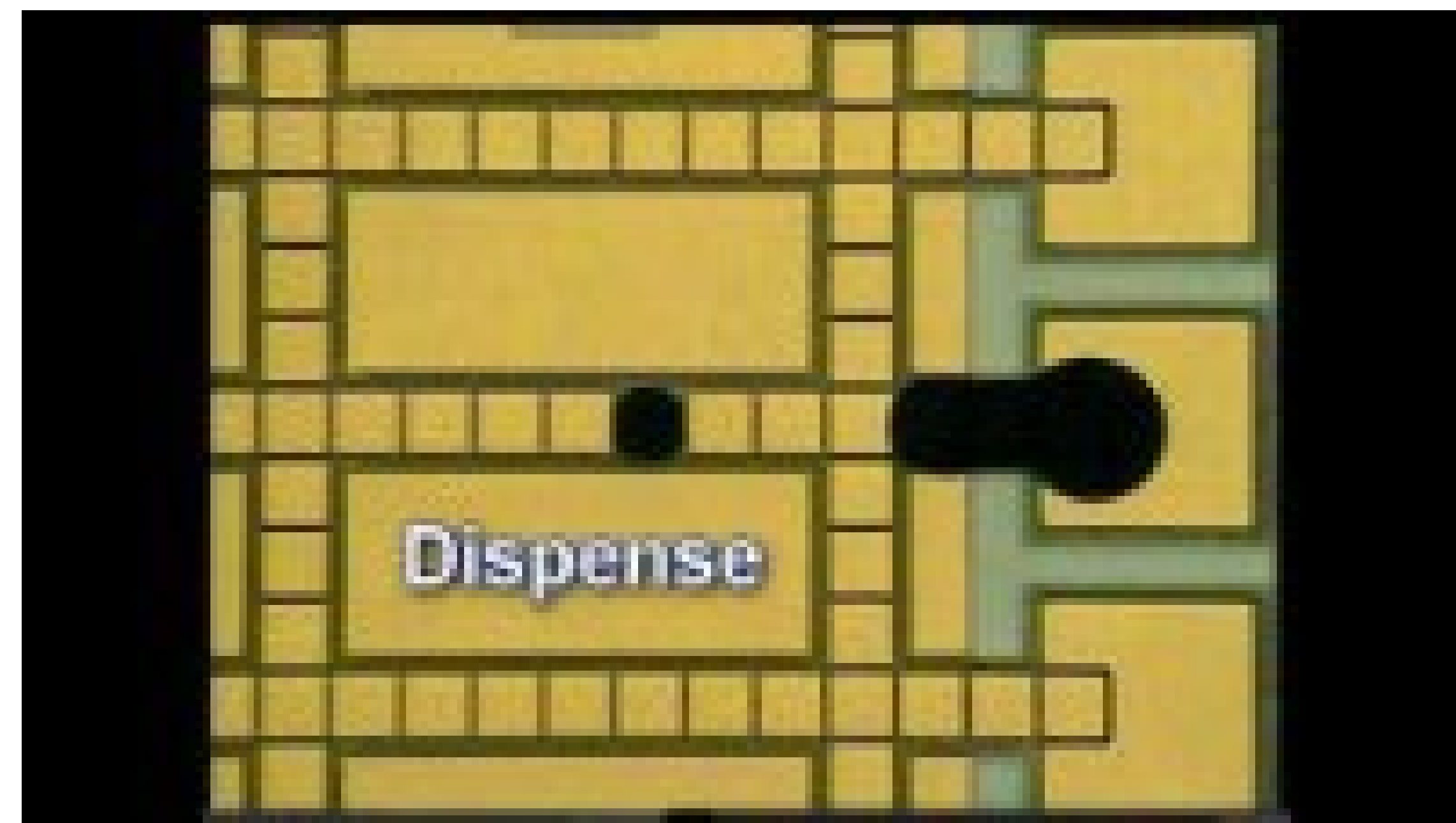
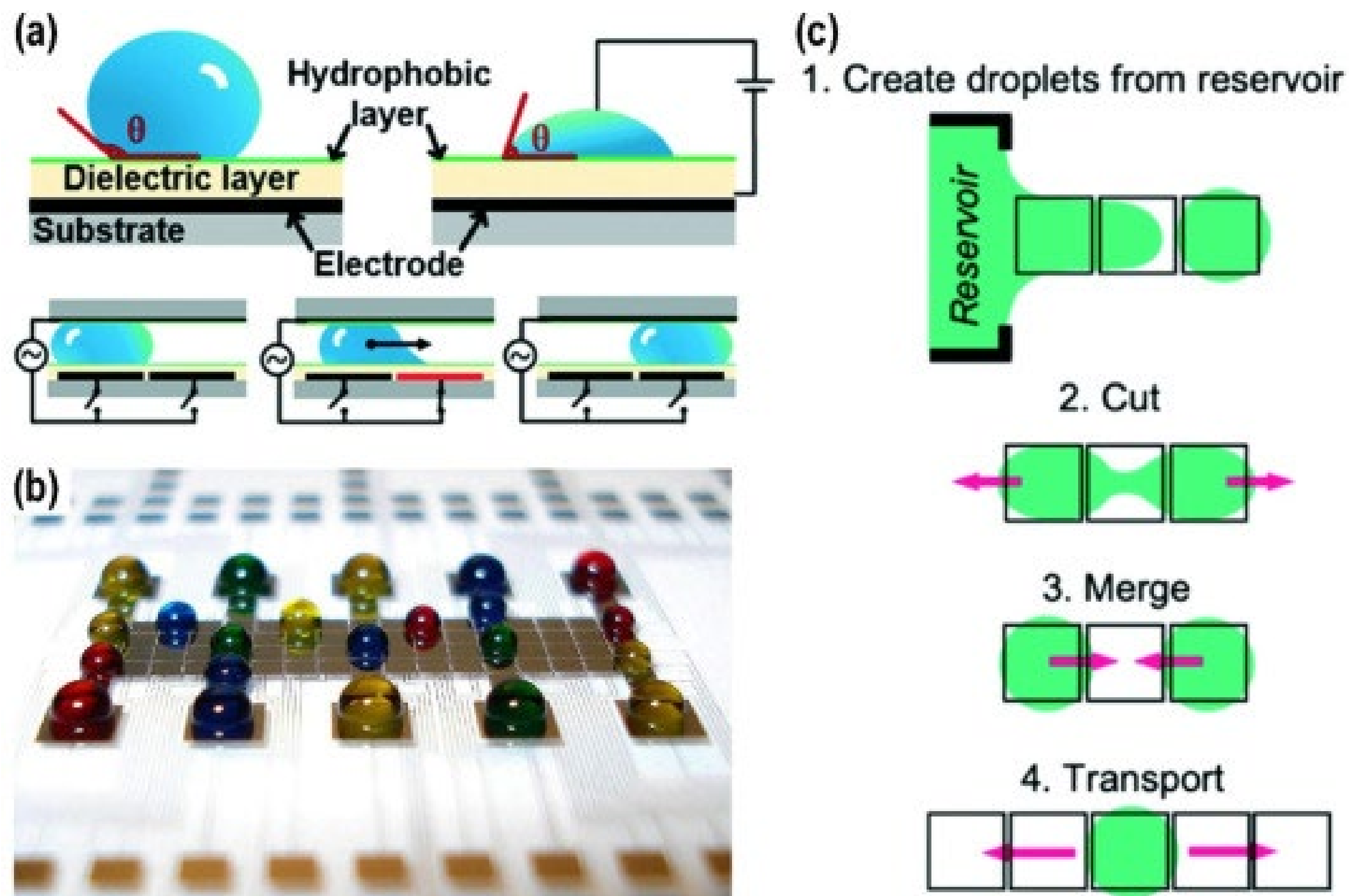
Valve-Based Microfluidics



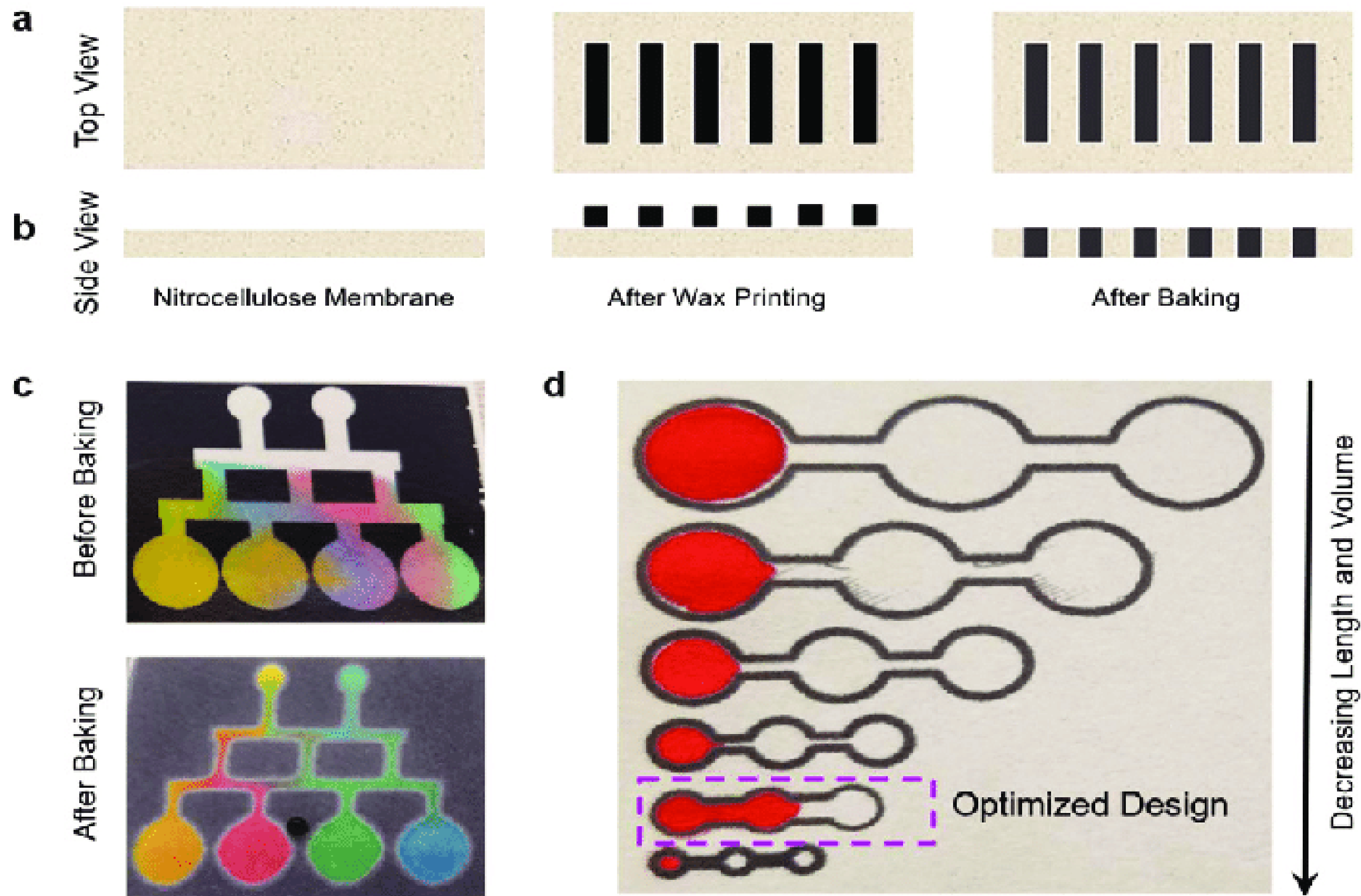
Droplet Microfluidics



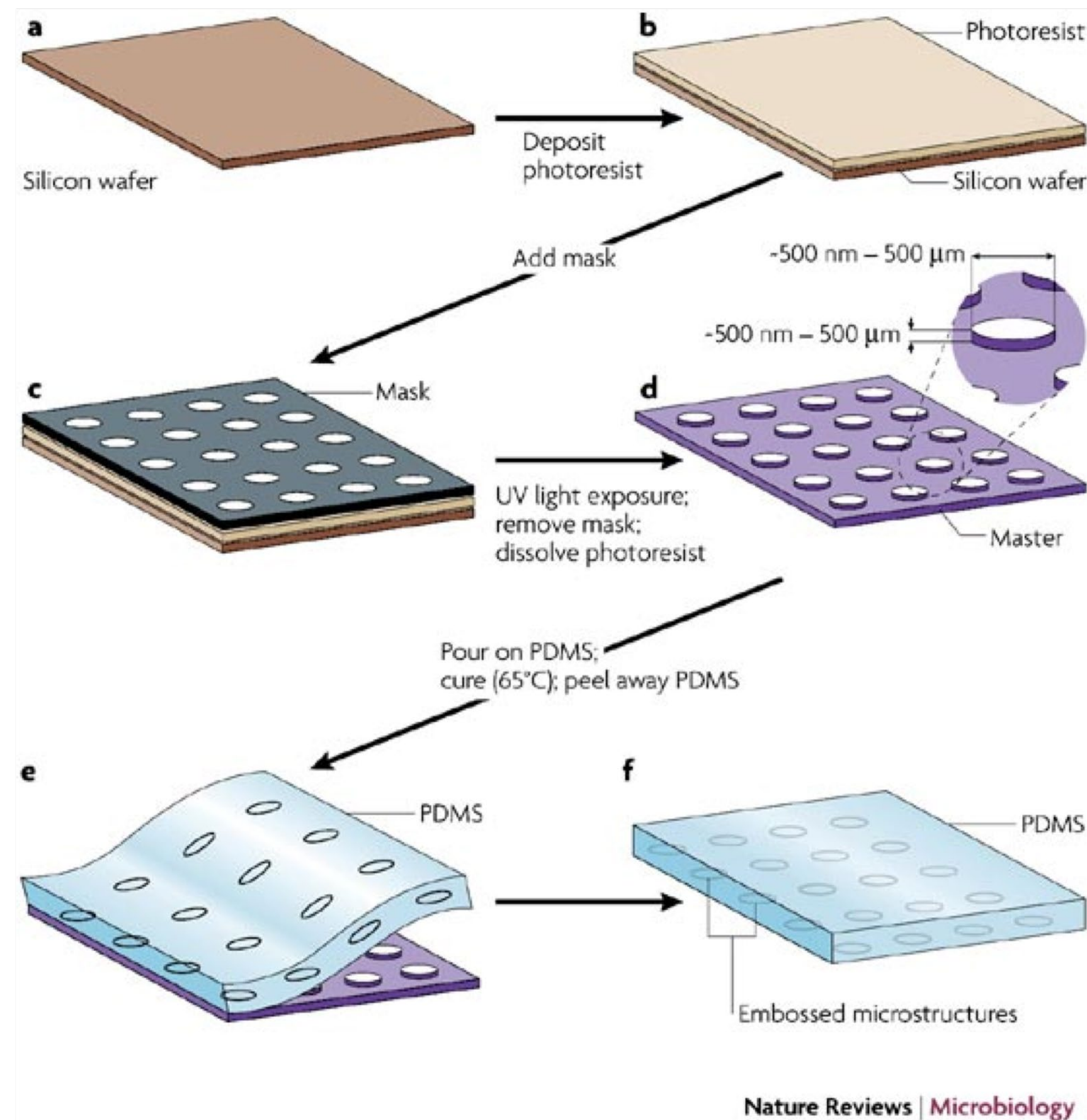
Digital Microfluidics



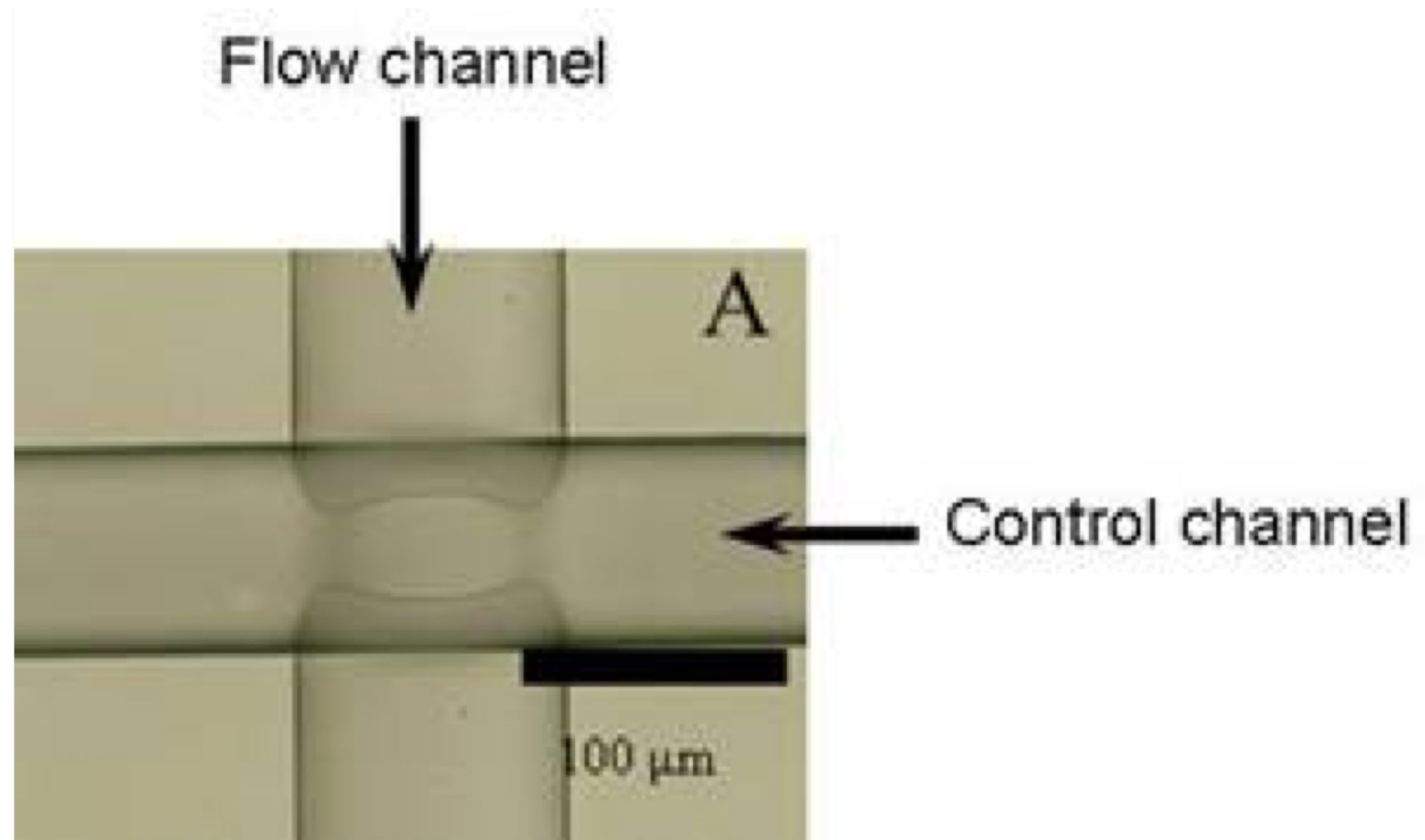
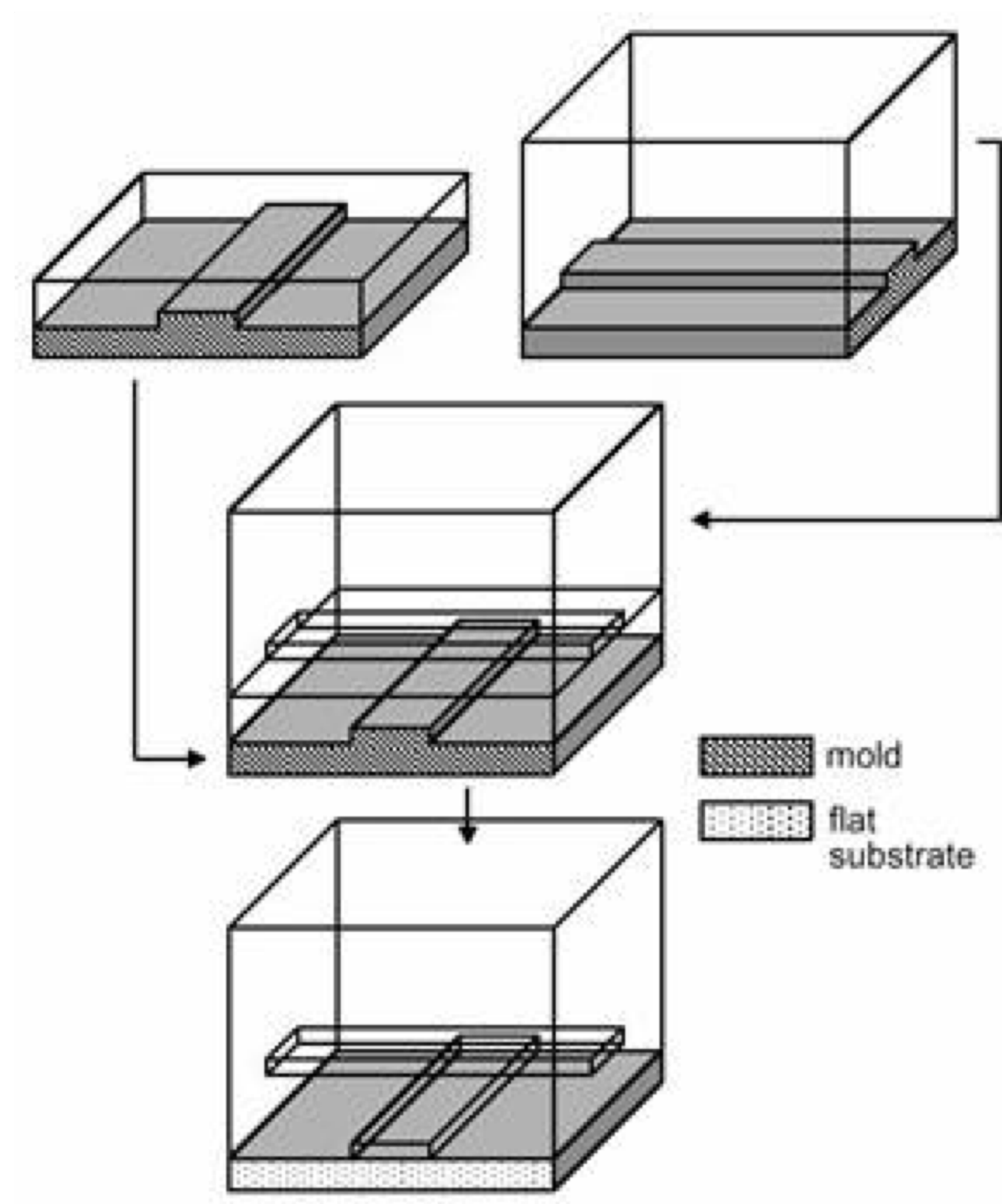
Paper Microfluidics



Soft-Lithography



Multilayer Soft-Lithography

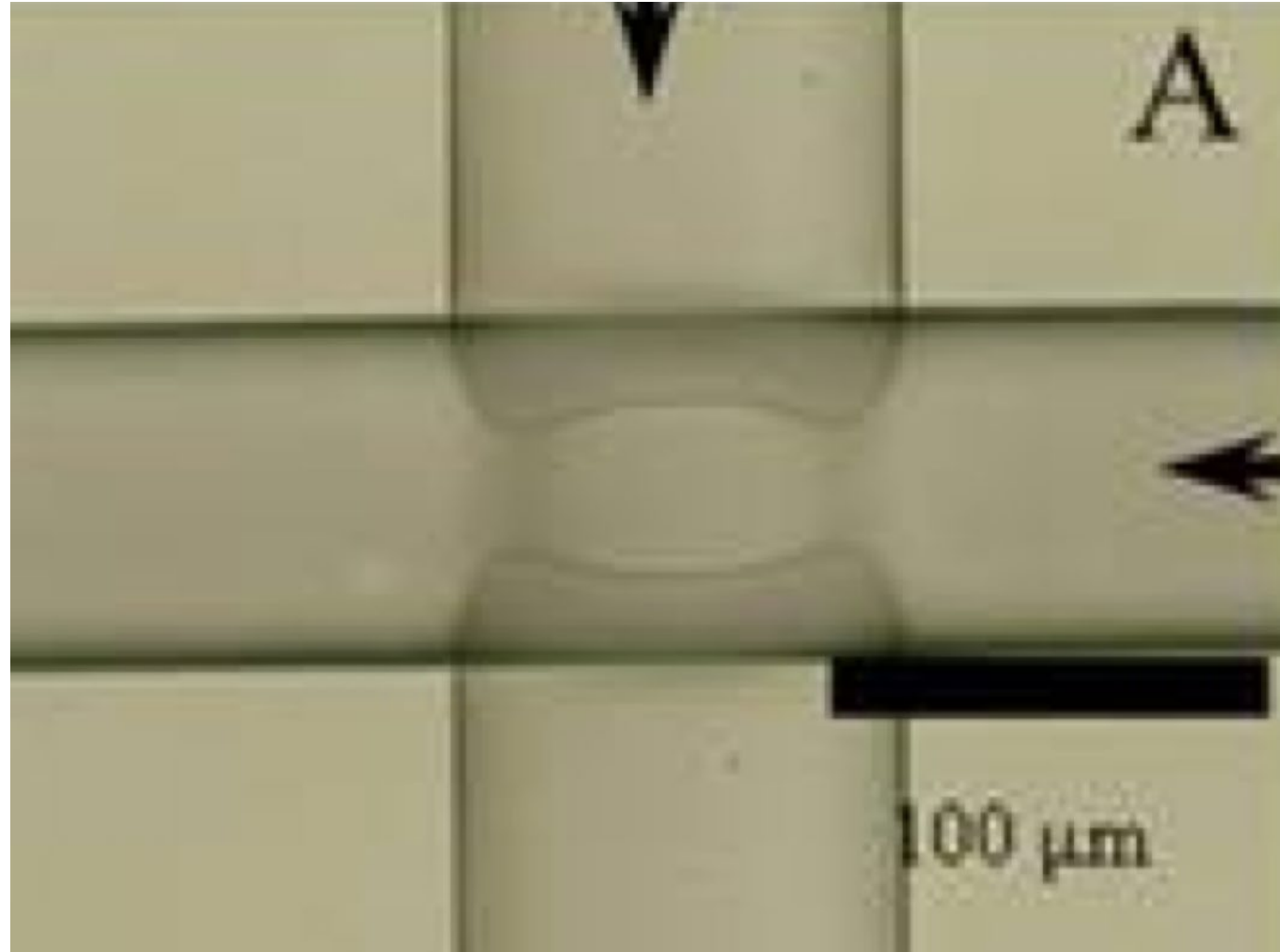


Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography

M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, S. R. Quake

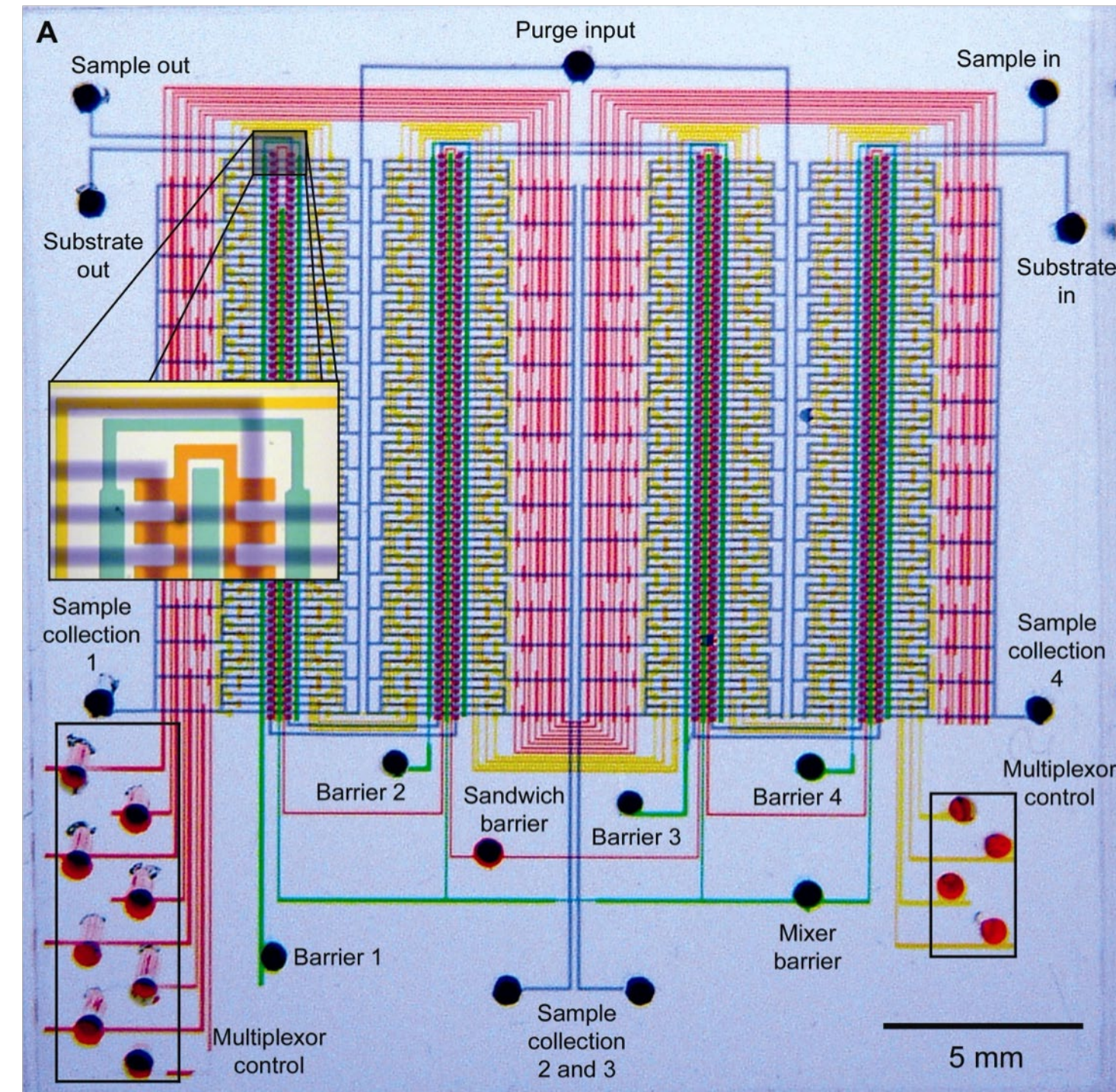
Science 288, 113 (2000).

Microfluidic Large-Scale Integration



1st Micromechanical Valve

M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, S. R. Quake
Science 288, 113 (2000).

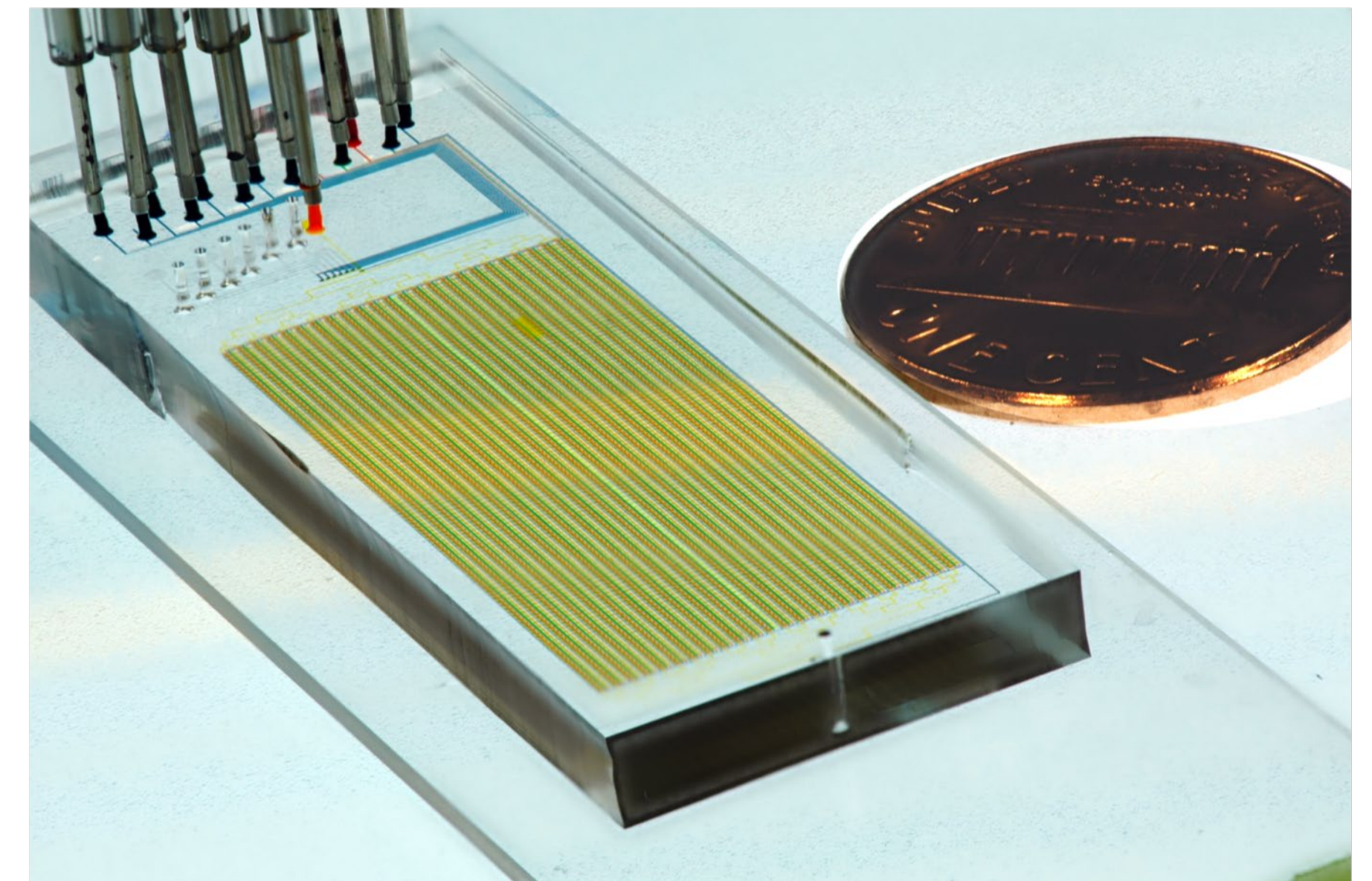


2056 Valves

T. Thorsen, S. J. Maerkl, S. R. Quake
Science 298, 580 (2002).

7233 Valves

S. J. Maerkl and S. R. Quake
Science 315, 233 (2007).



Chip Design

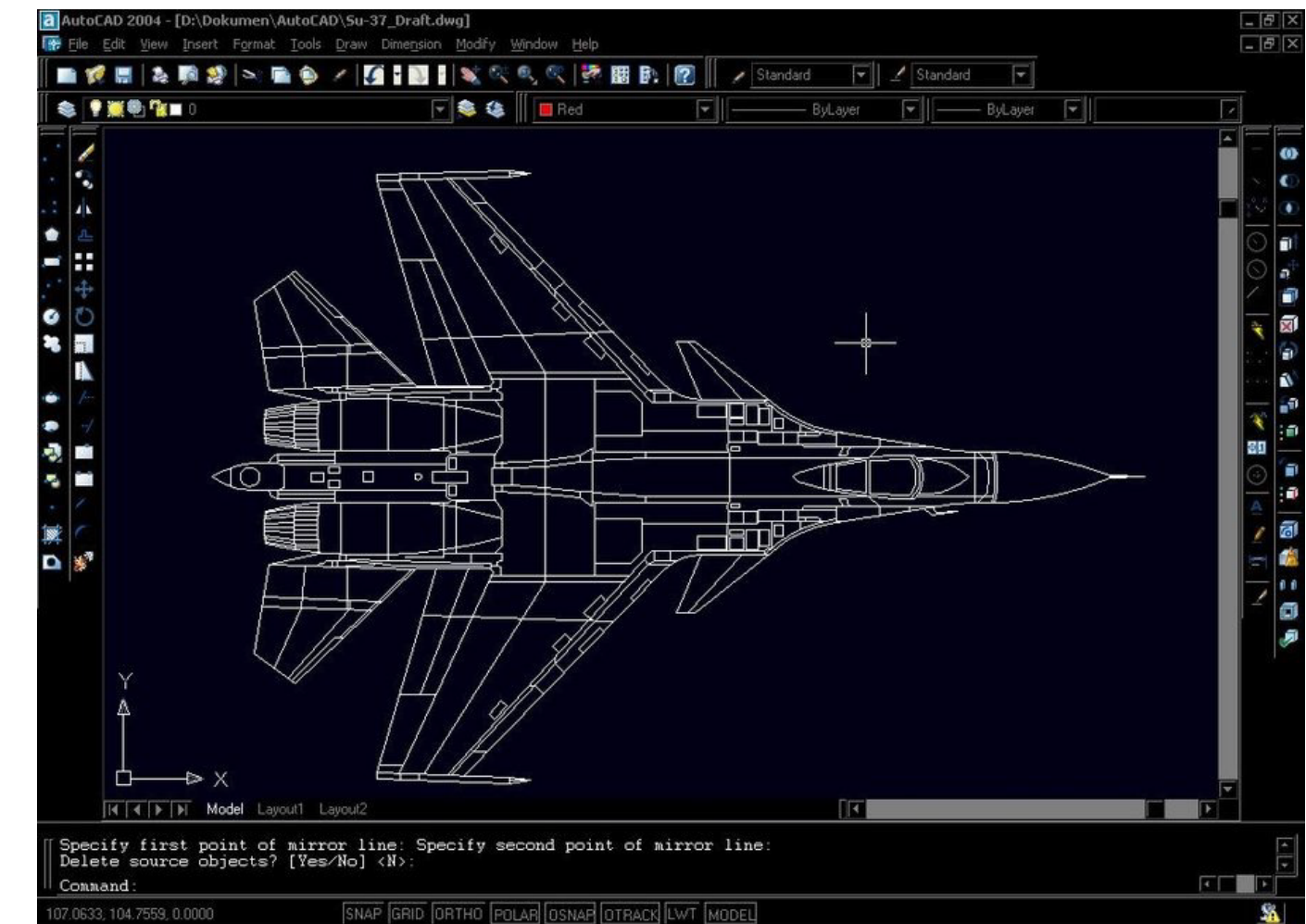
Idea



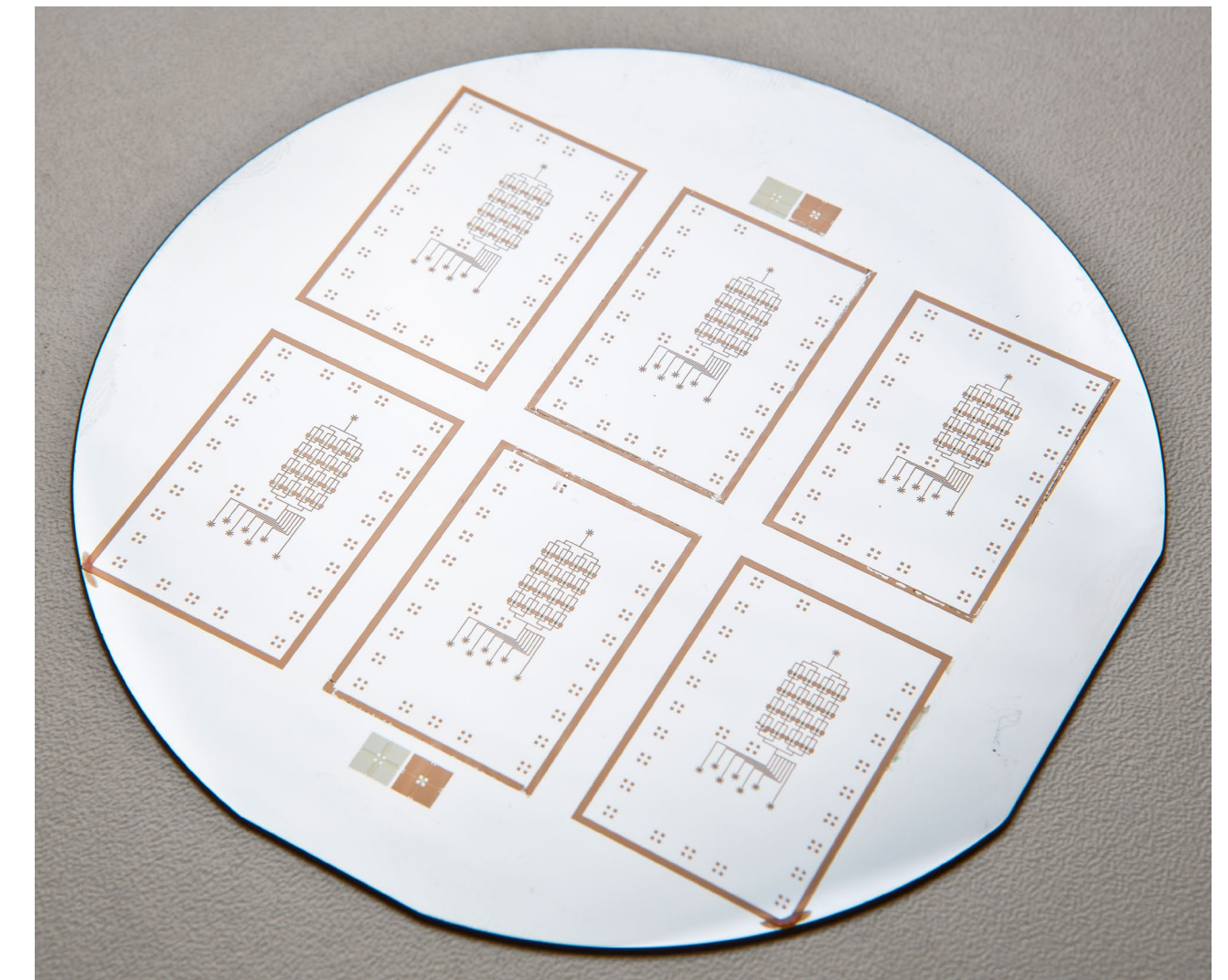
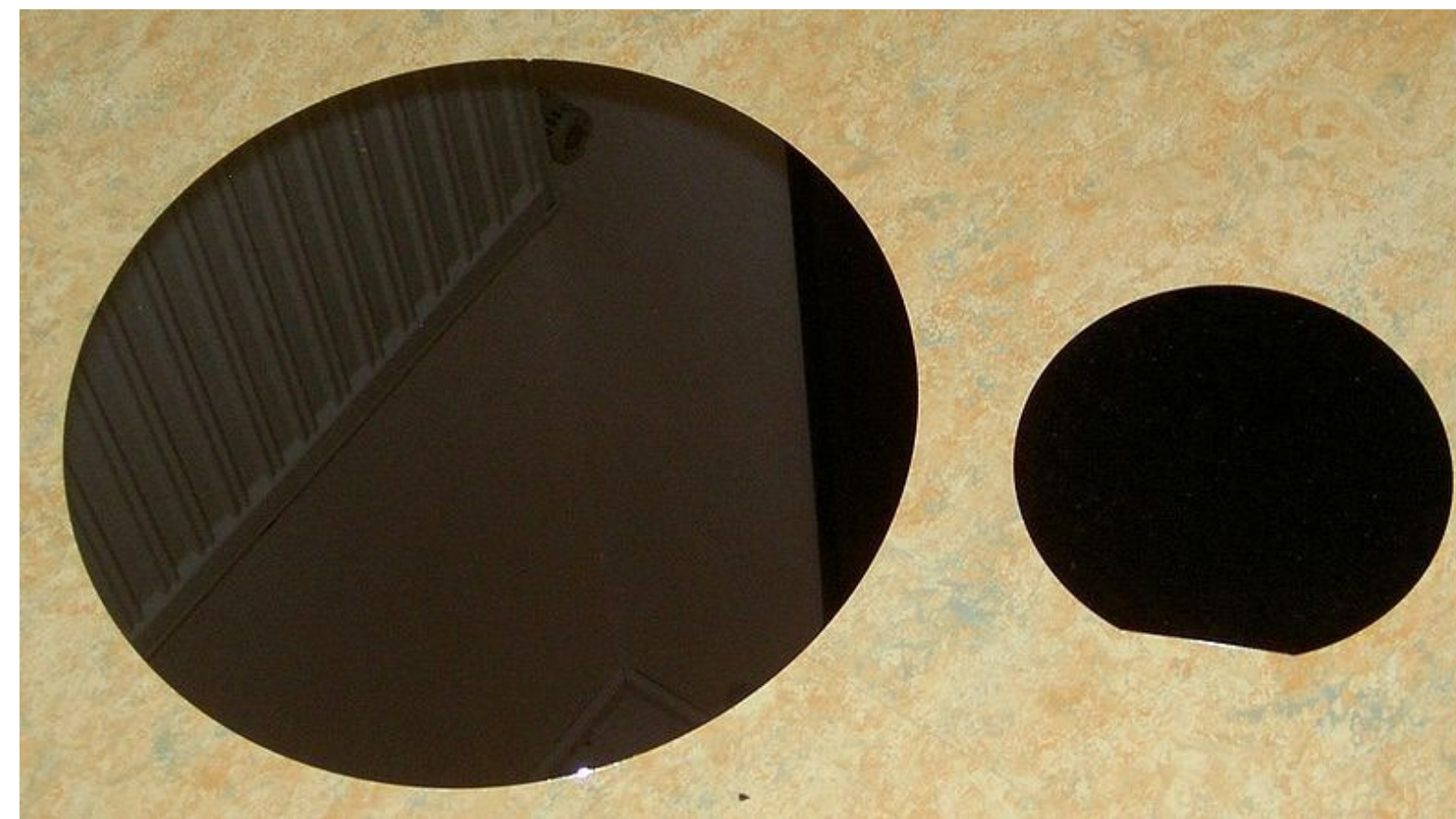
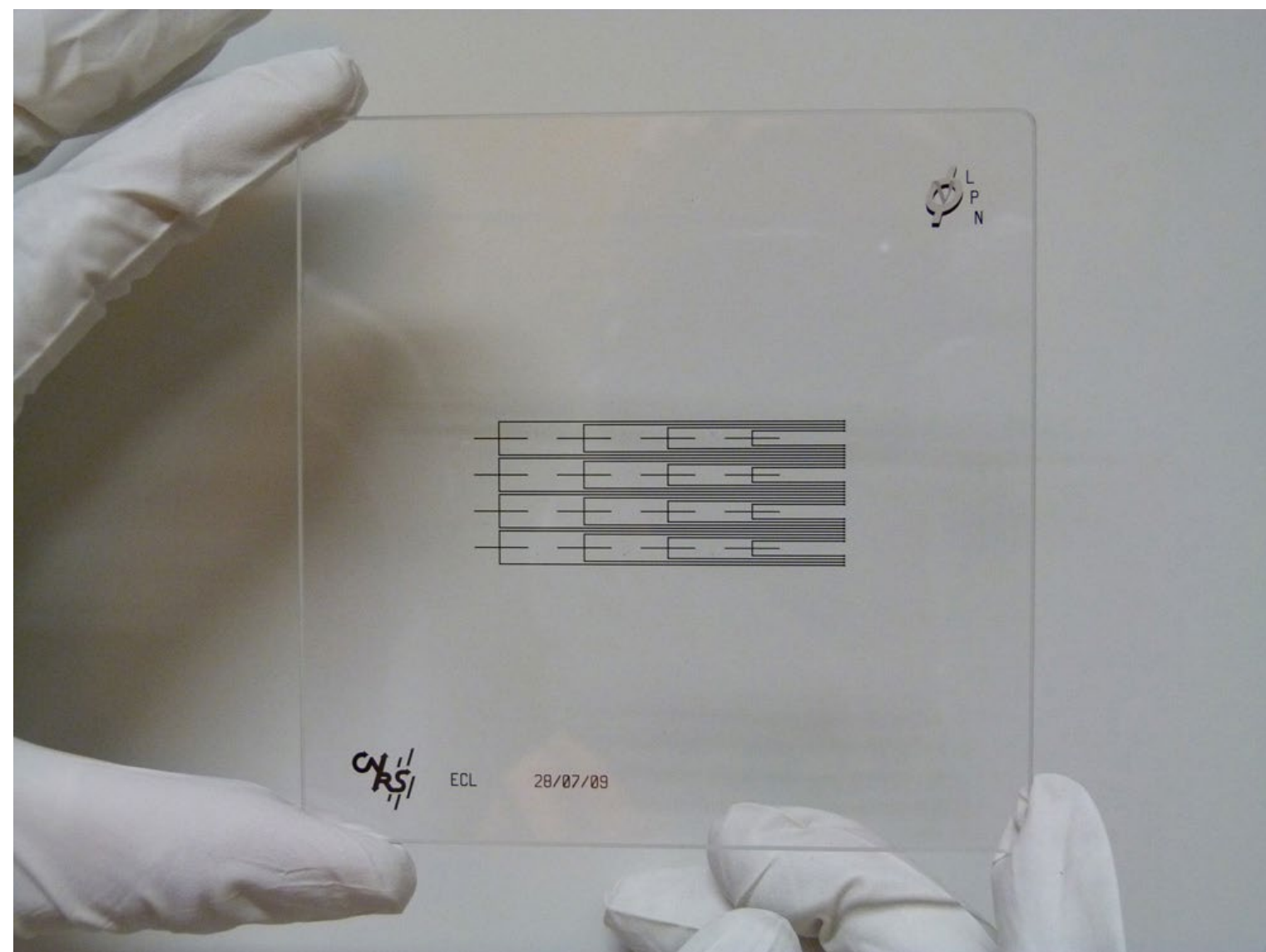
Pen & Paper



CAD



Mold Fabrication

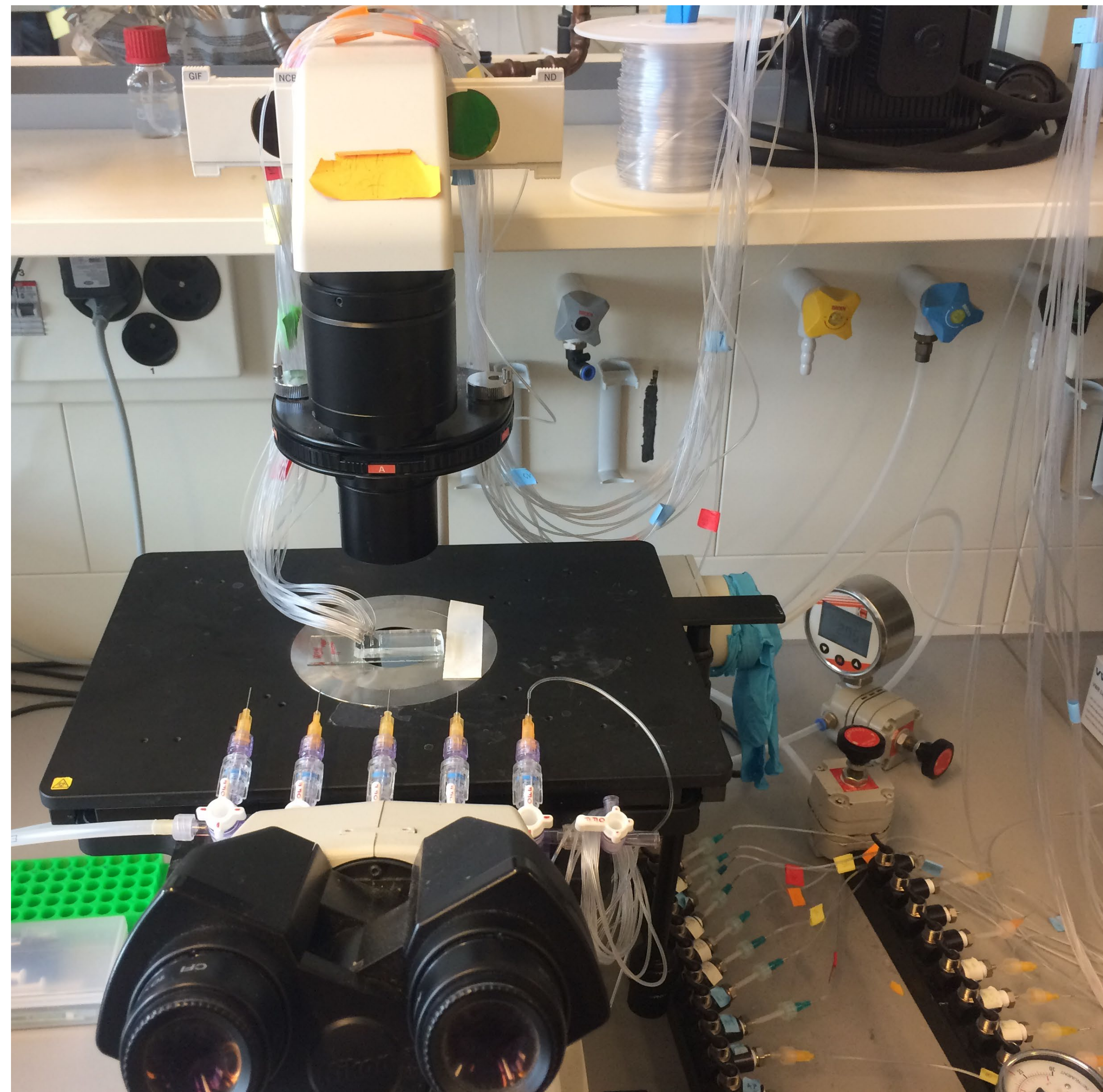


Chip Fabrication

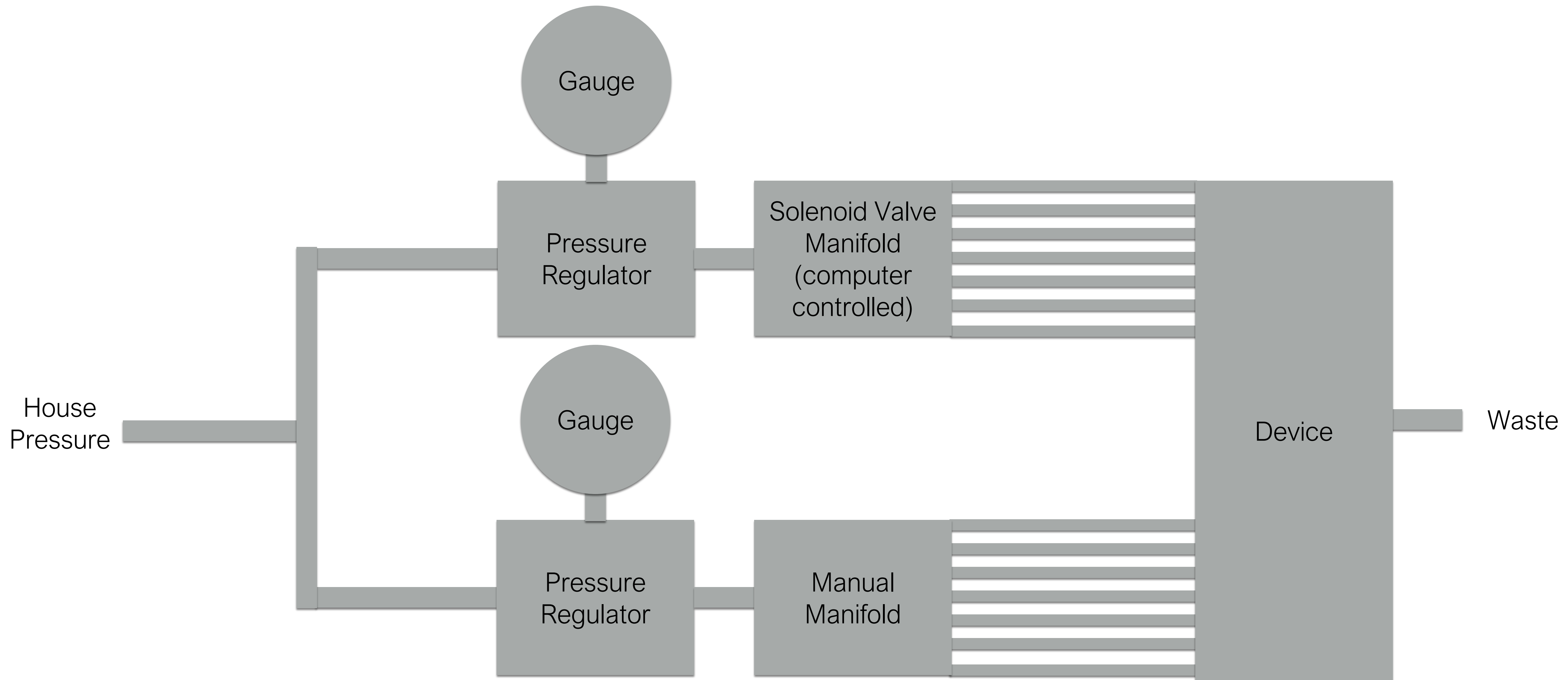


- ~ 2.5 hours to fabricate a batch of chips (4-6 devices)

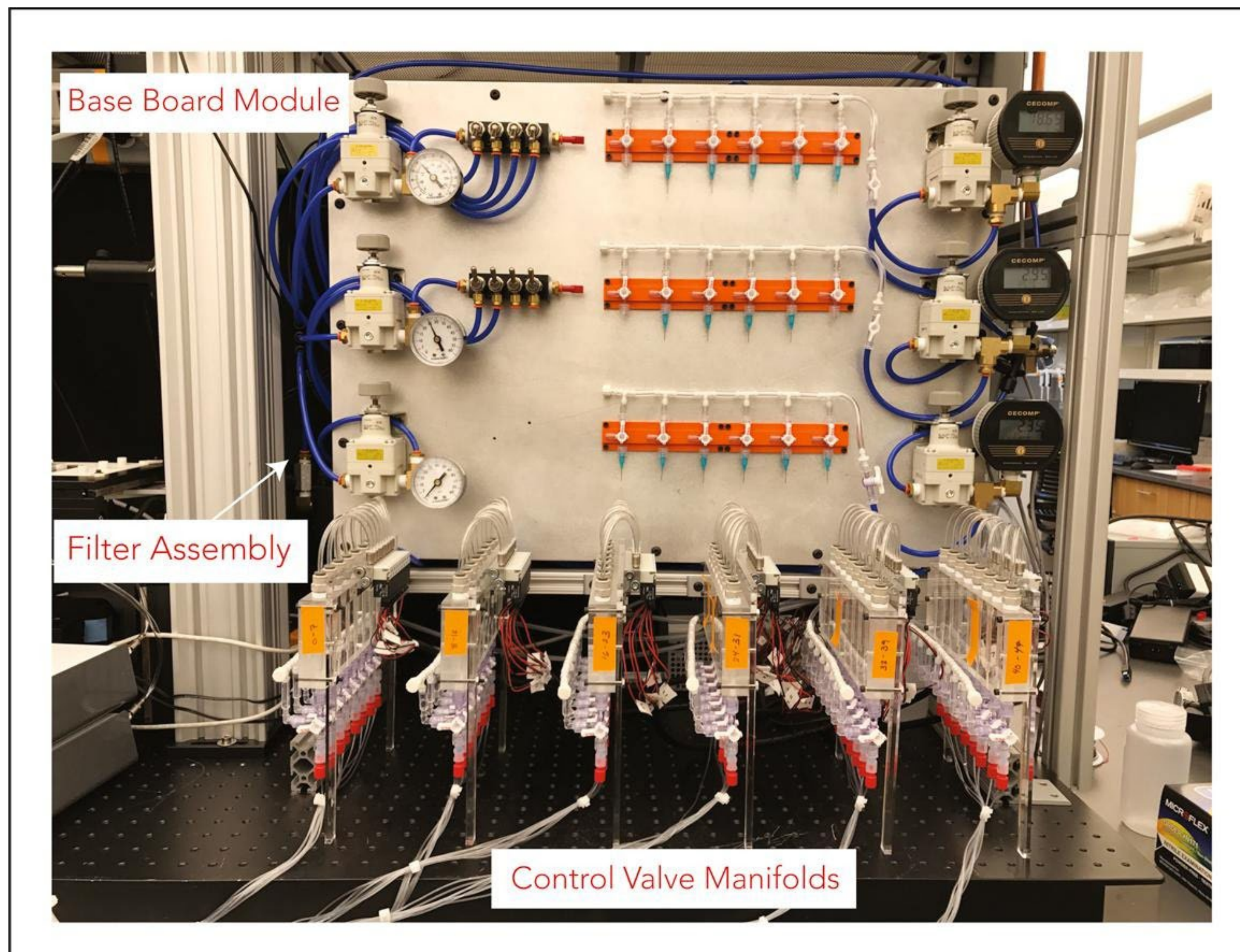
Microfluidic Setup



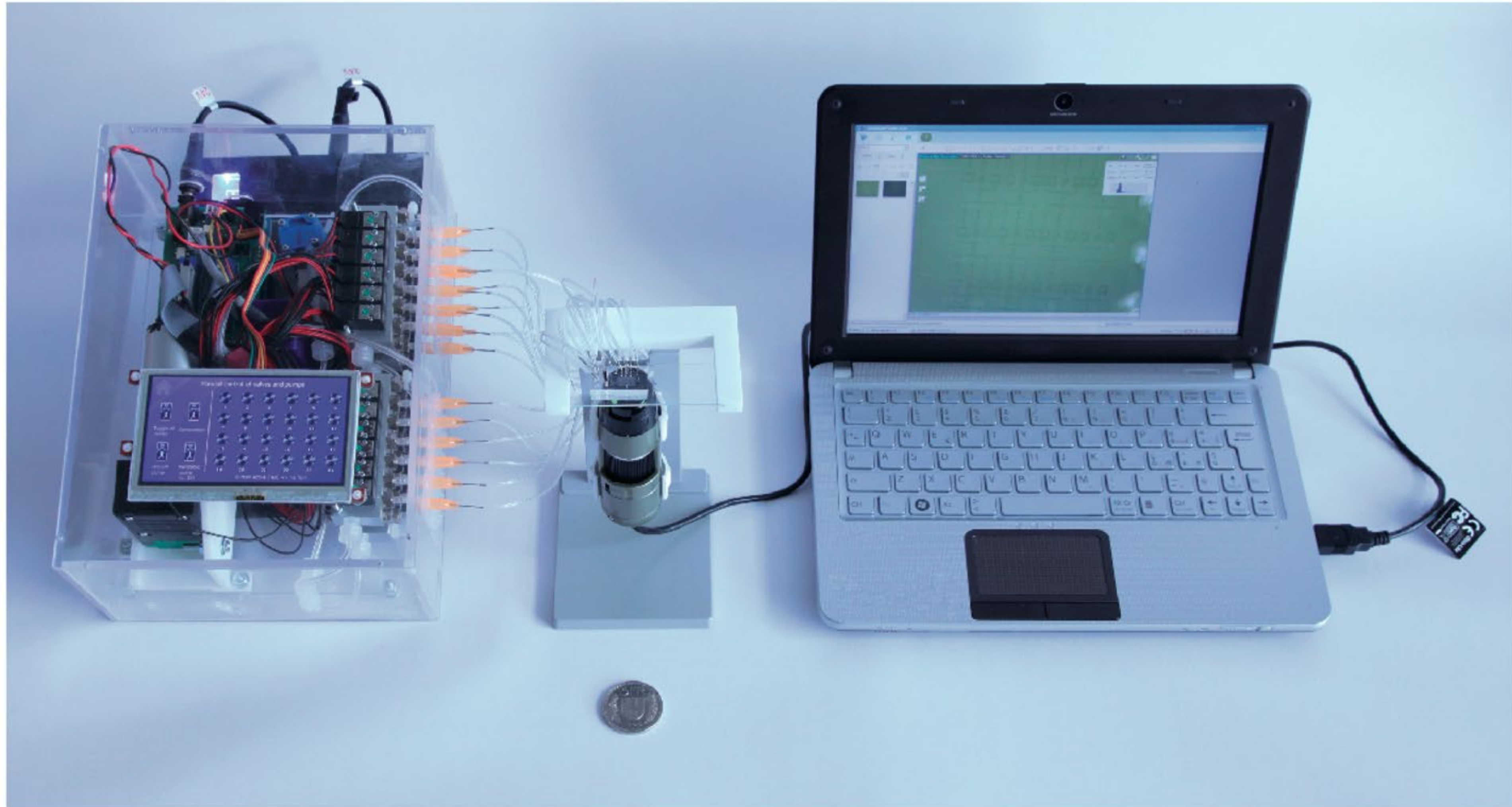
Device Control



Device Control



Device Control

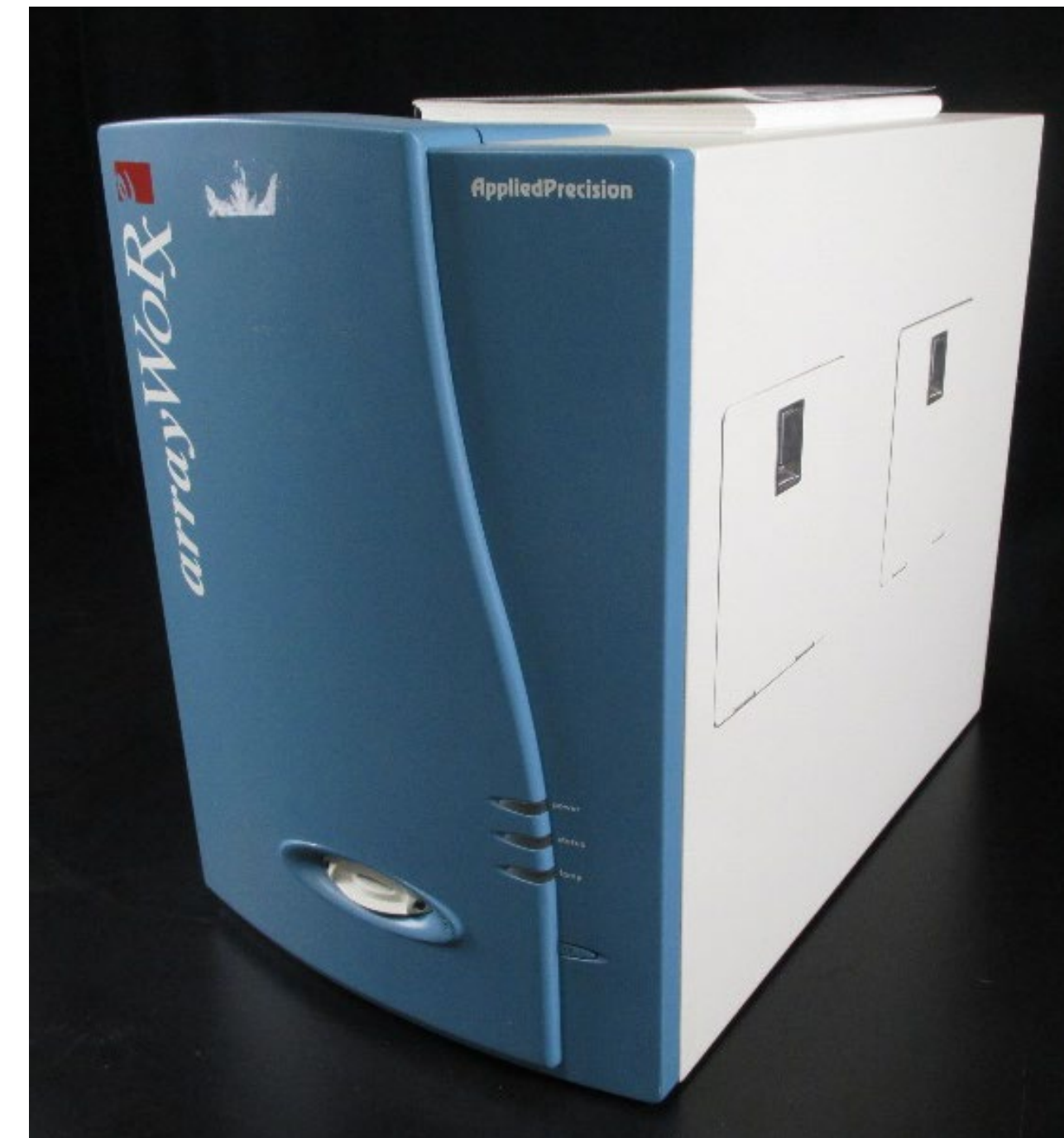


Device Readout

Microscopy



DNA Array Scanners



On-Chip Elements

Active

Valves

Peristaltic Pumps

Mixers

Freestanding Membranes

Multiplexers

Passive

Fluid Trees

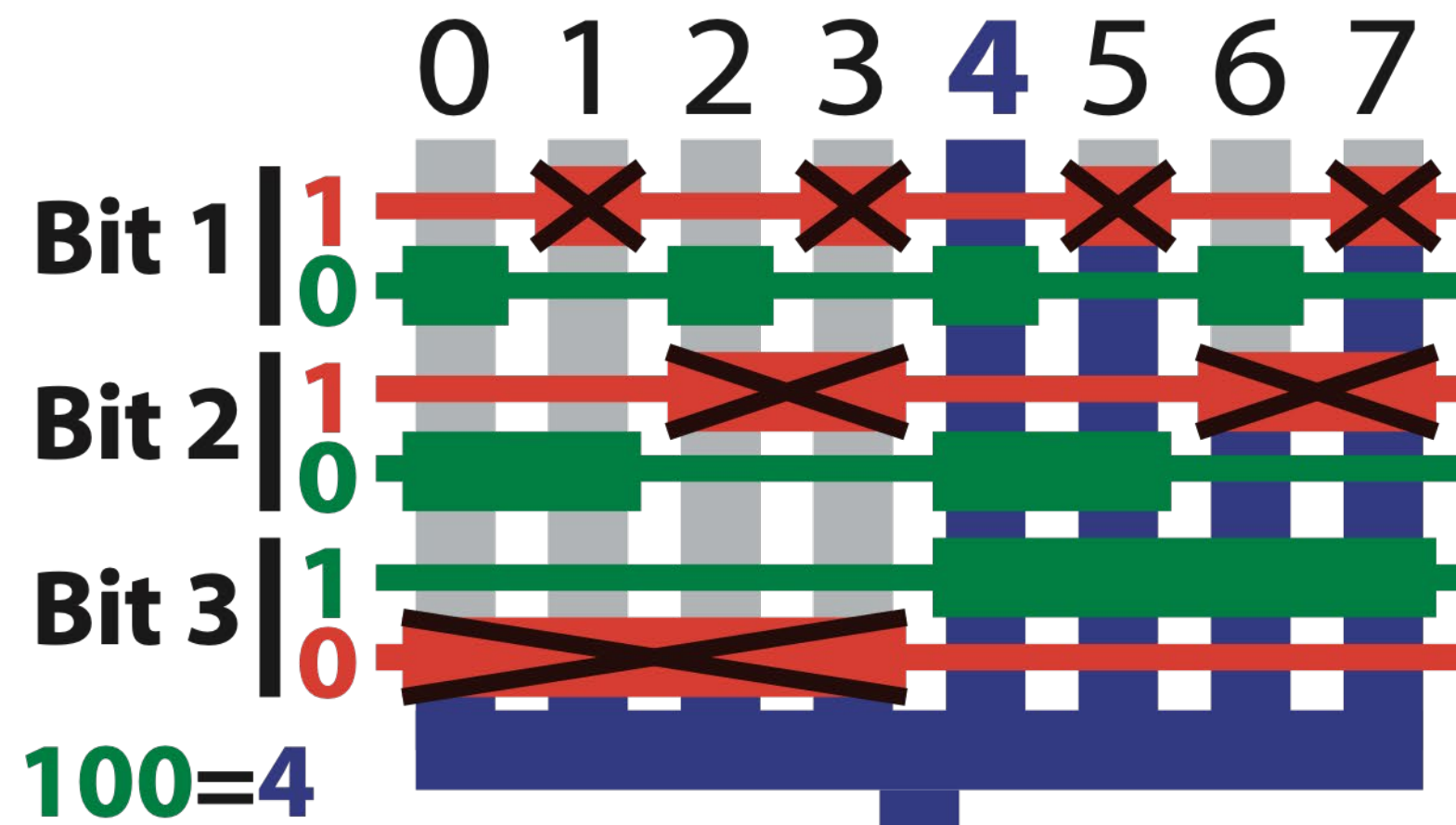
Cell Traps

Sieve Channels

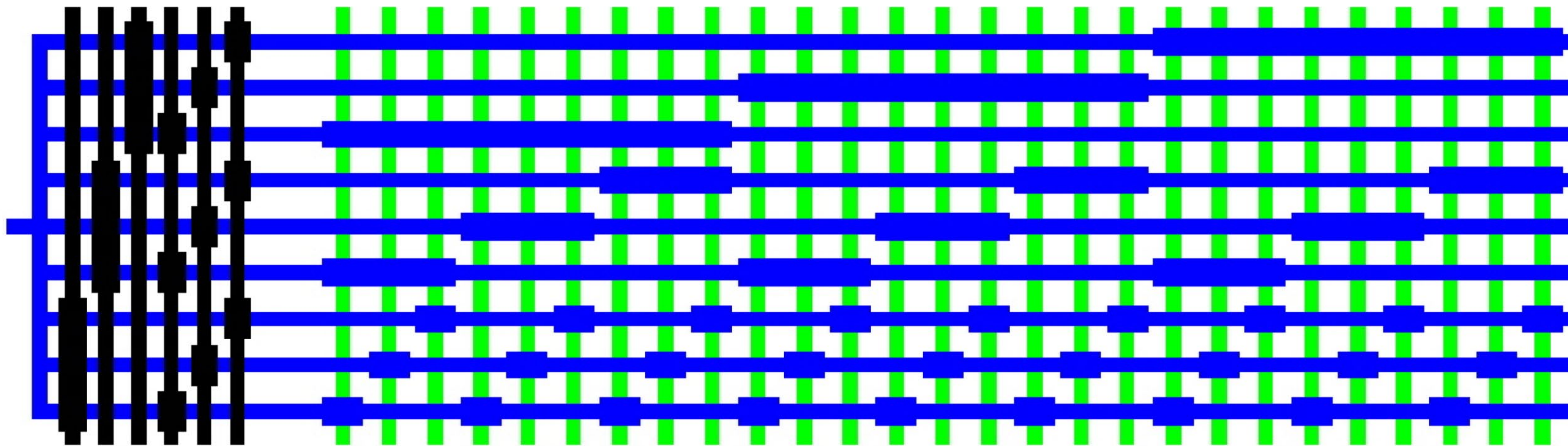
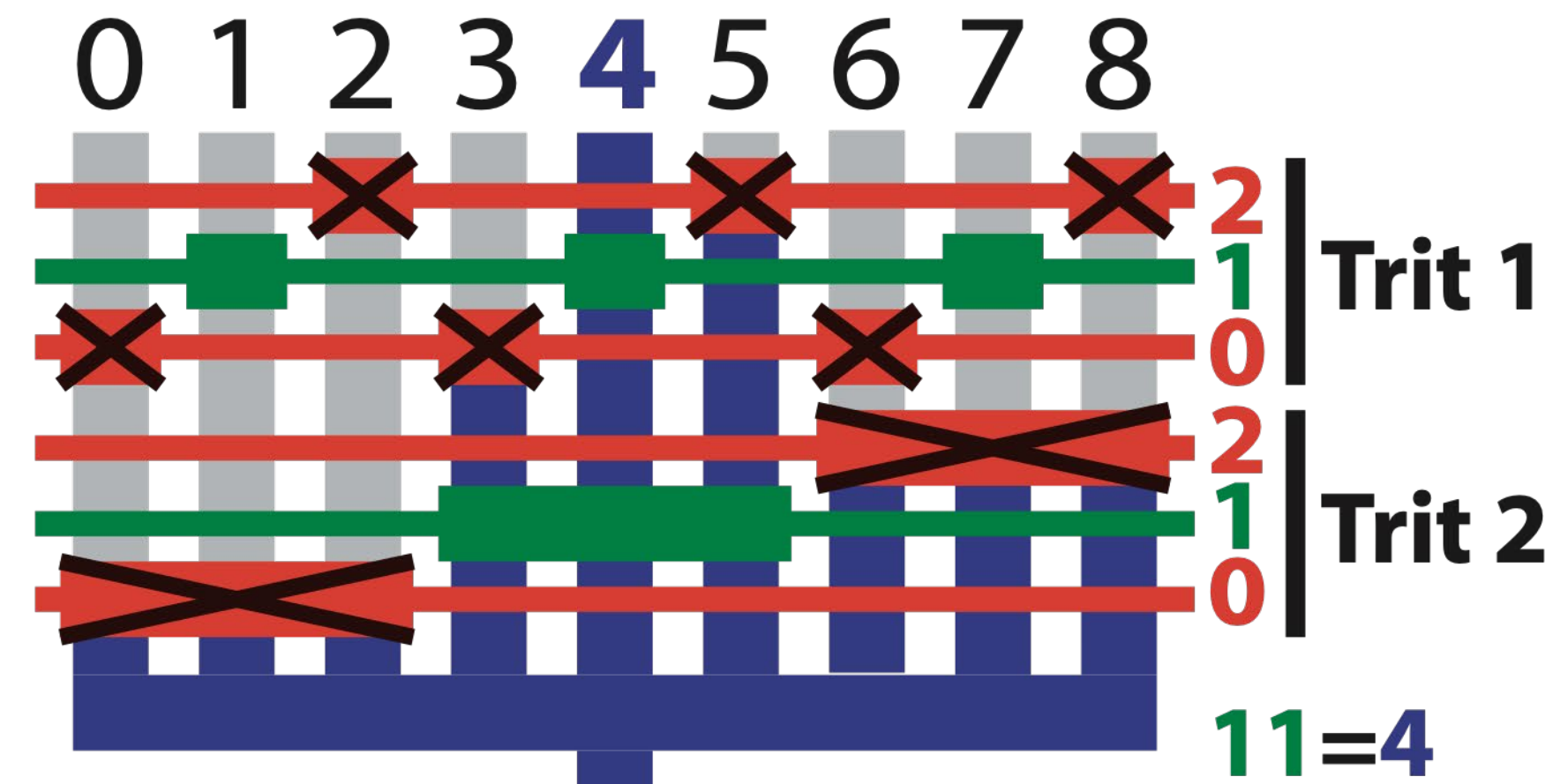
Chaotic Mixers

Multiplexers and Valve Cascades

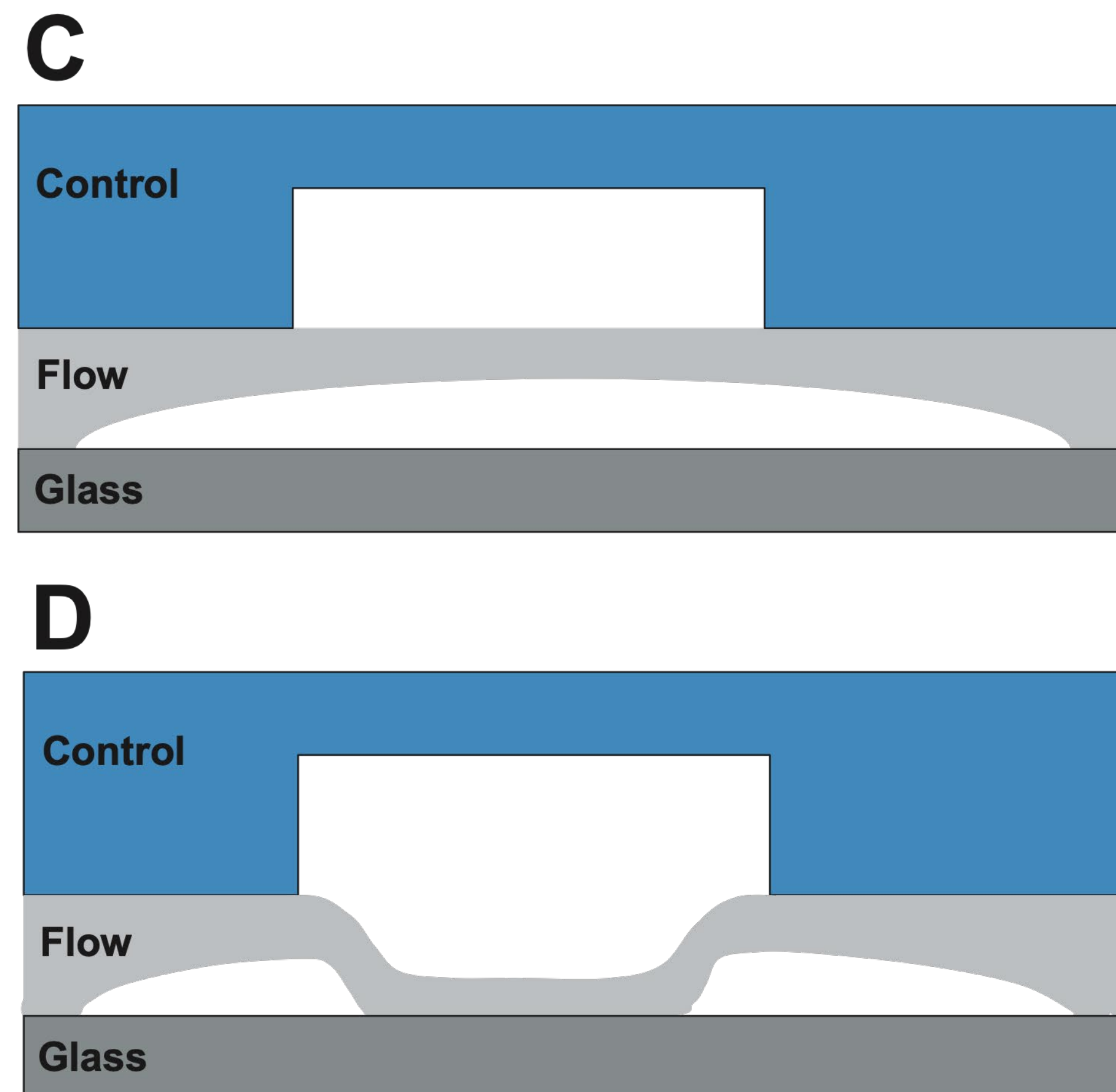
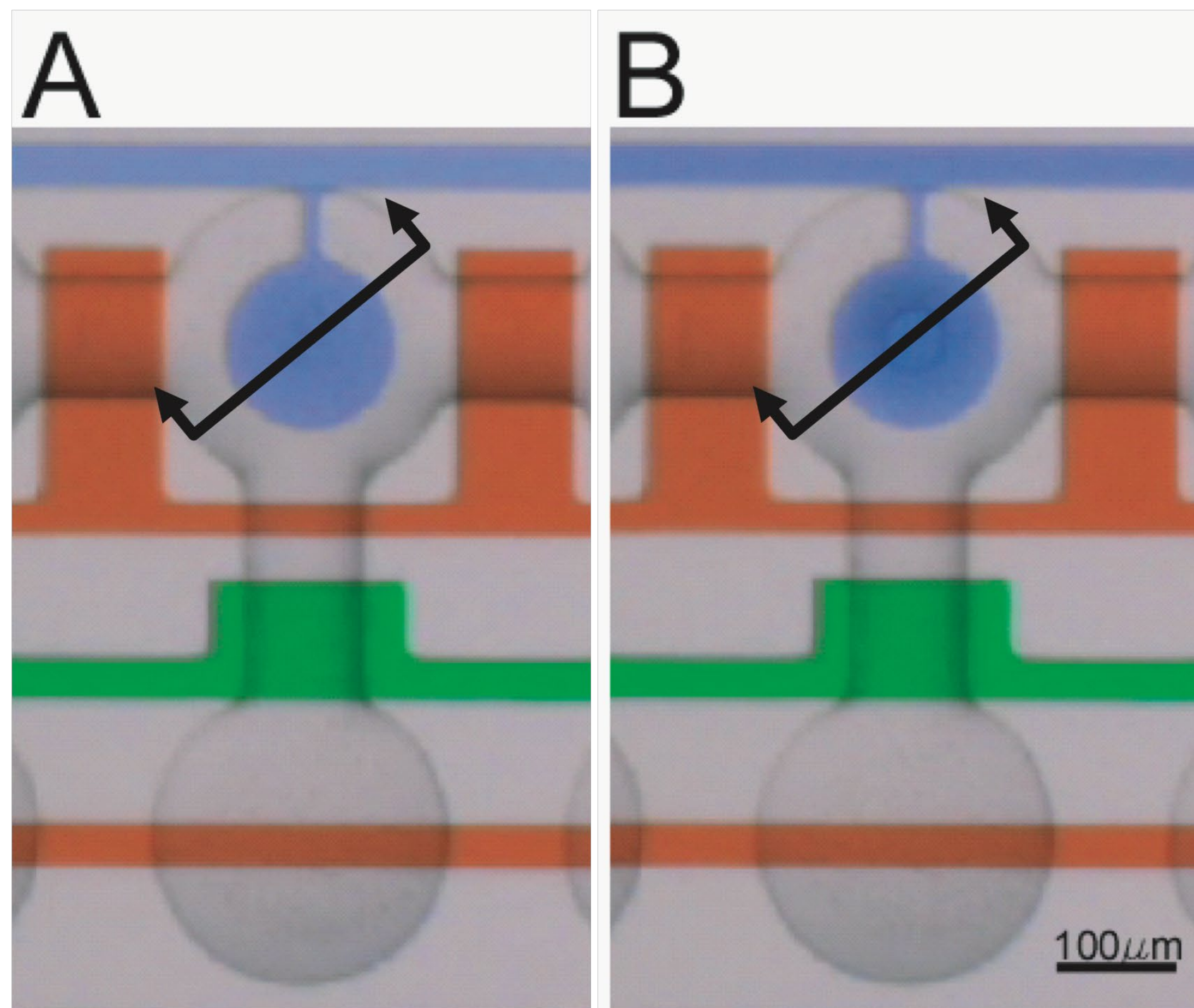
Binary Multiplexer



Ternary Multiplexer

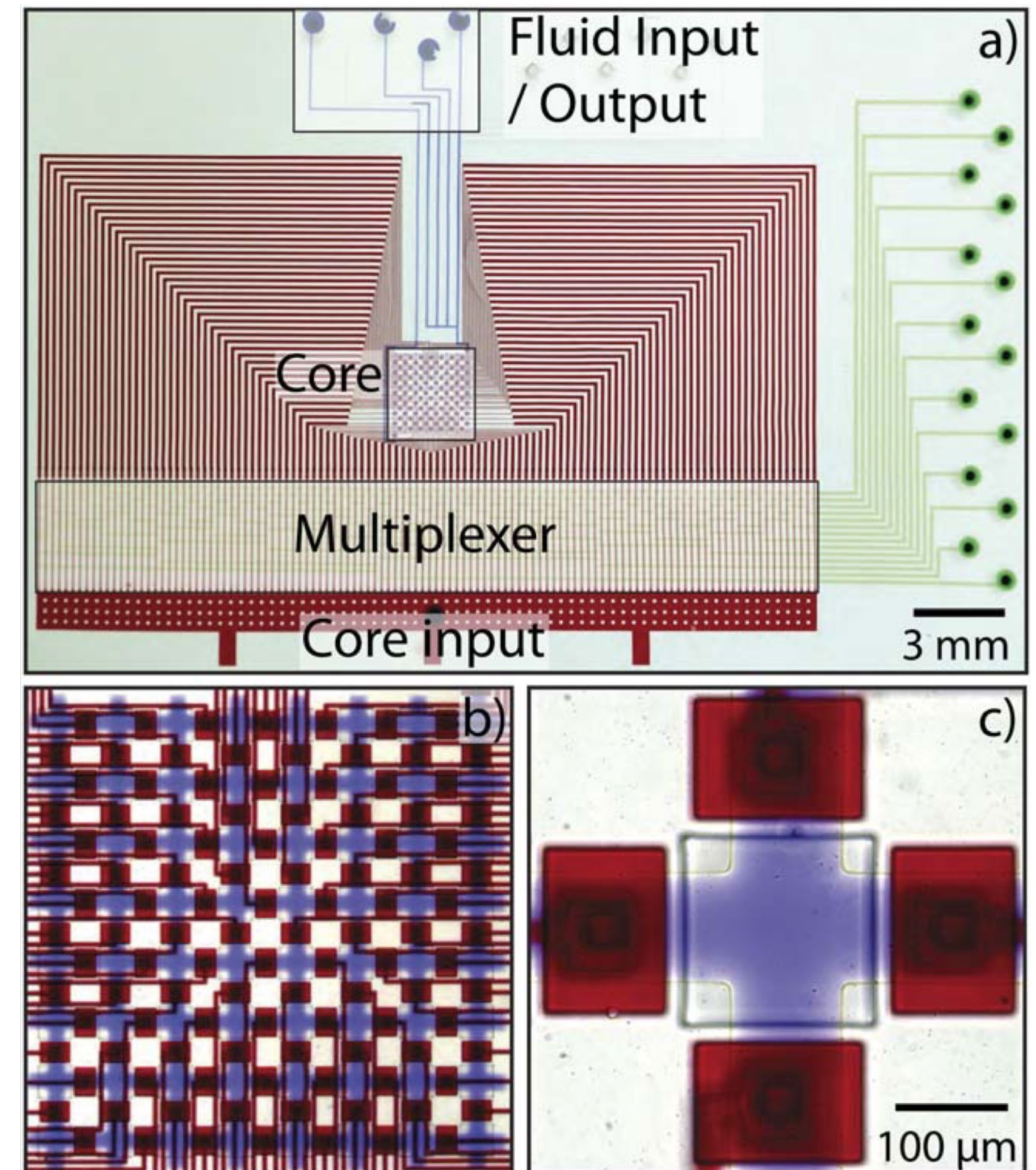


Free-standing Membranes (MITOMI Button)

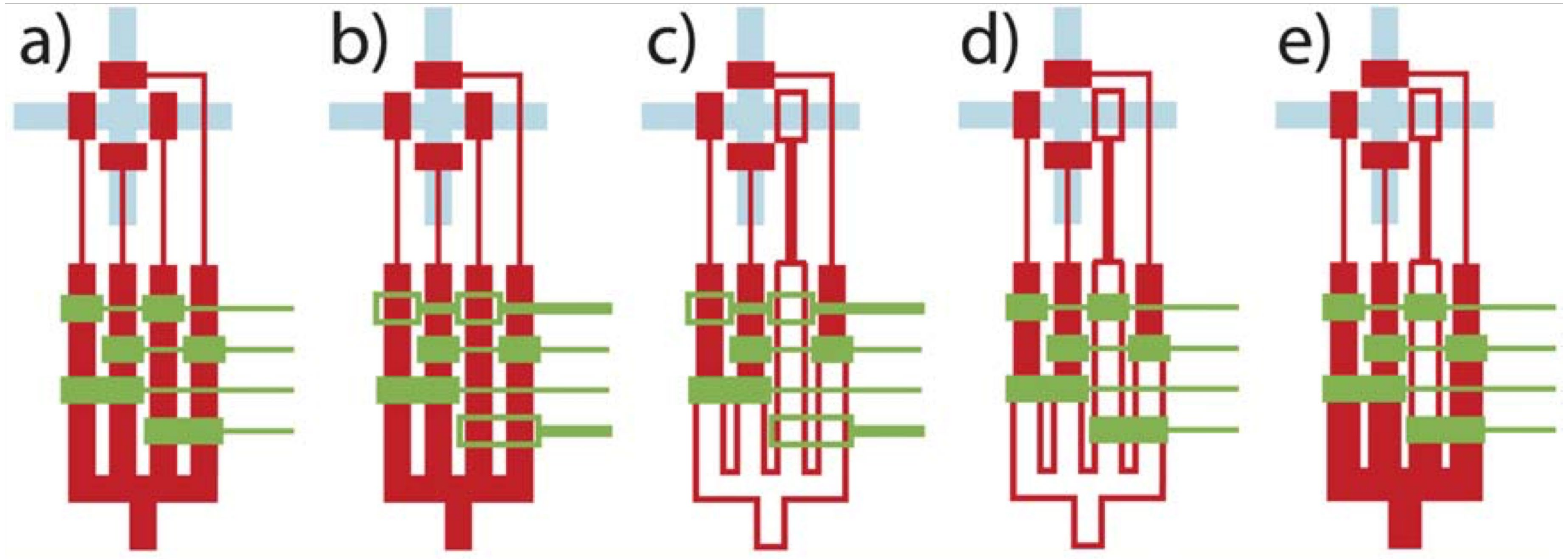


A software reconfigurable device (FPGA)

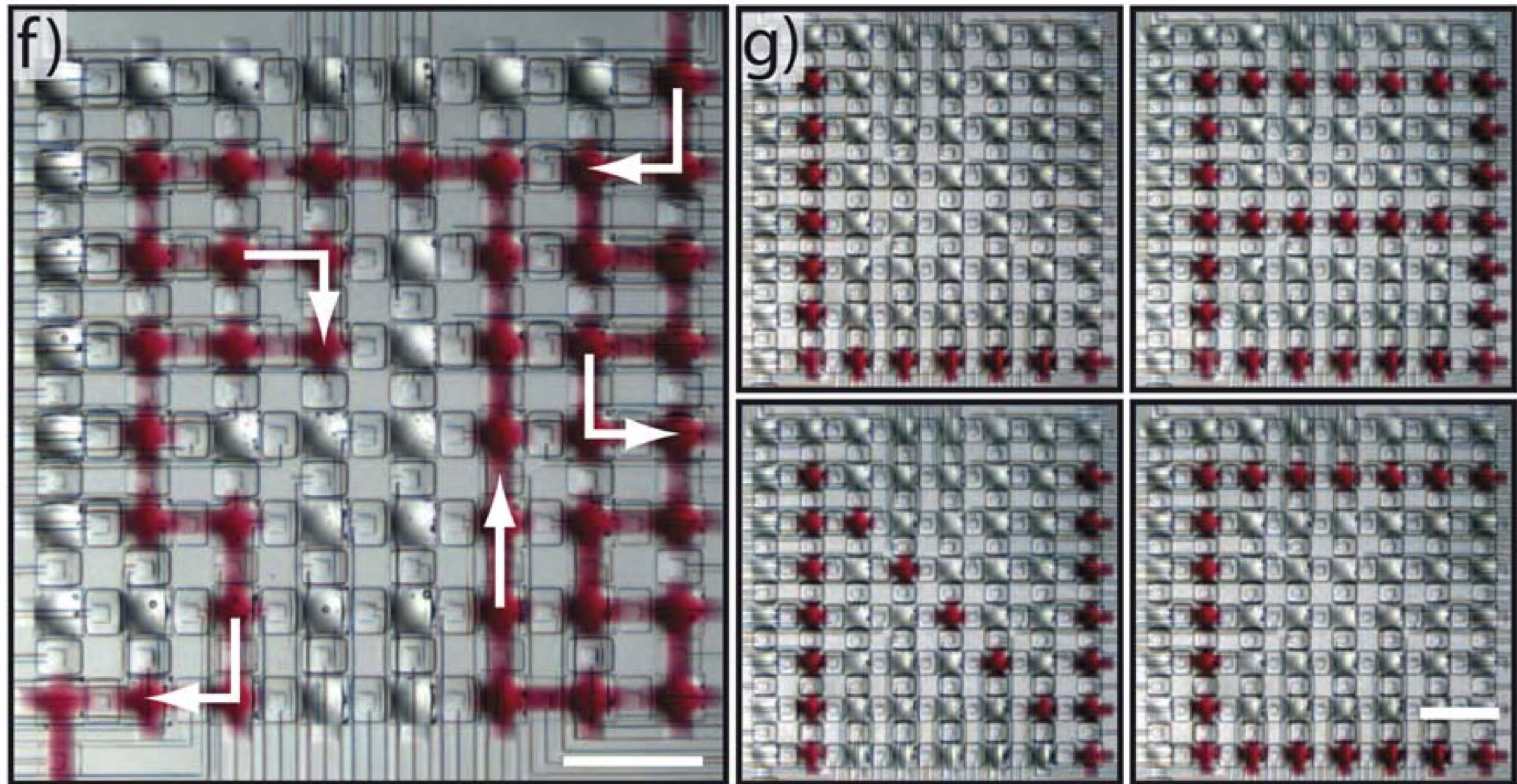
- Software programmable device architecture
- Reconfiguration can be done on the fly
- Core consists of 8x8 columns / rows
- Intersections are controlled by 114 individually controllable valves



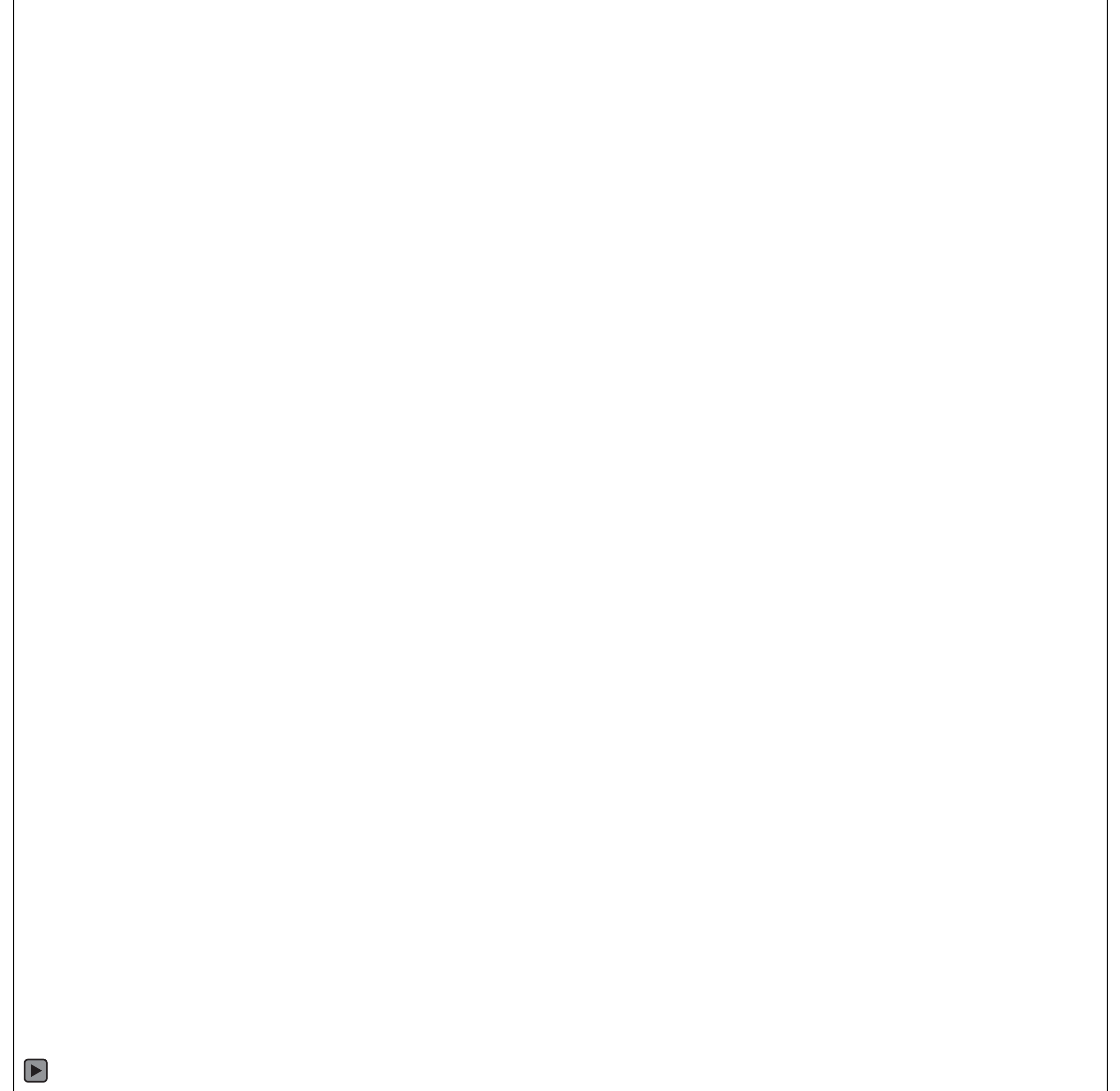
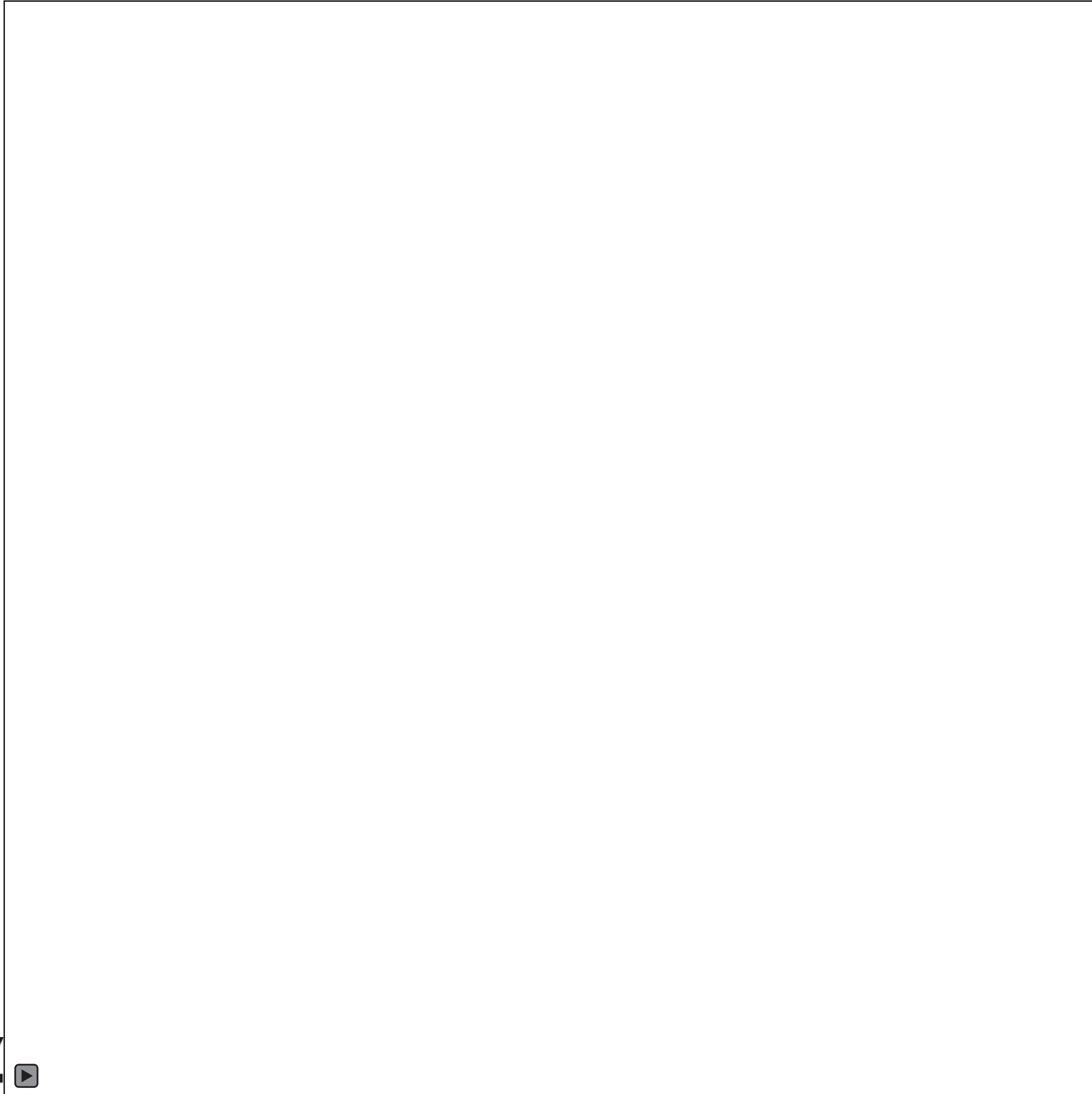
Multiplexor Control Scheme



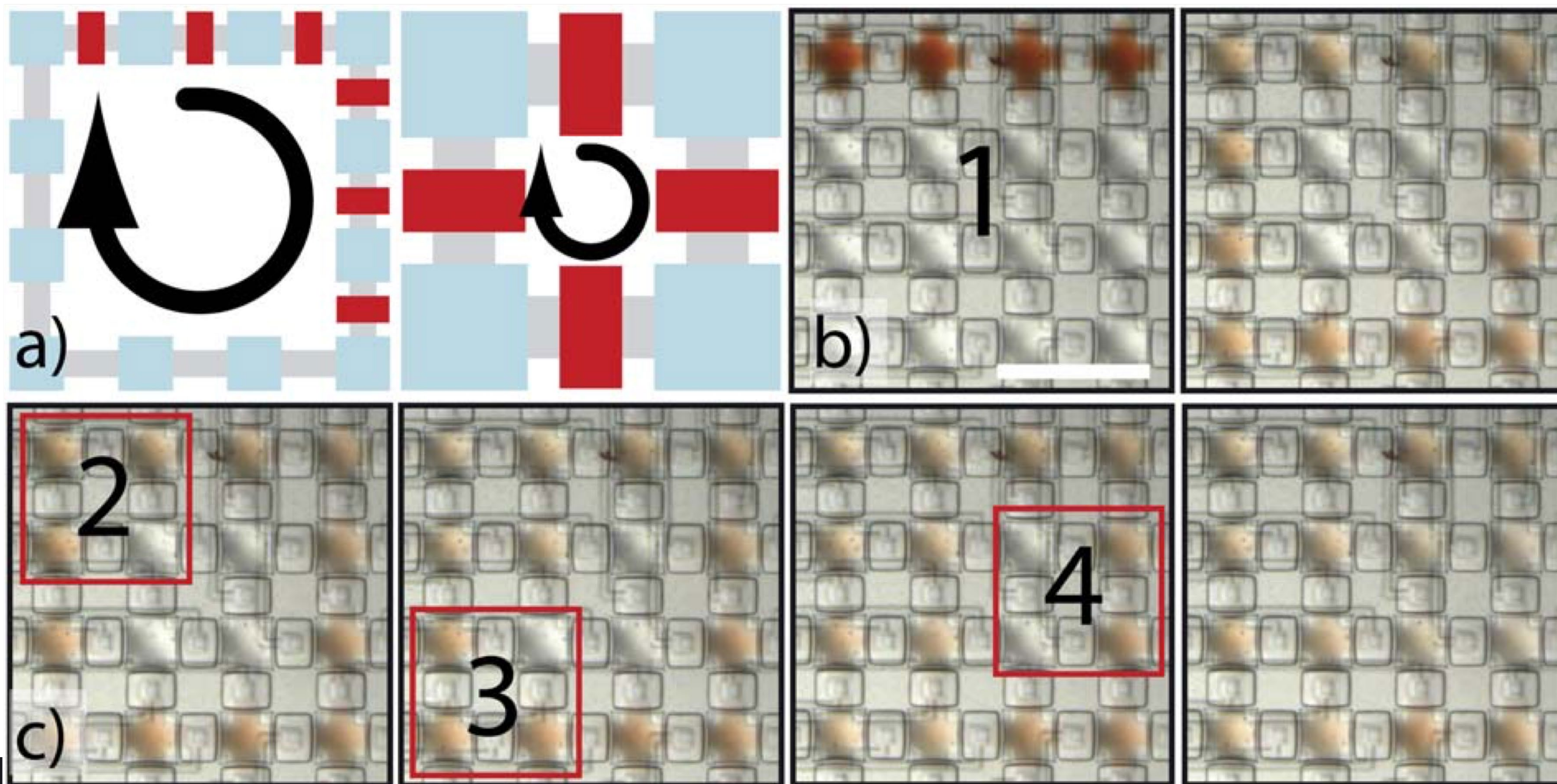
Microfluidic State Reconfiguration



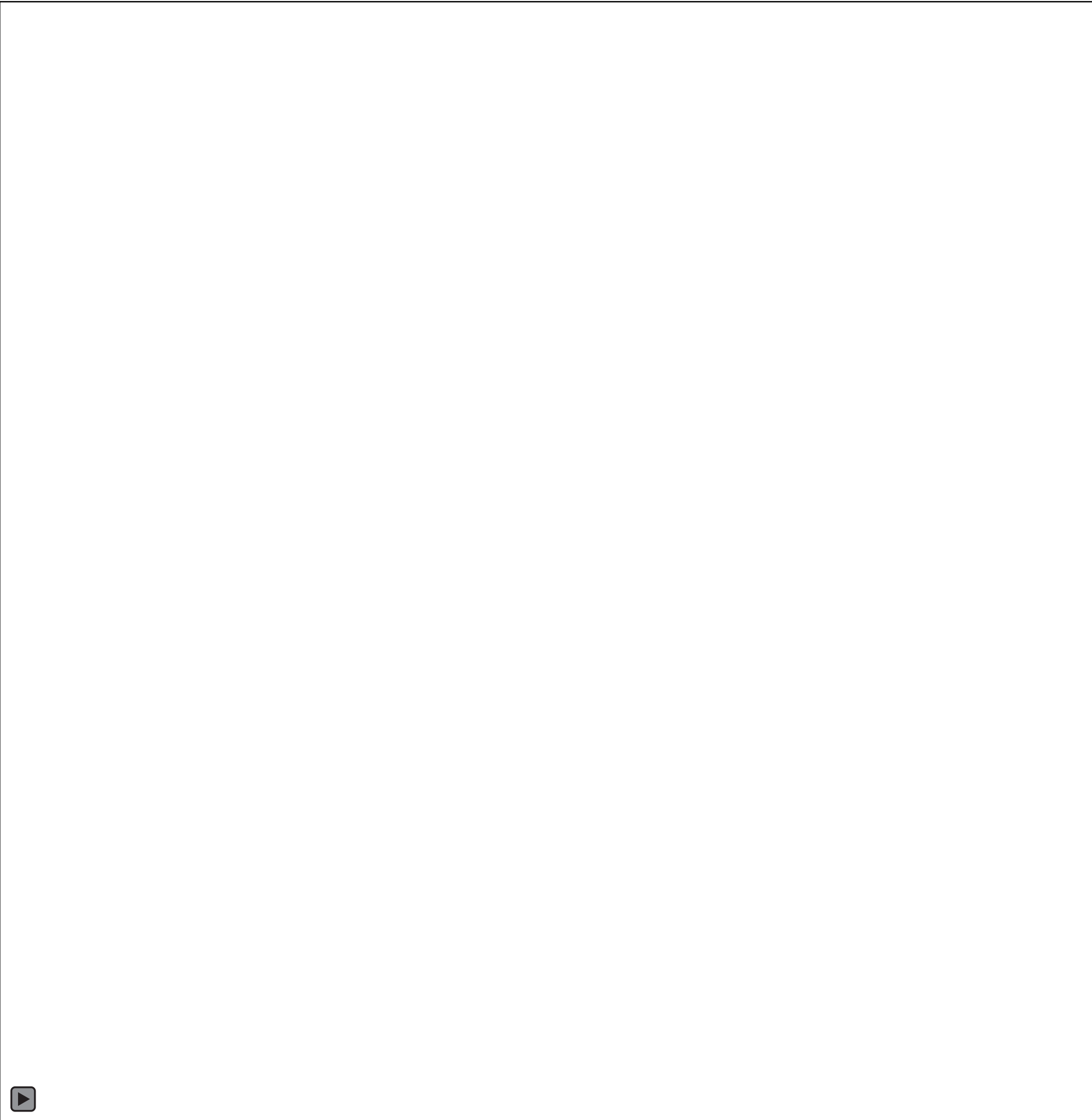
Writing Paths and Liquid Displays



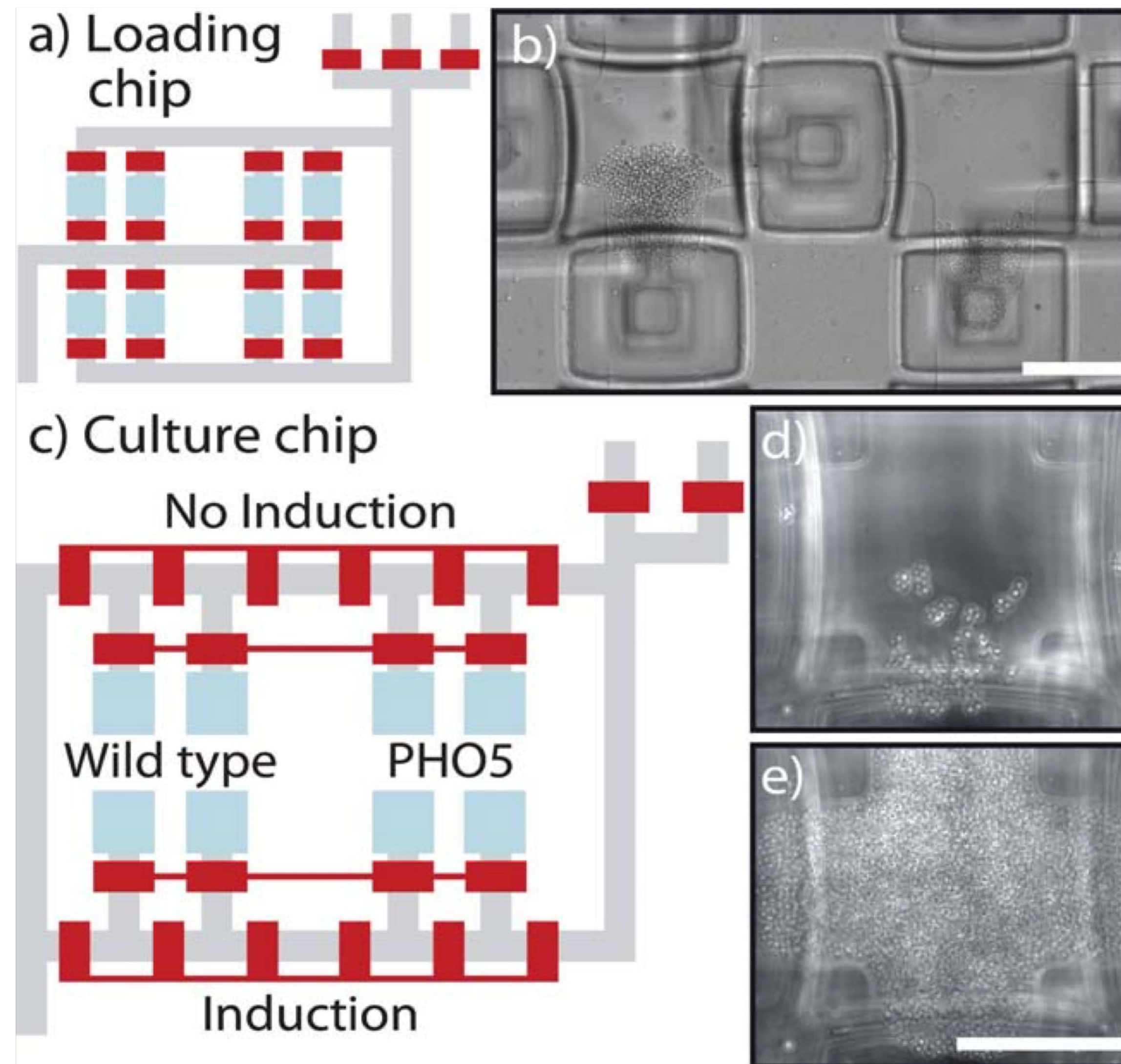
Mixing and Metering



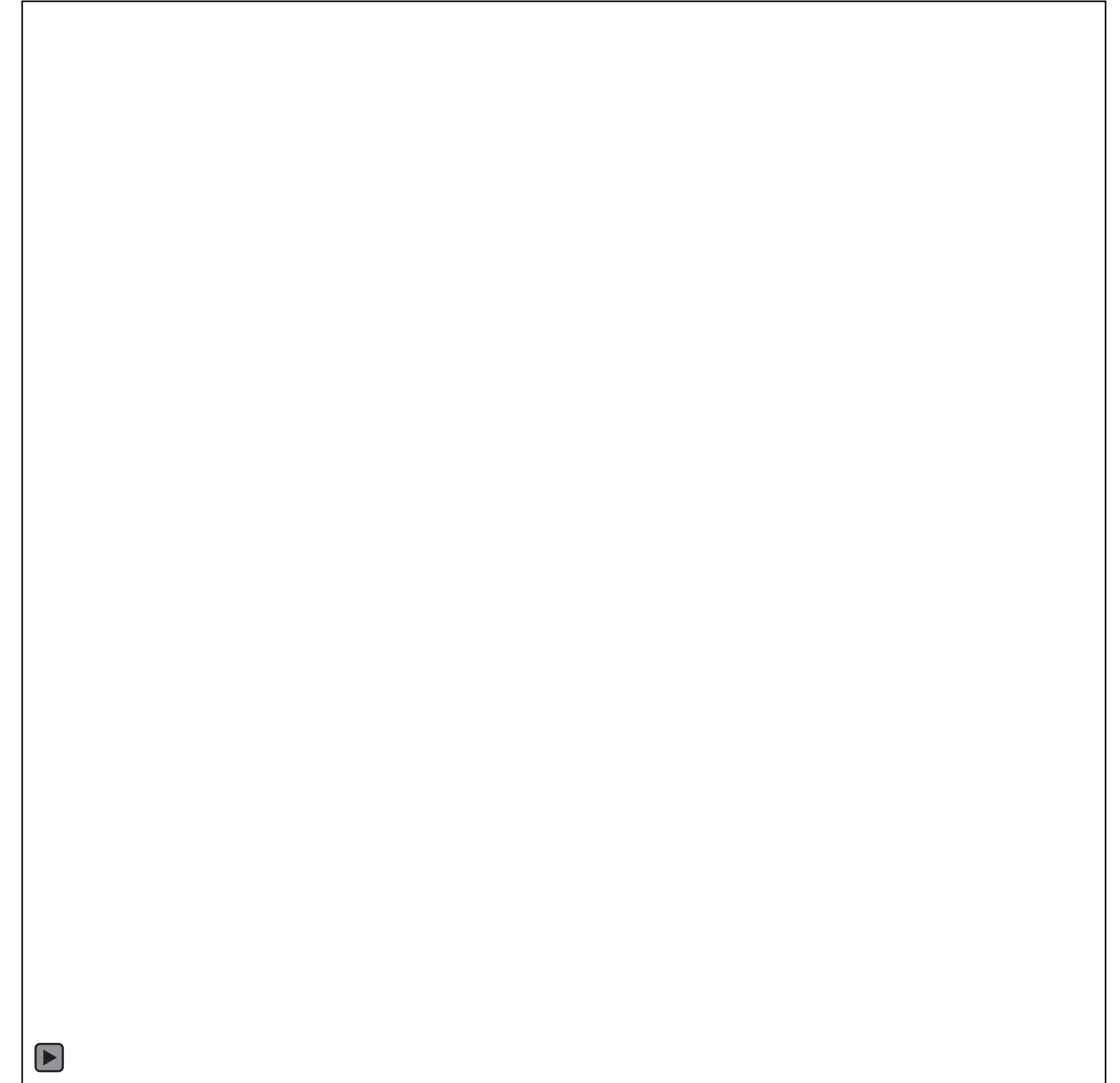
Mixing and Metering



Application Example: Cell Culturing



Cell-Loading and Culturing

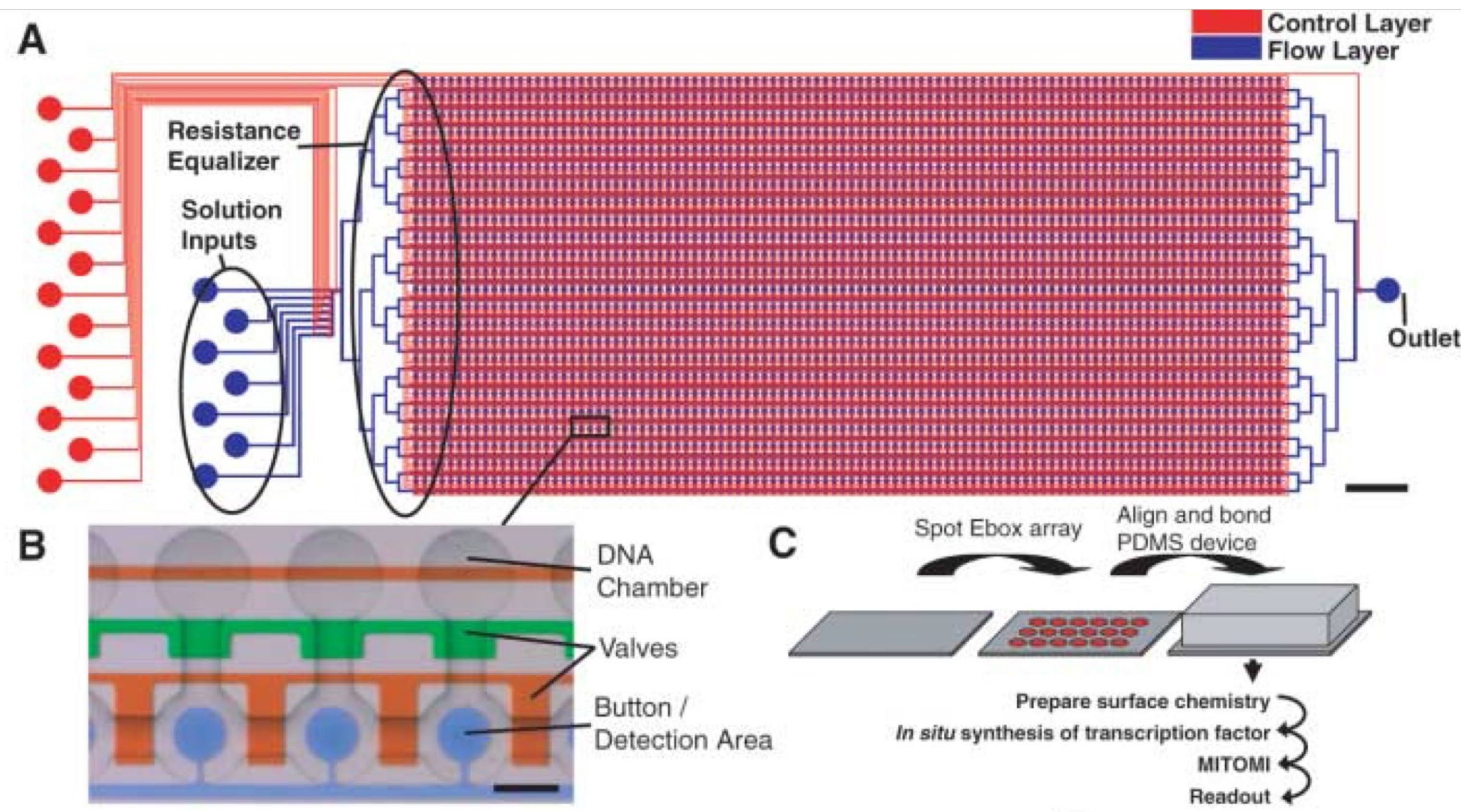


Protein Biochemistry

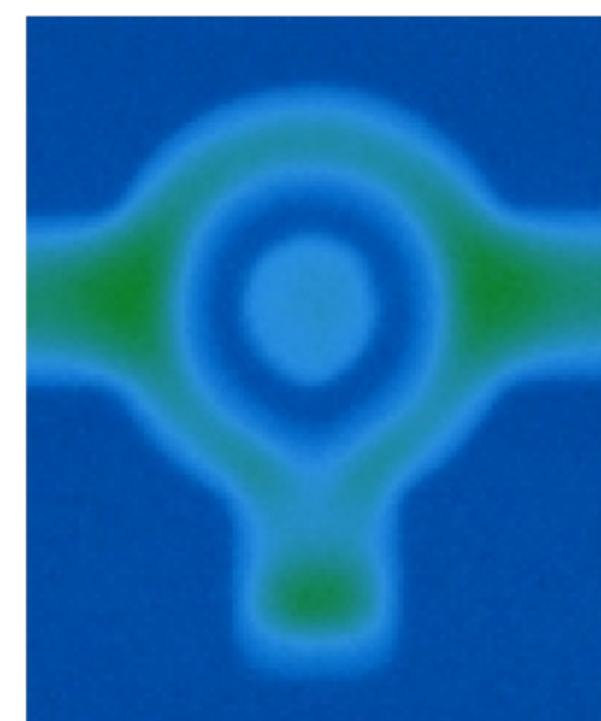
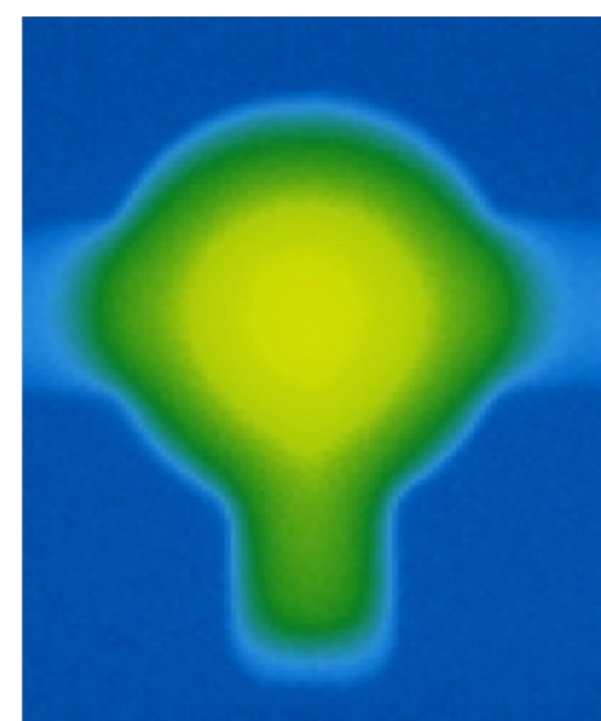
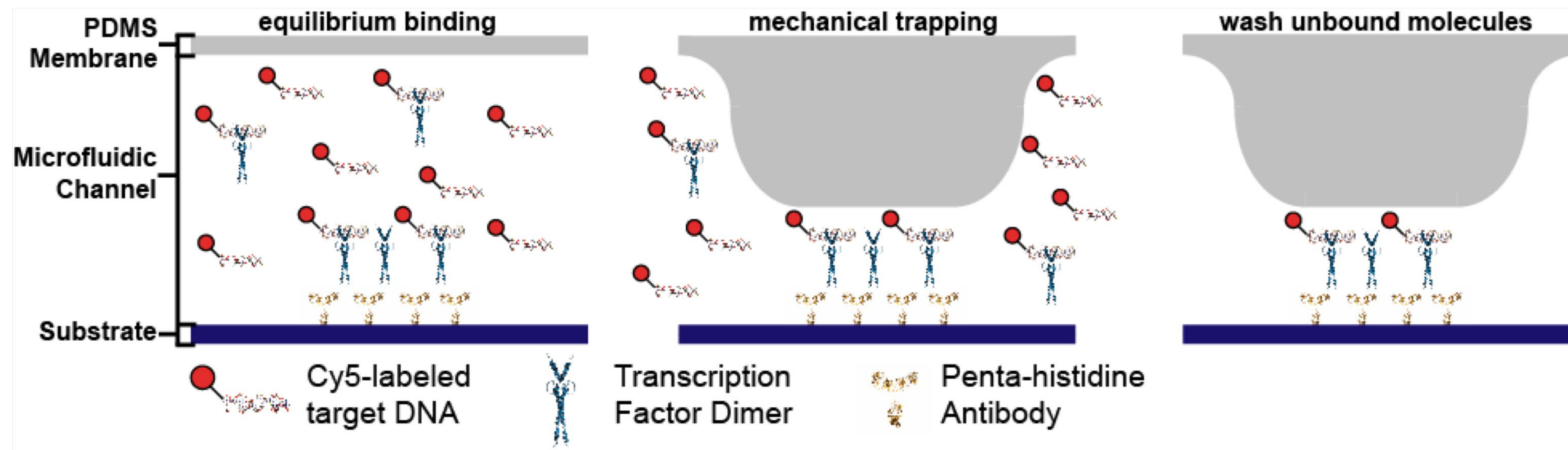
Mechanically Induced Trapping of Molecular Interactions

MITOMI Devices

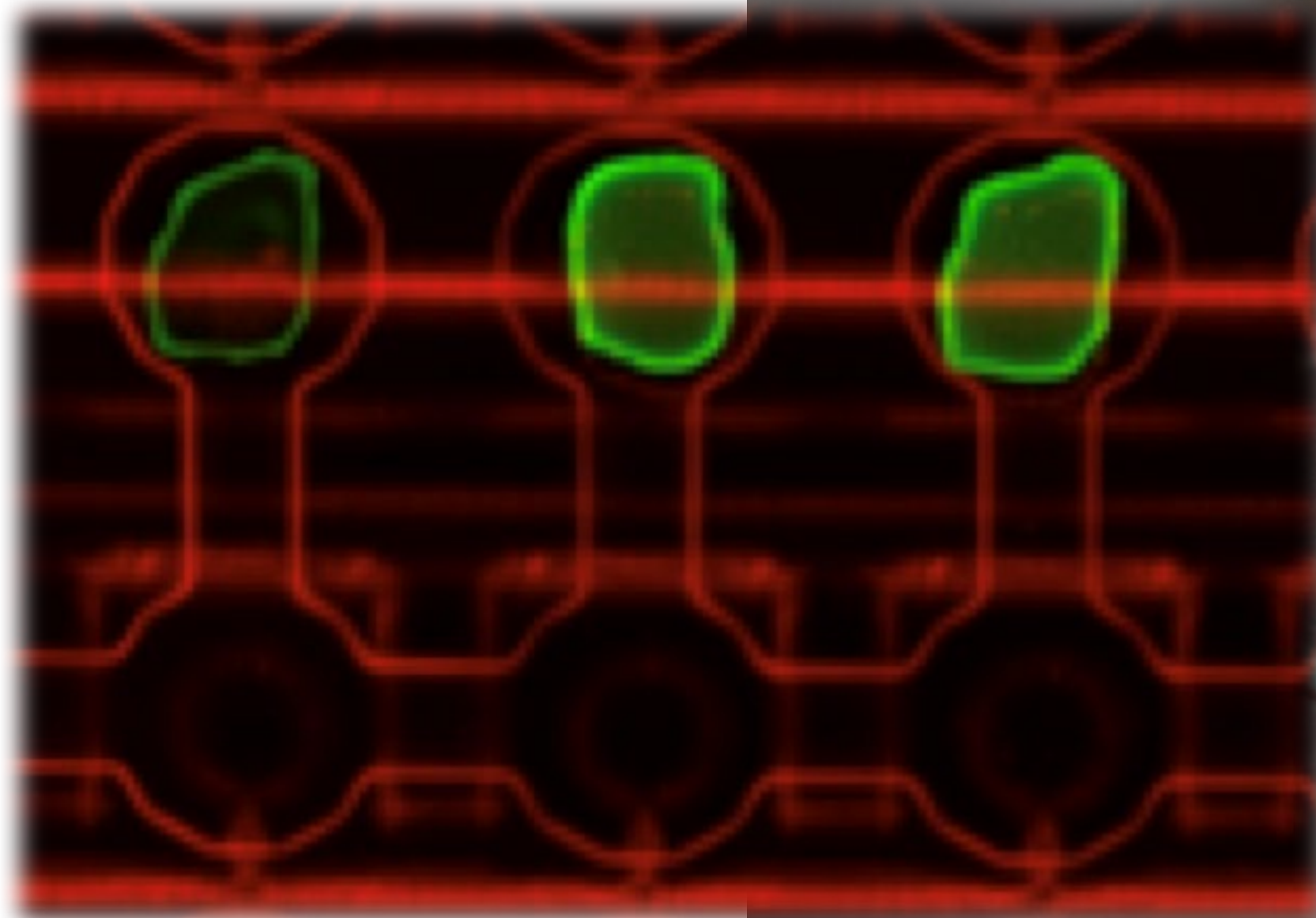
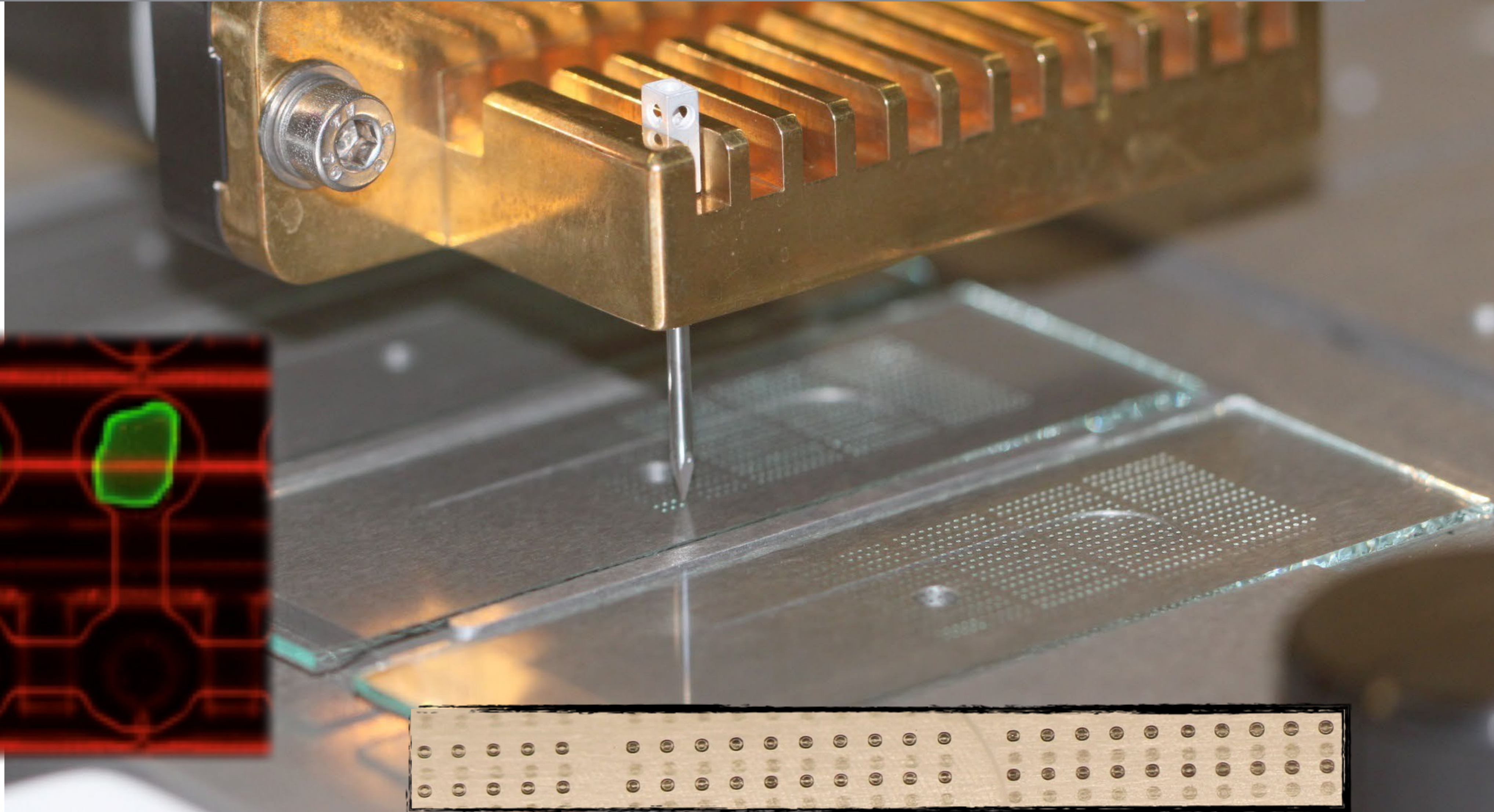
- 768 - 4096 experiments per device
- new detection mechanism (MITOMI)
- integrated protein expression, purification and characterization



Mechanically Induced Trapping of Molecular Interactions (MITOMI)



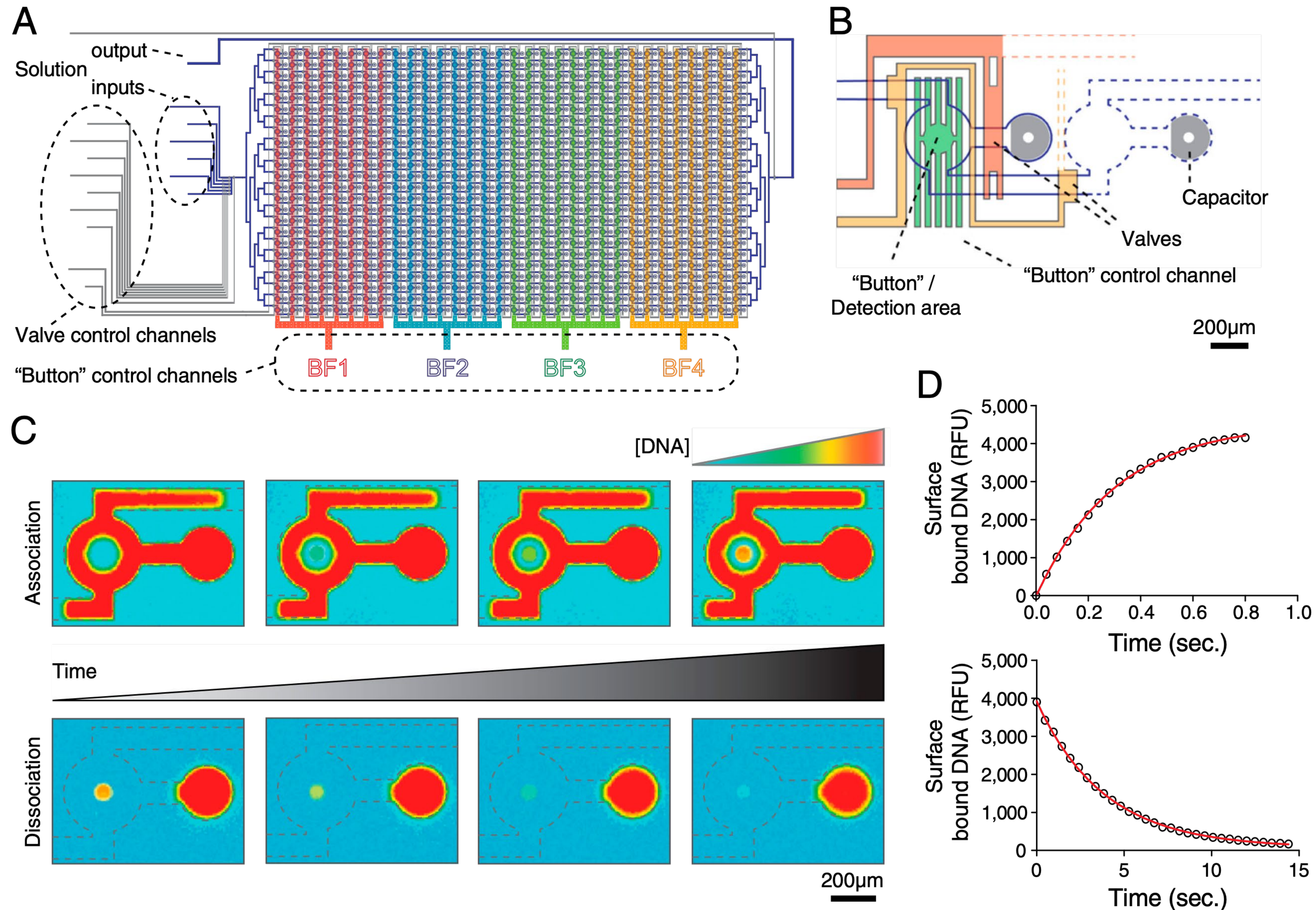
Solving the world to chip interface problem



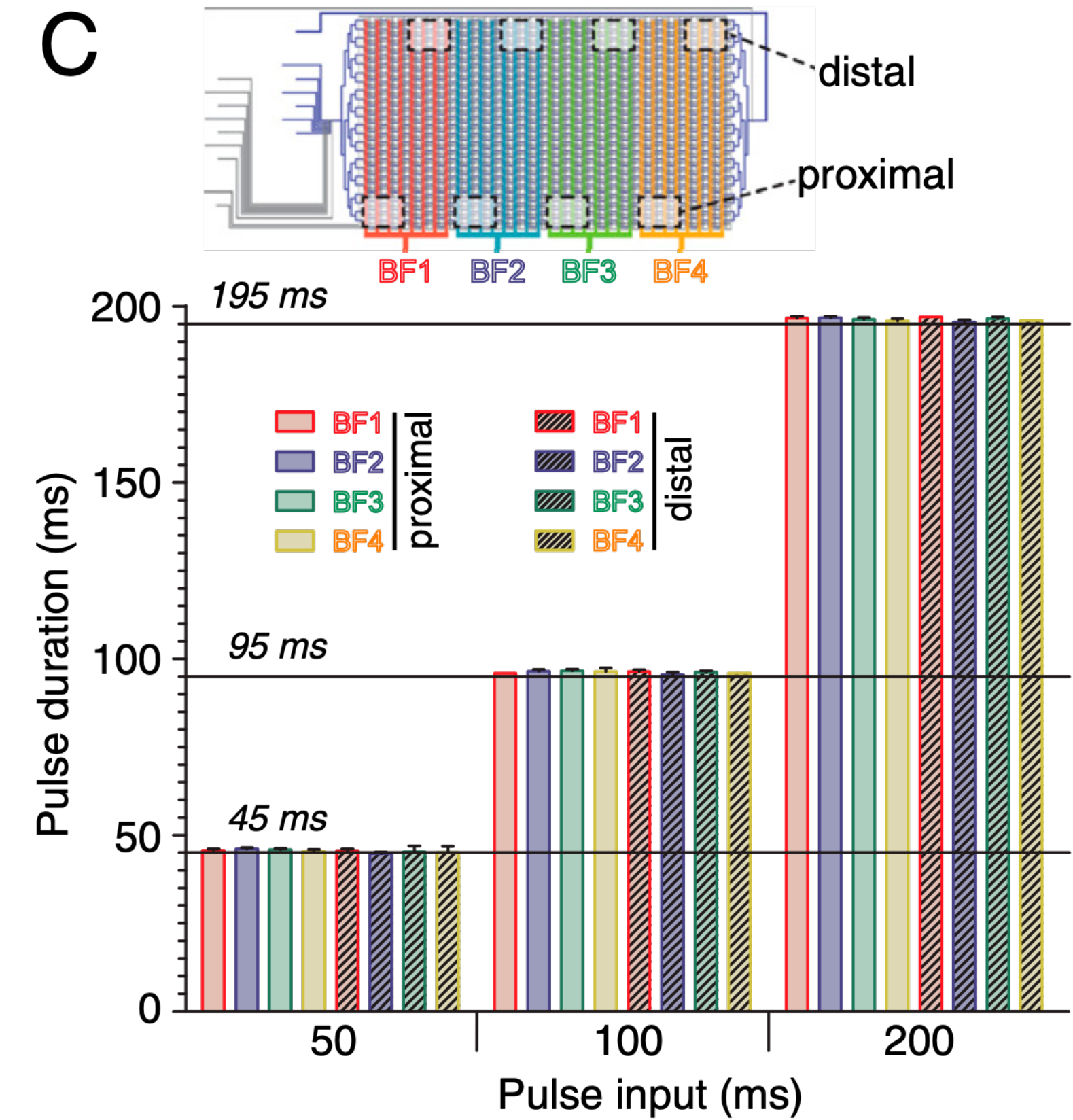
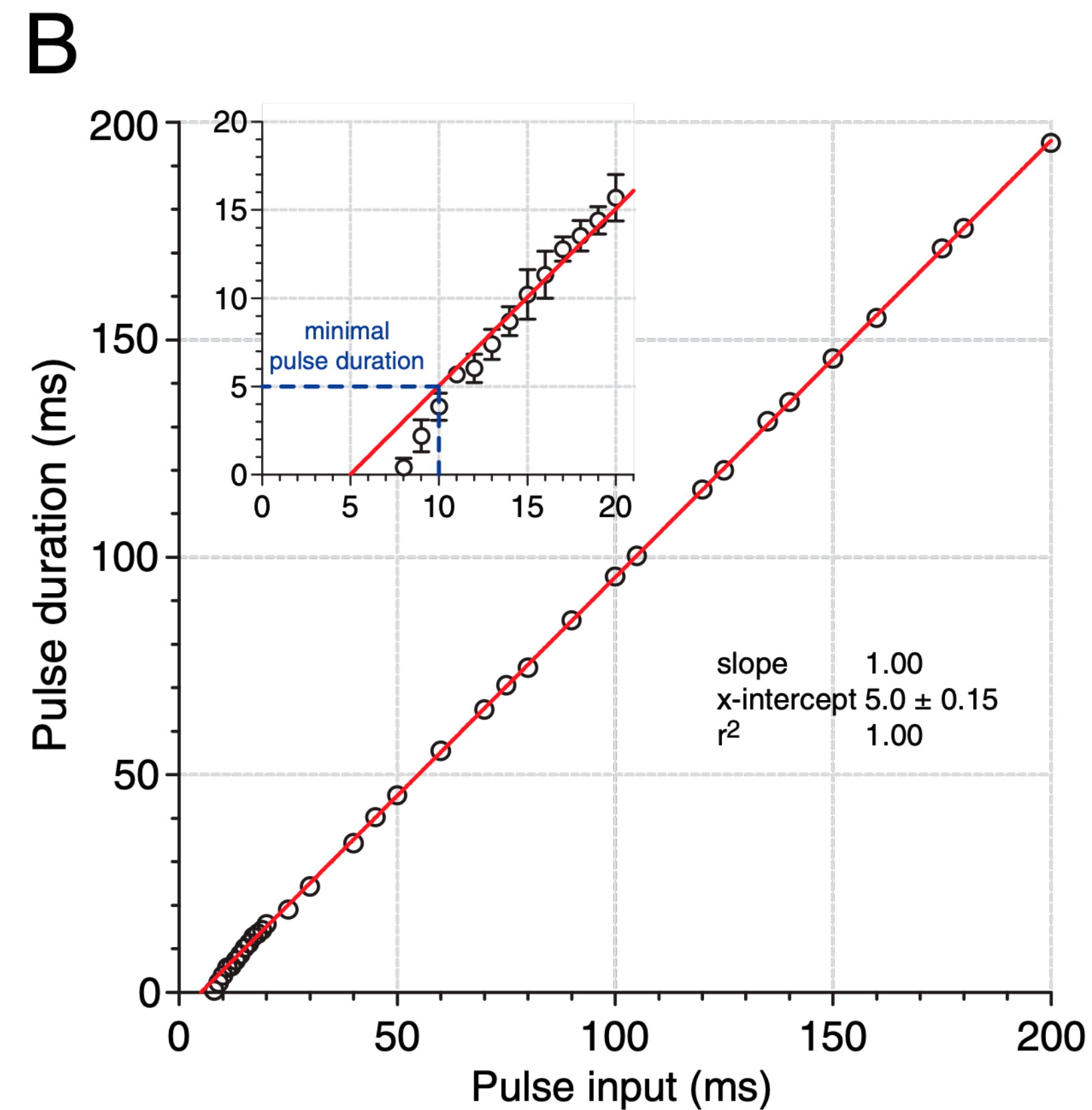
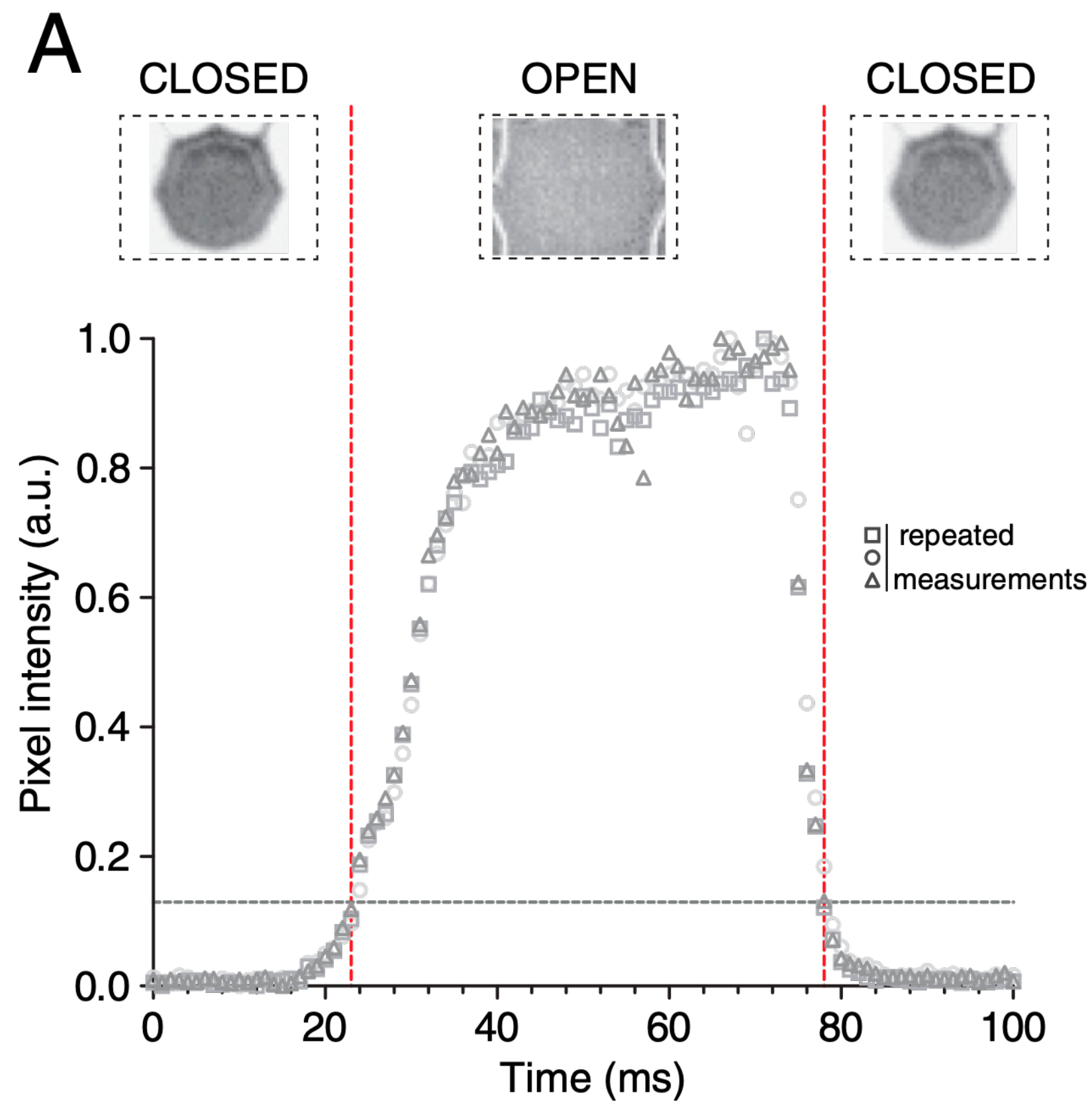
MITOMI Applications

- Protein - DNA
- Protein - RNA
- Protein - Protein
- Protein - small molecule
- HT kinetics
- Immunoassays
- Protein Engineering

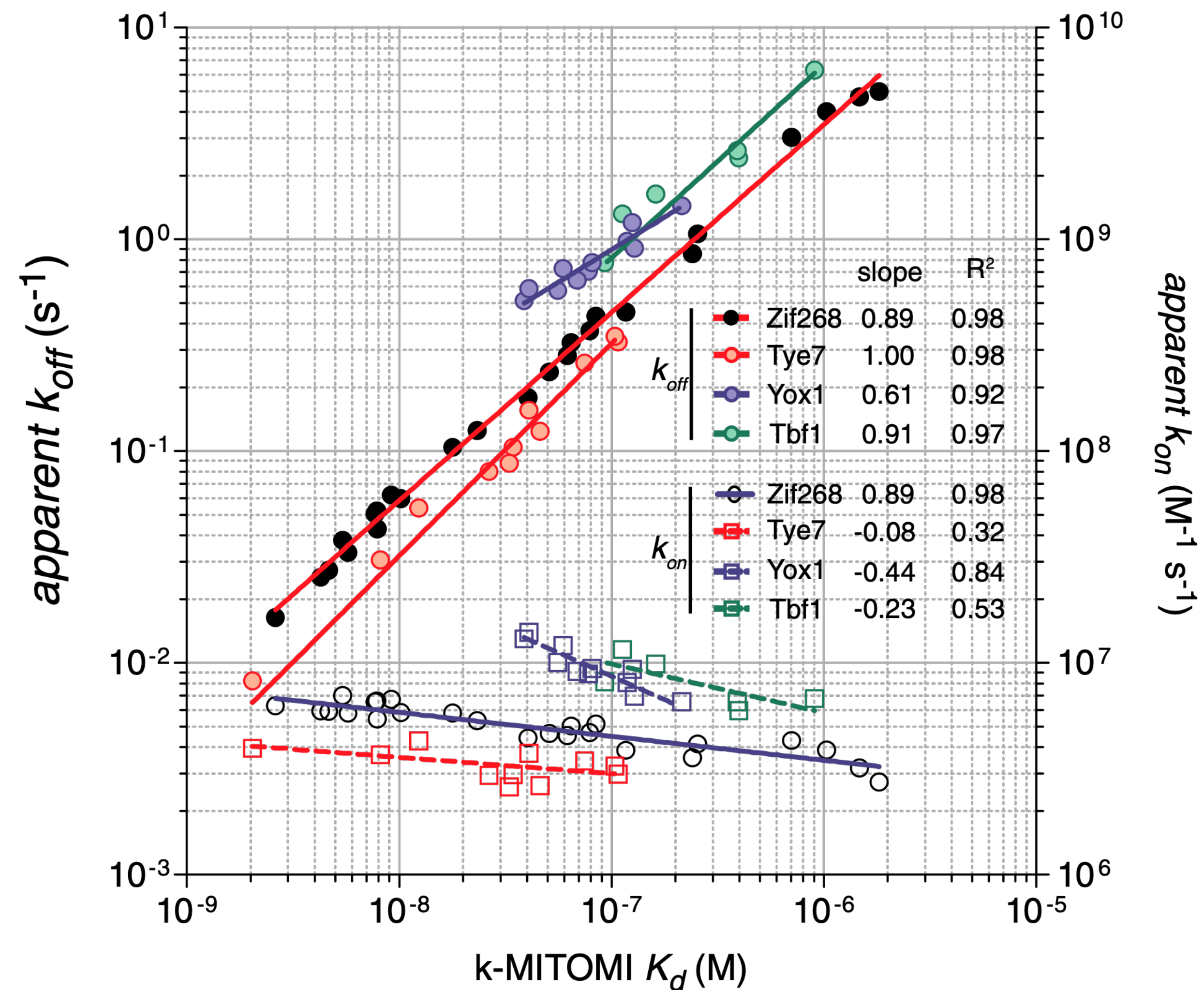
Kinetic Rate Measurements



Rapid and Uniform Button Actuation

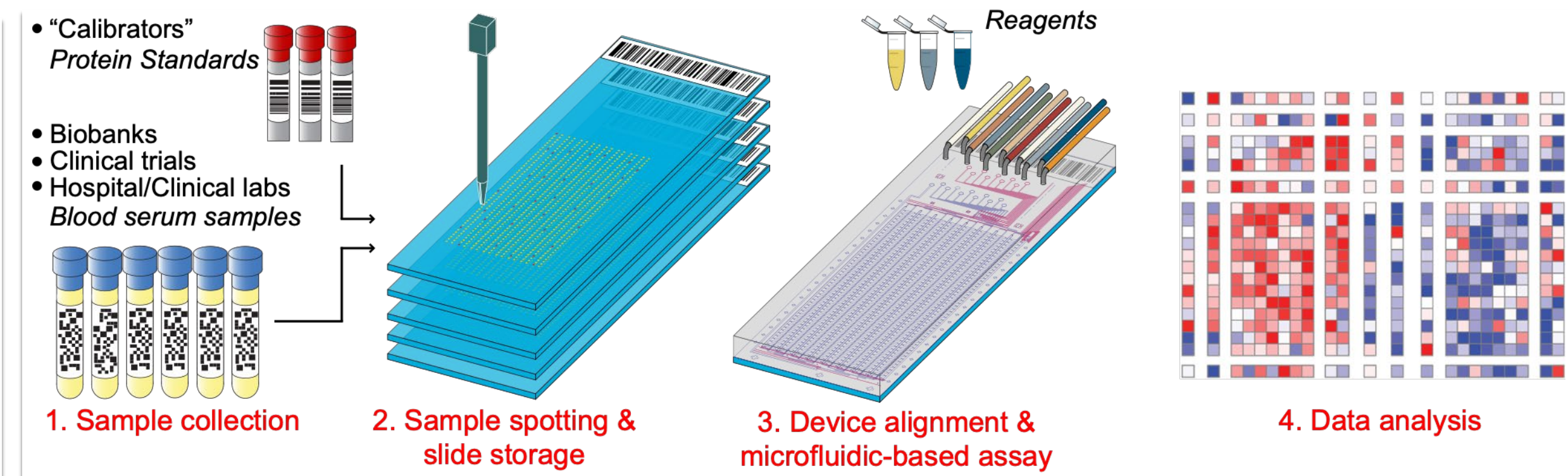
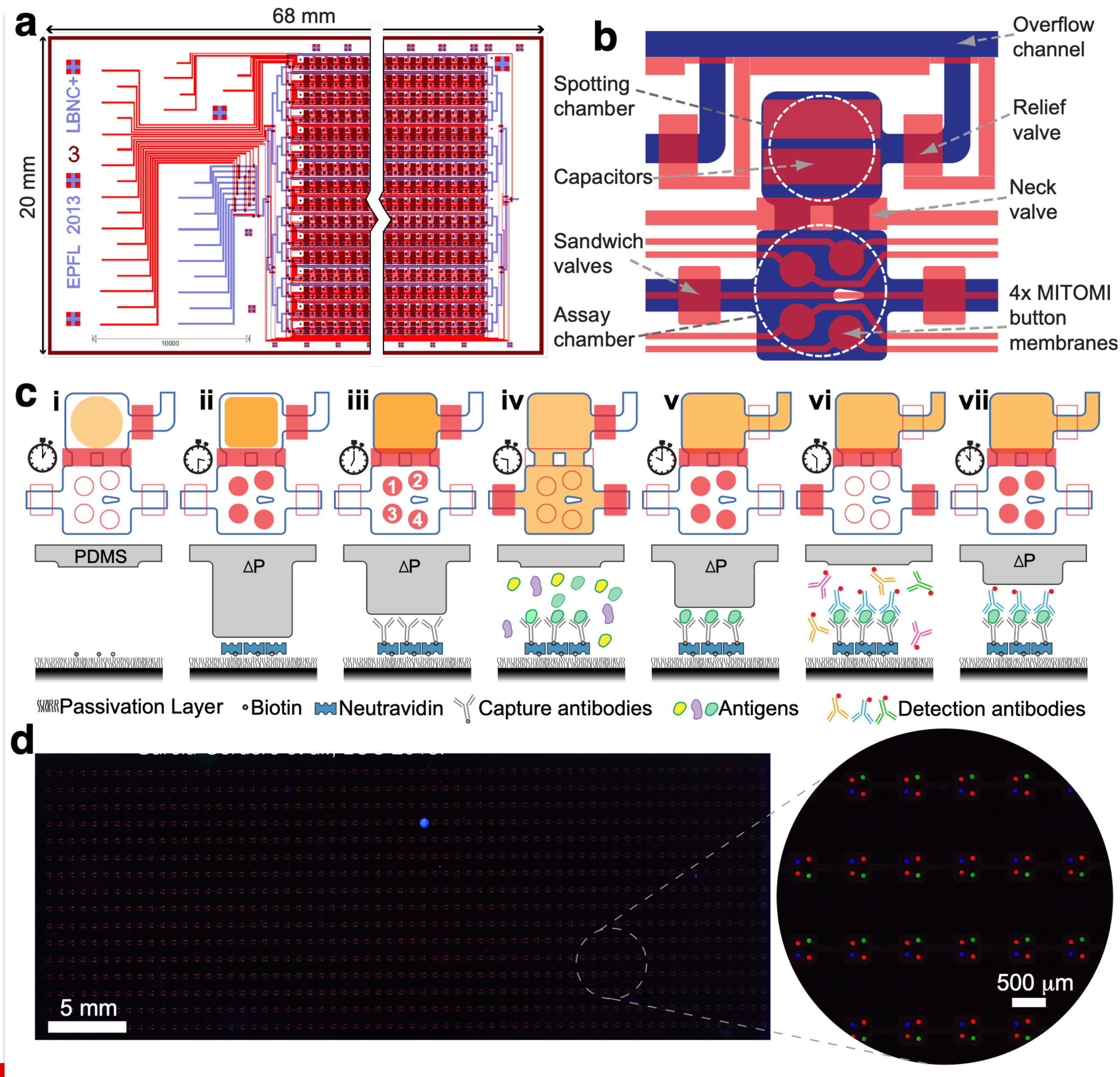


Transcription Factor Kinetics



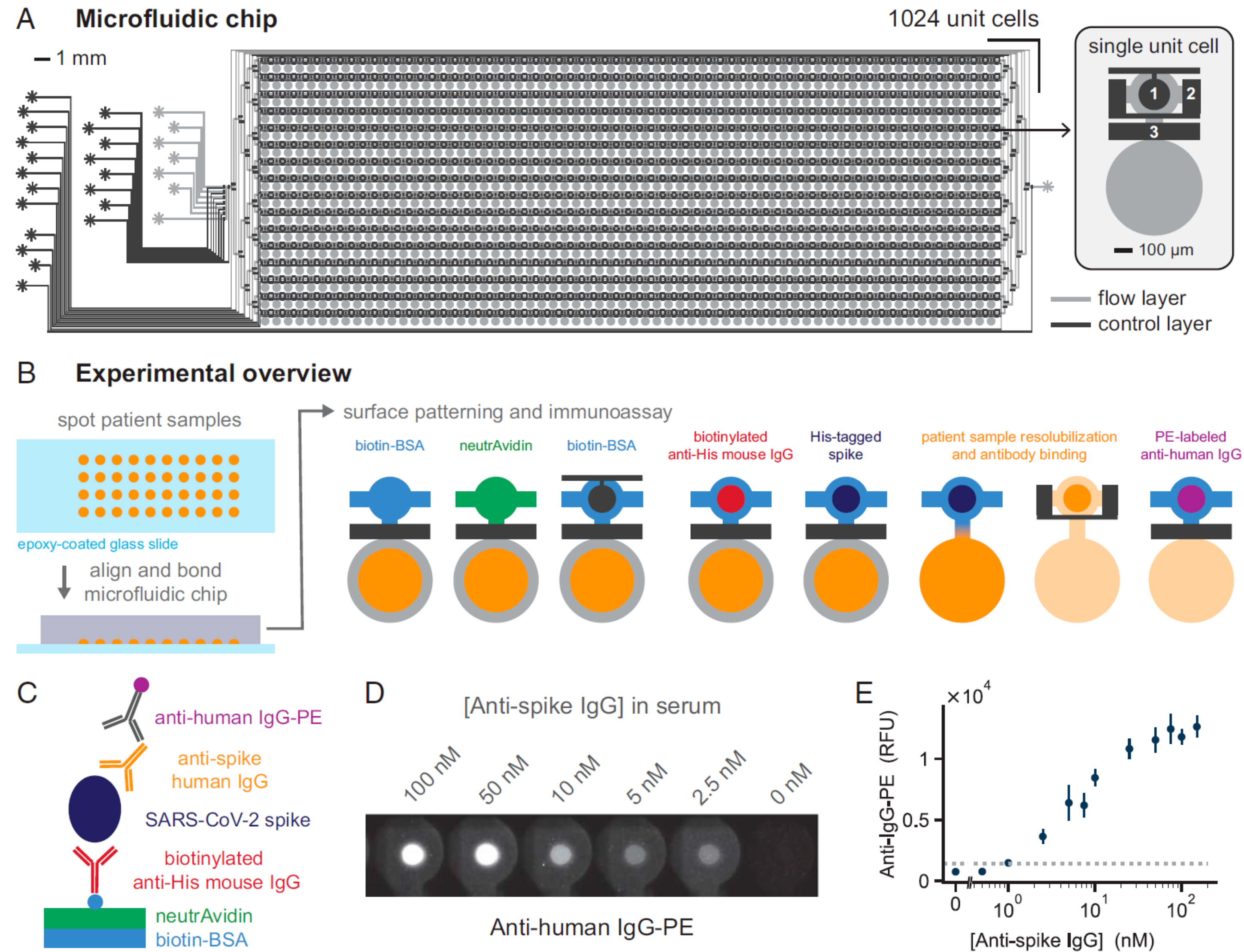
Molecular Diagnostics

4'096 Immunoassays on a Single Device

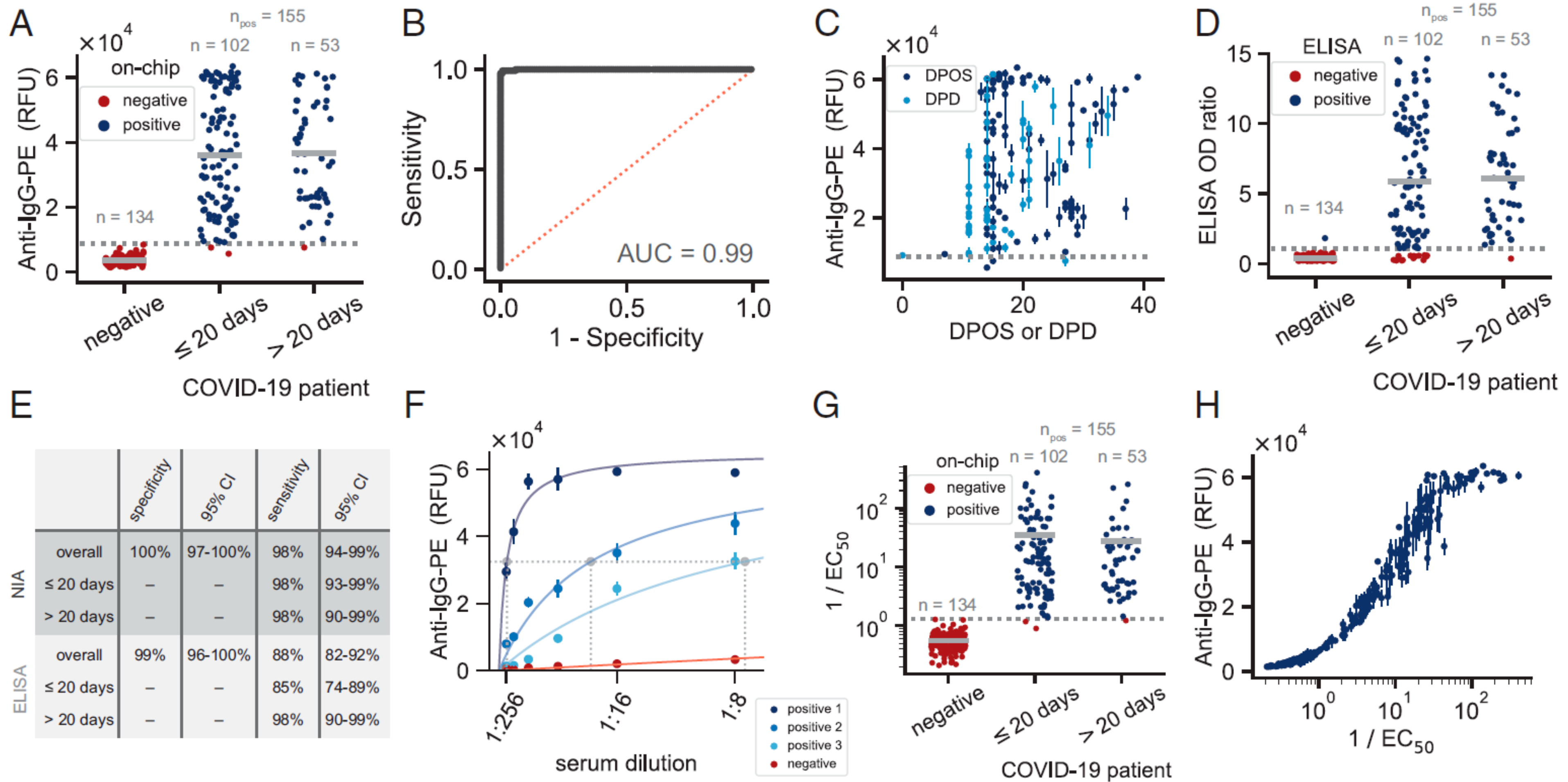


- **1,024** samples on **10 cm²**
- Multiplexed - **4** biomarkers per sample
- **4,096** assays per device
- Sensitivity - **100's fM** from **nL-volume samples (zmol)**
- Matrix insensitive - **human serum, cell culture media, buffers, etc.**
- Total cost of reagents per assay = **€ 0.0001**
- Total cost per chip (reagents) = **€ 0.1**

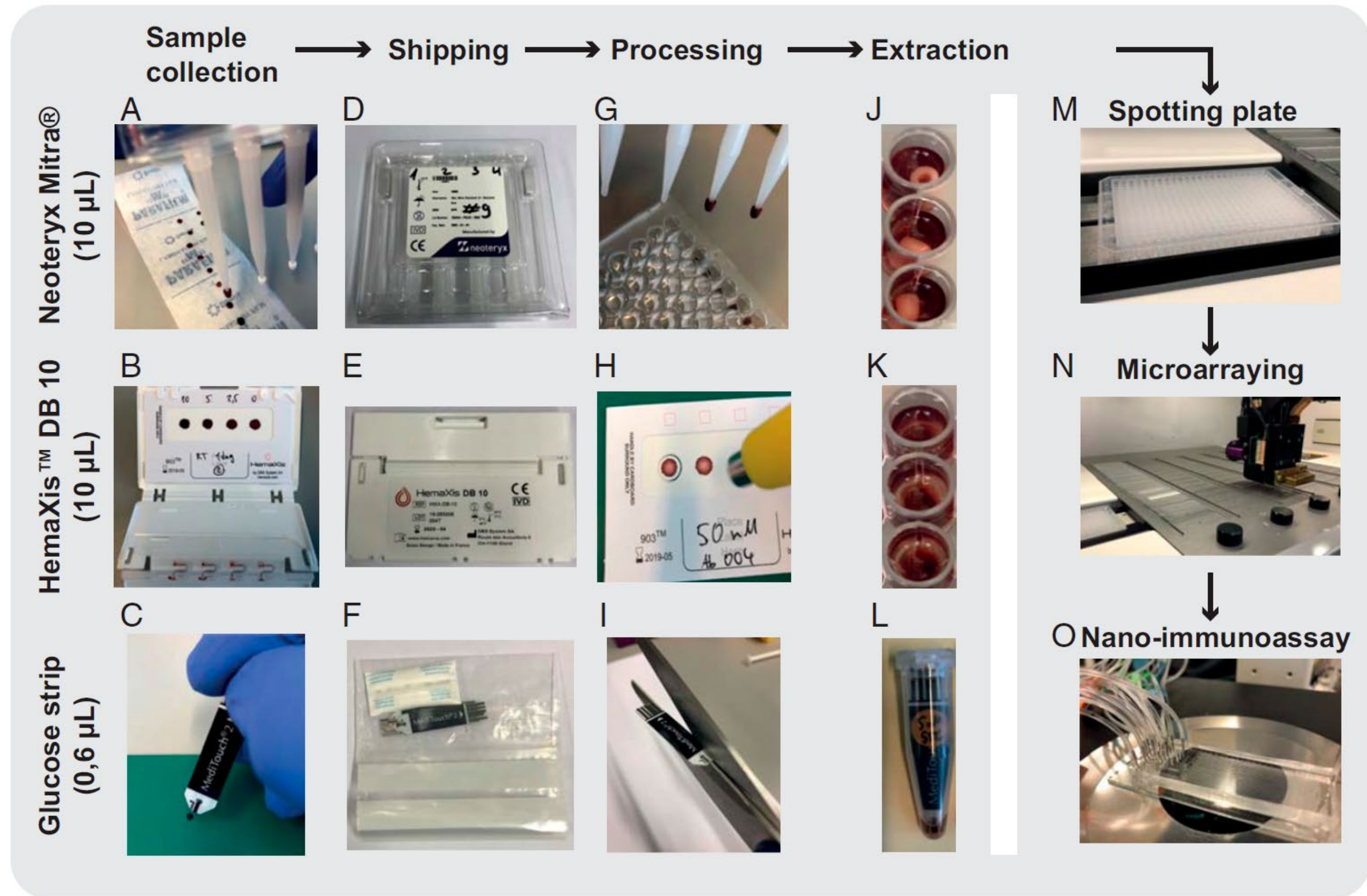
High-throughput SARS-CoV-2 Serology



High-throughput SARS-CoV-2 Serology



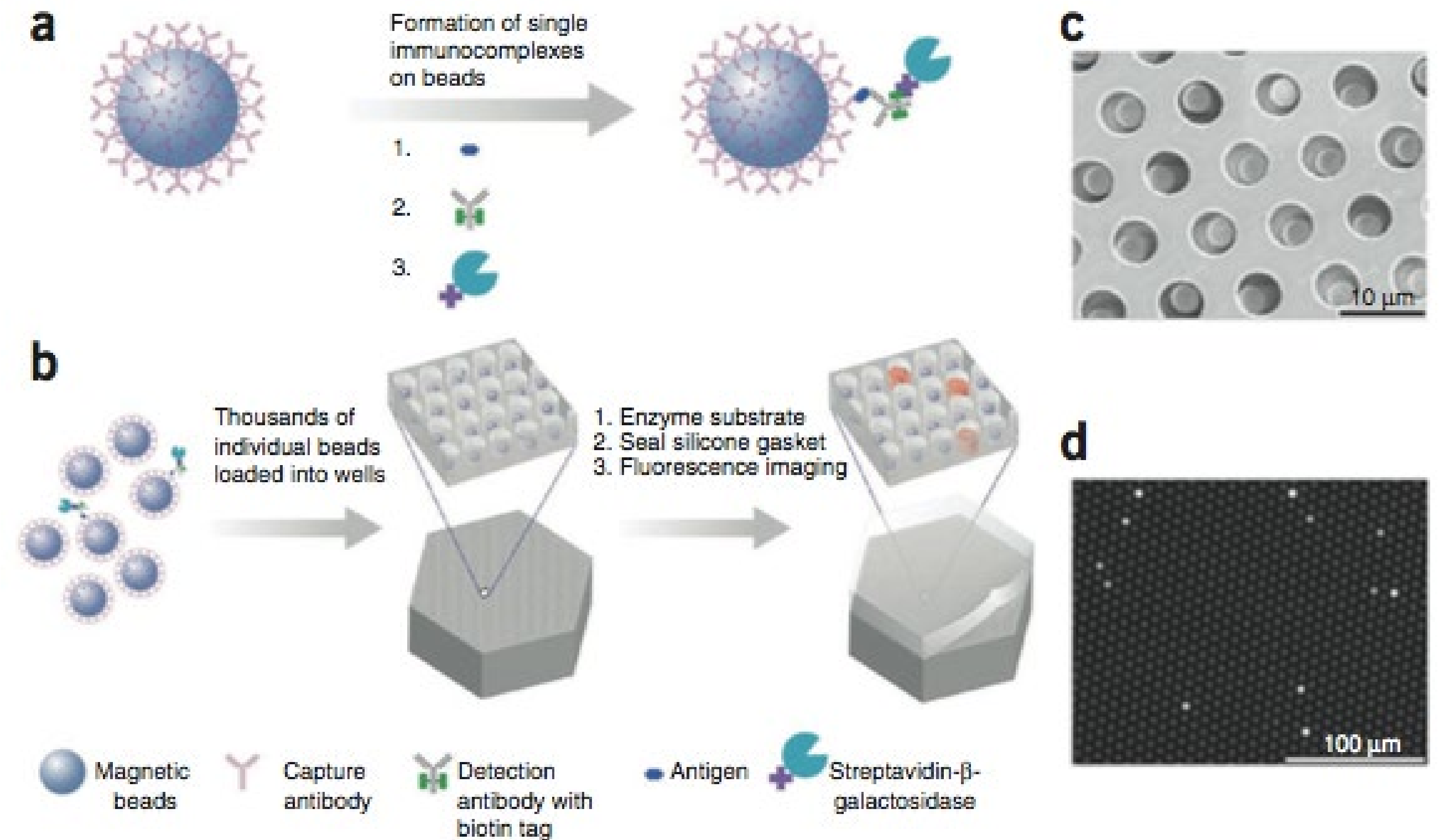
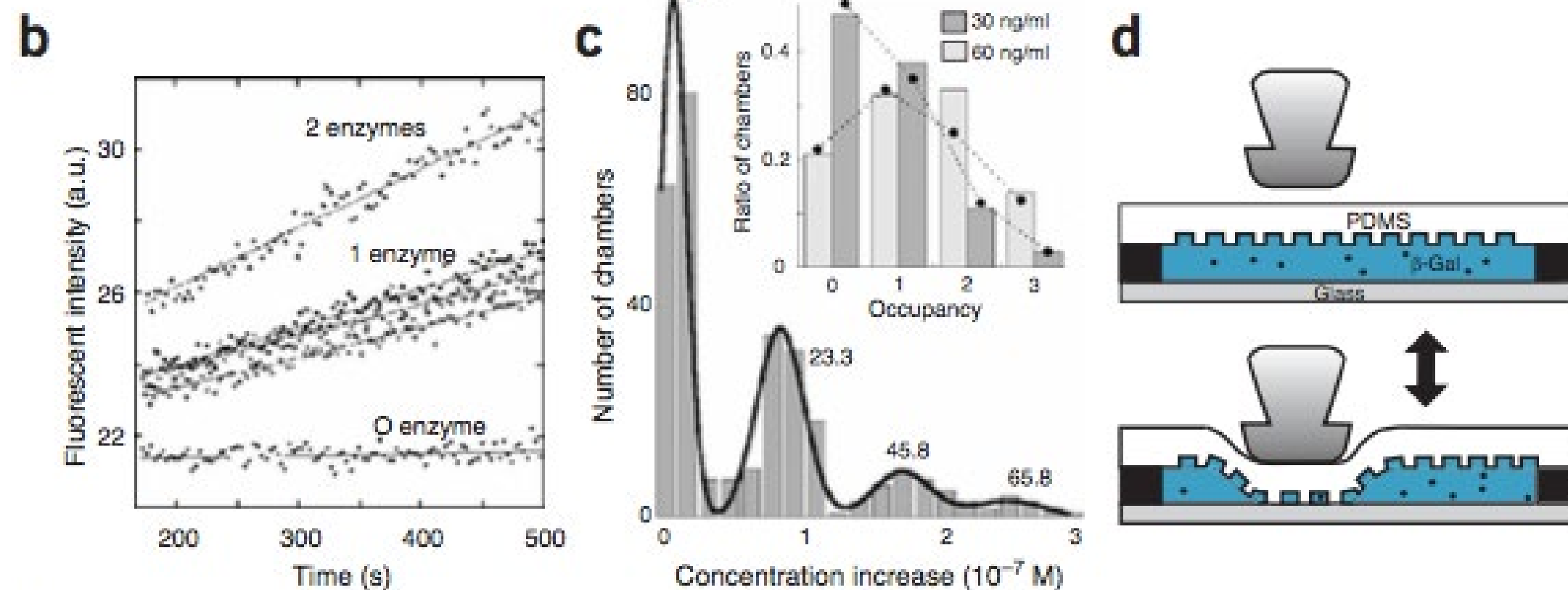
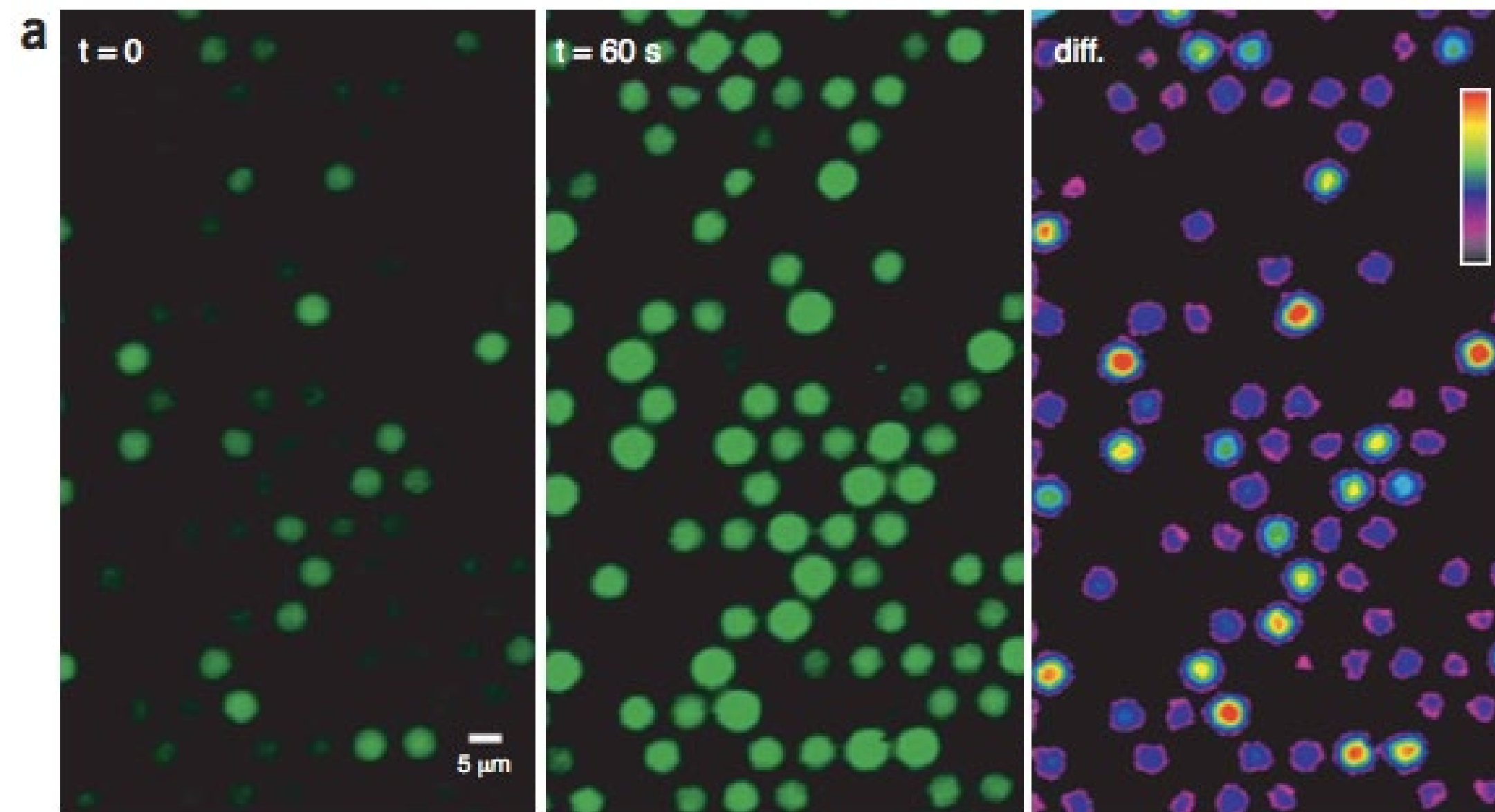
High-throughput SARS-CoV-2 Serology



How long can we go?

Concentration	# Molecules in 1uL
100 fM	60000
10 fM	6000
1 fM	600
100 aM	60
10 aM	6

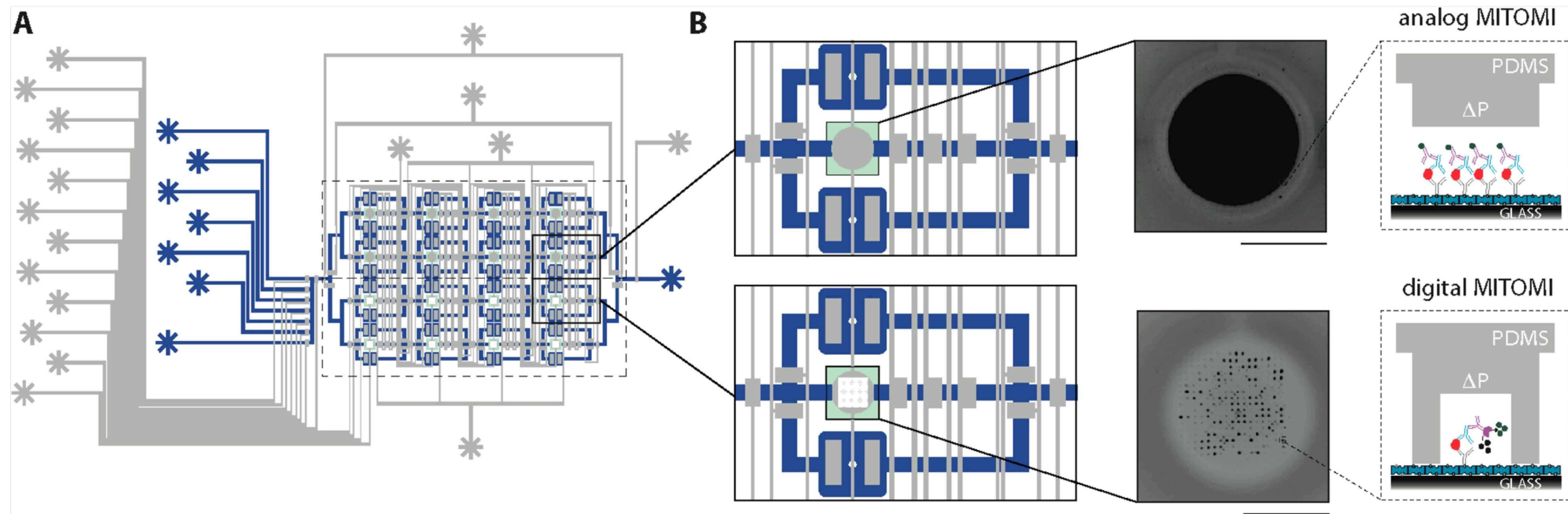
Single Molecule Detection with Digital ELISA



Rissin et al, Nature Biotechnology 2010.

LOD: 0.4 fM for PSA in serum (100uL)

Next Generation Diagnostic Device

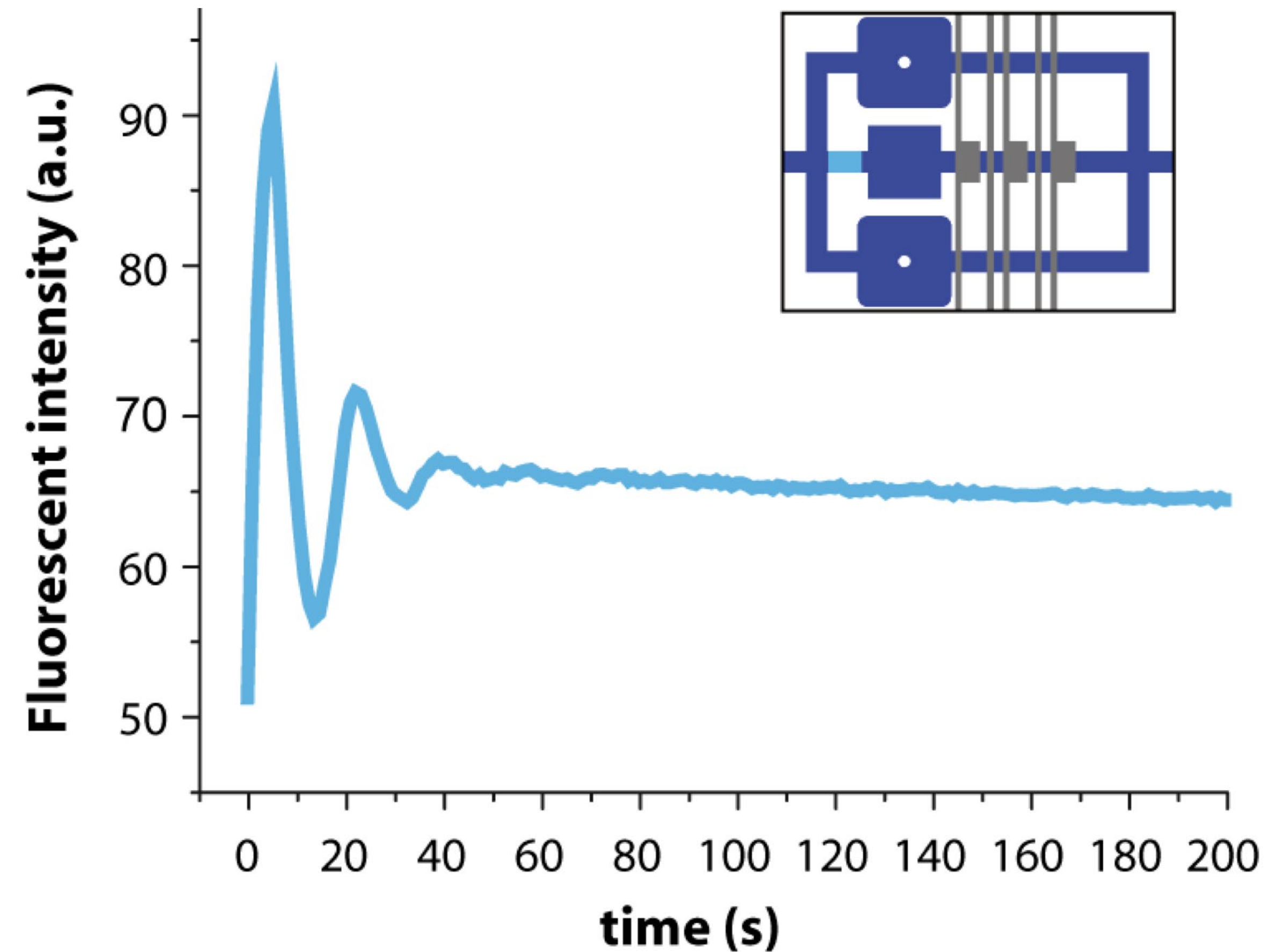
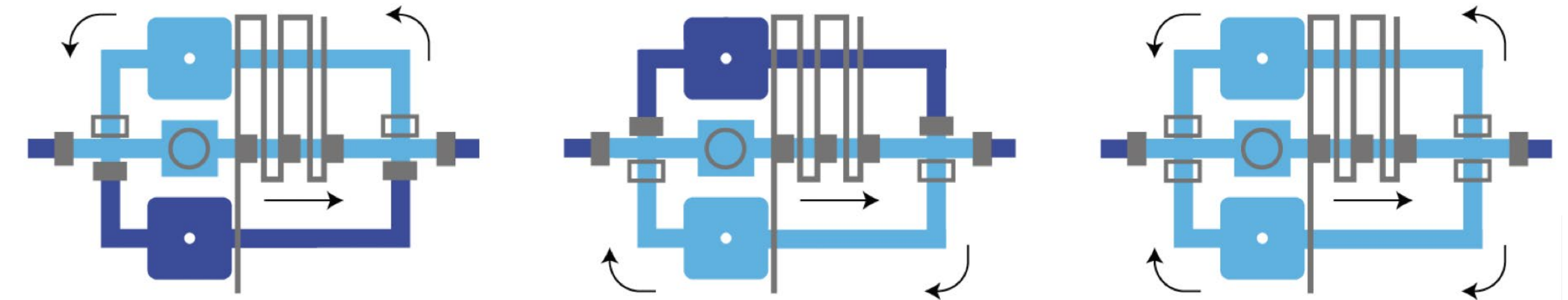
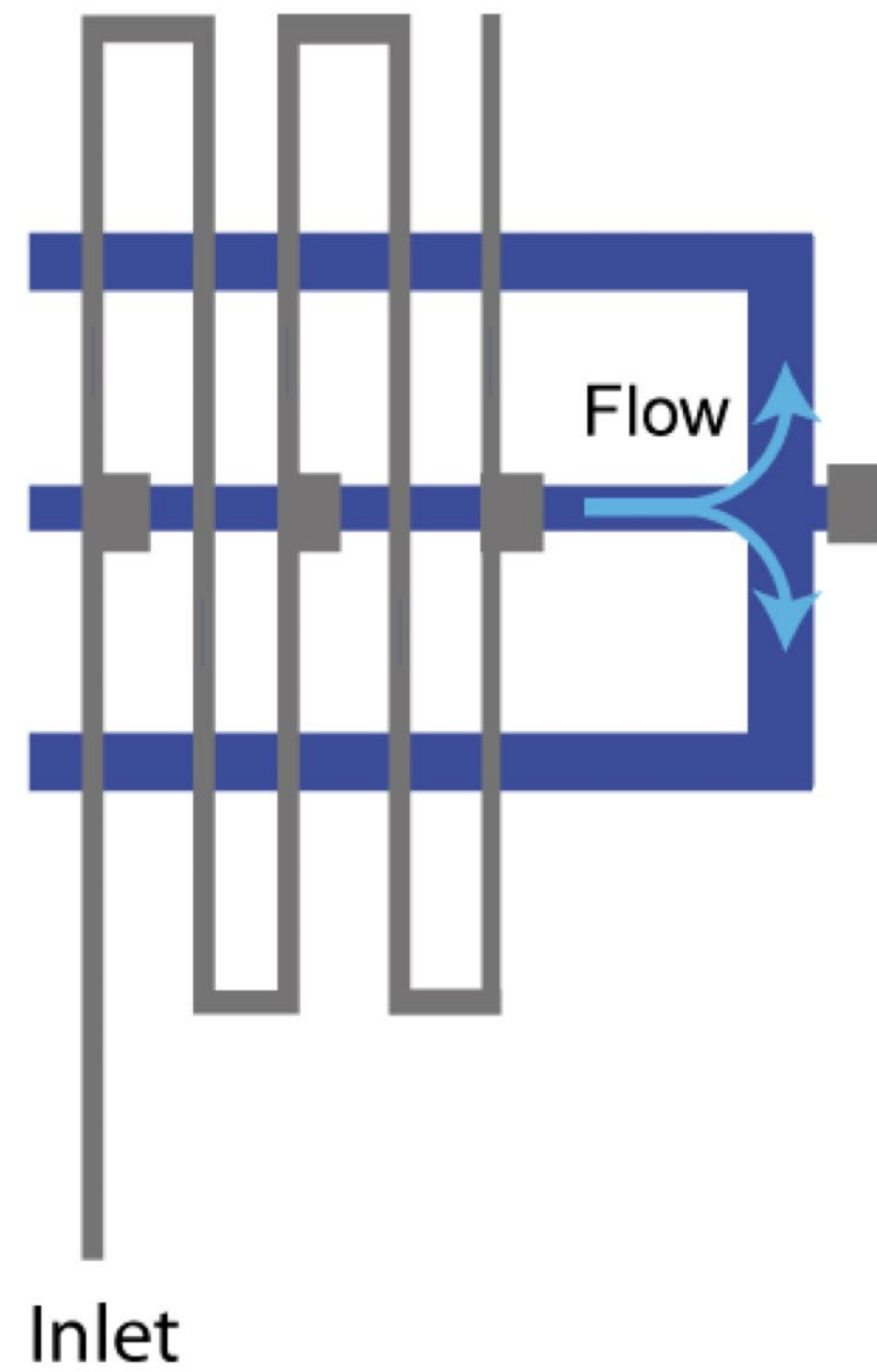
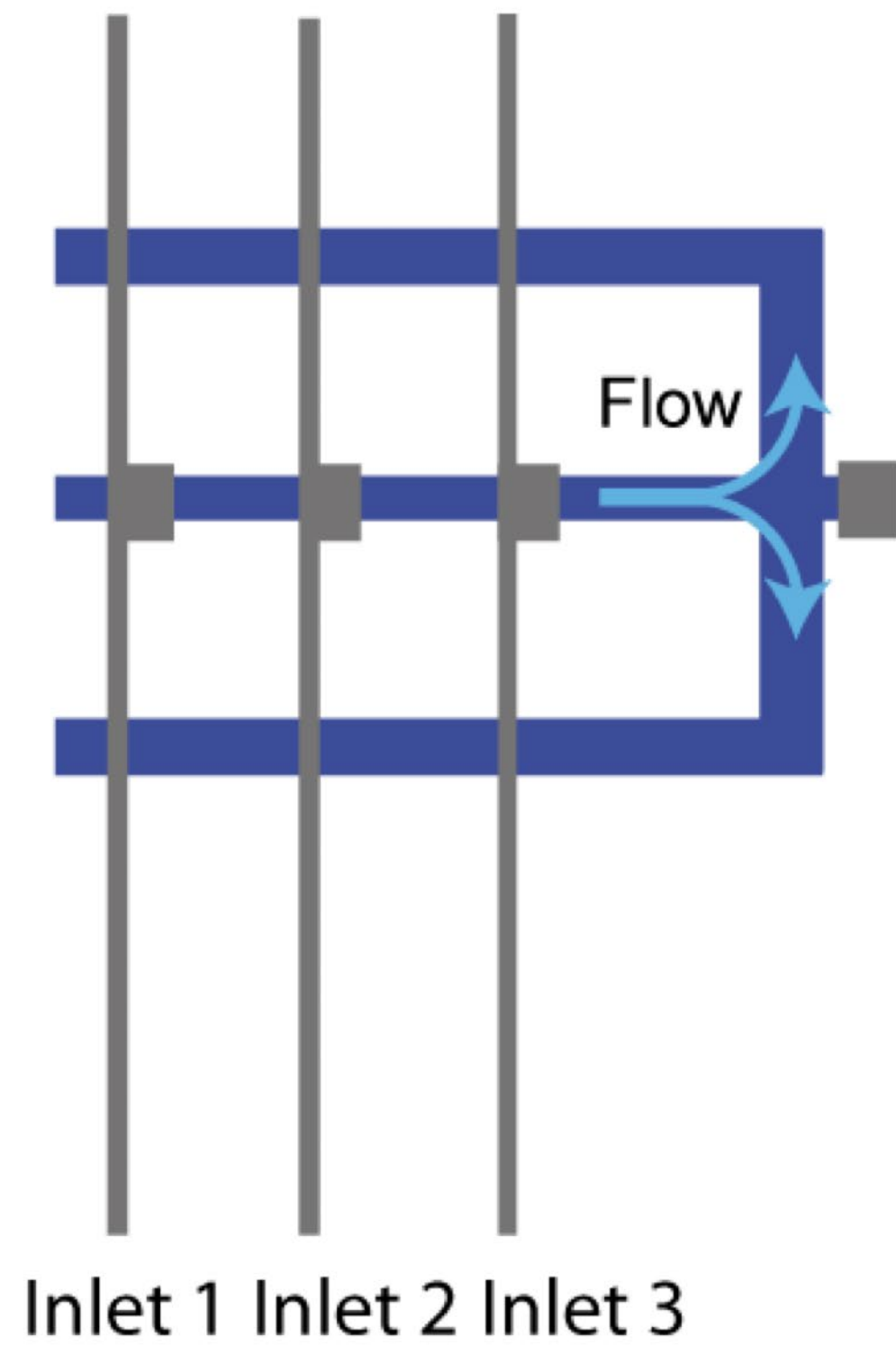


Analog/Digital Hybrid Device
Active Mixing of Unit Cells

On-chip Mixing for Faster Assay Speed

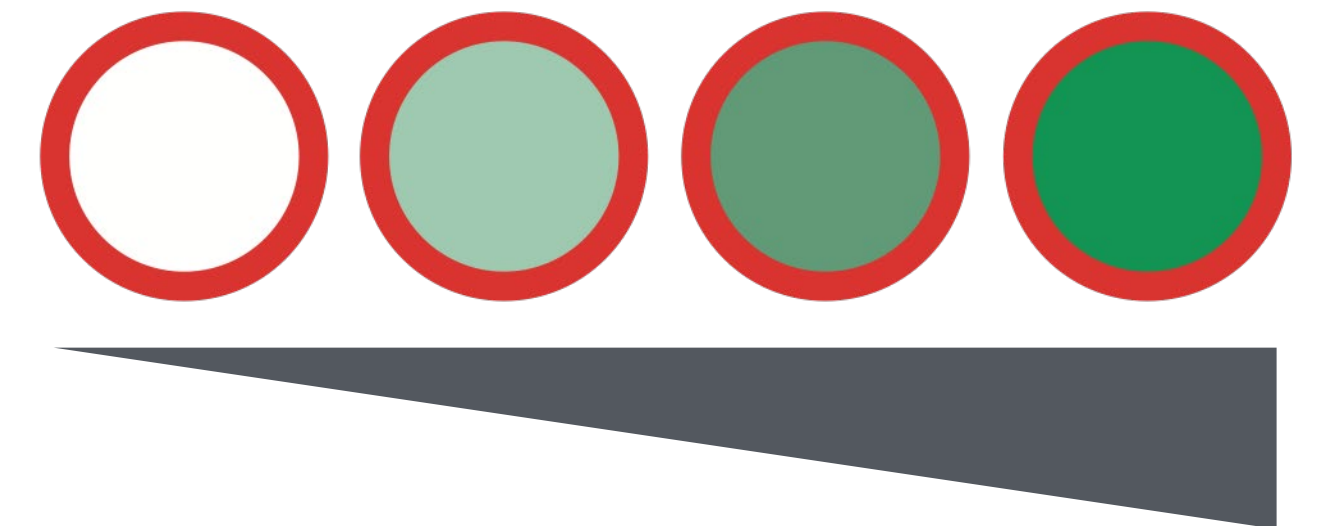
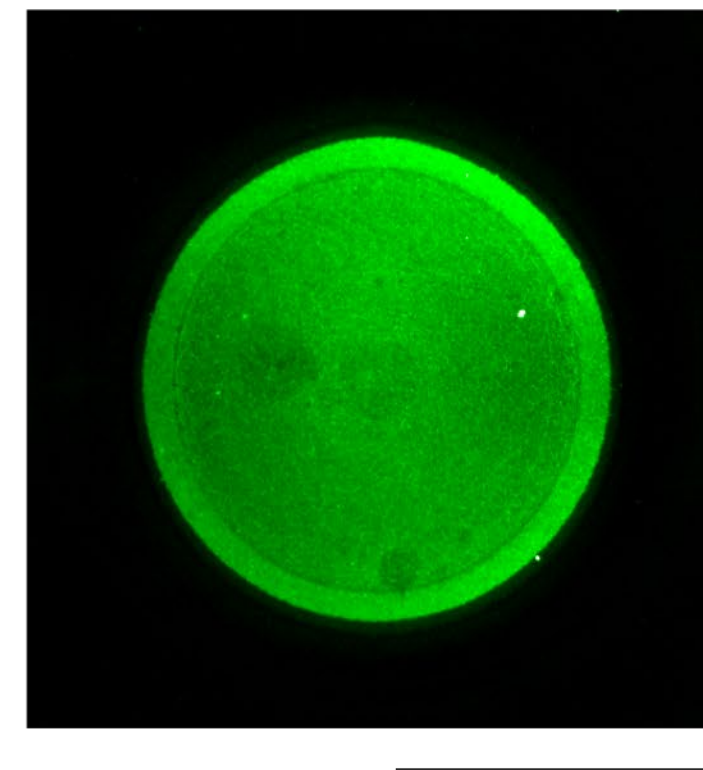
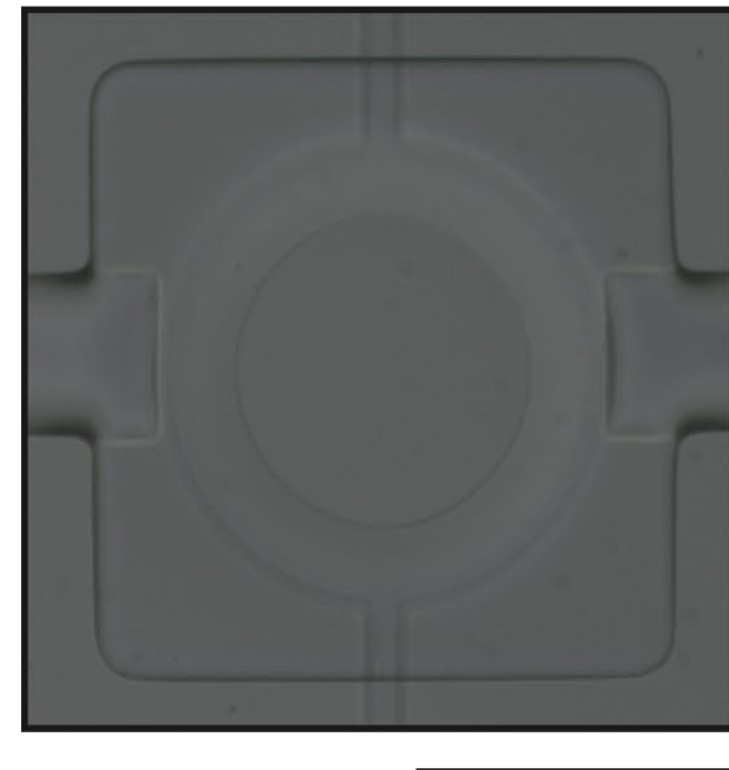
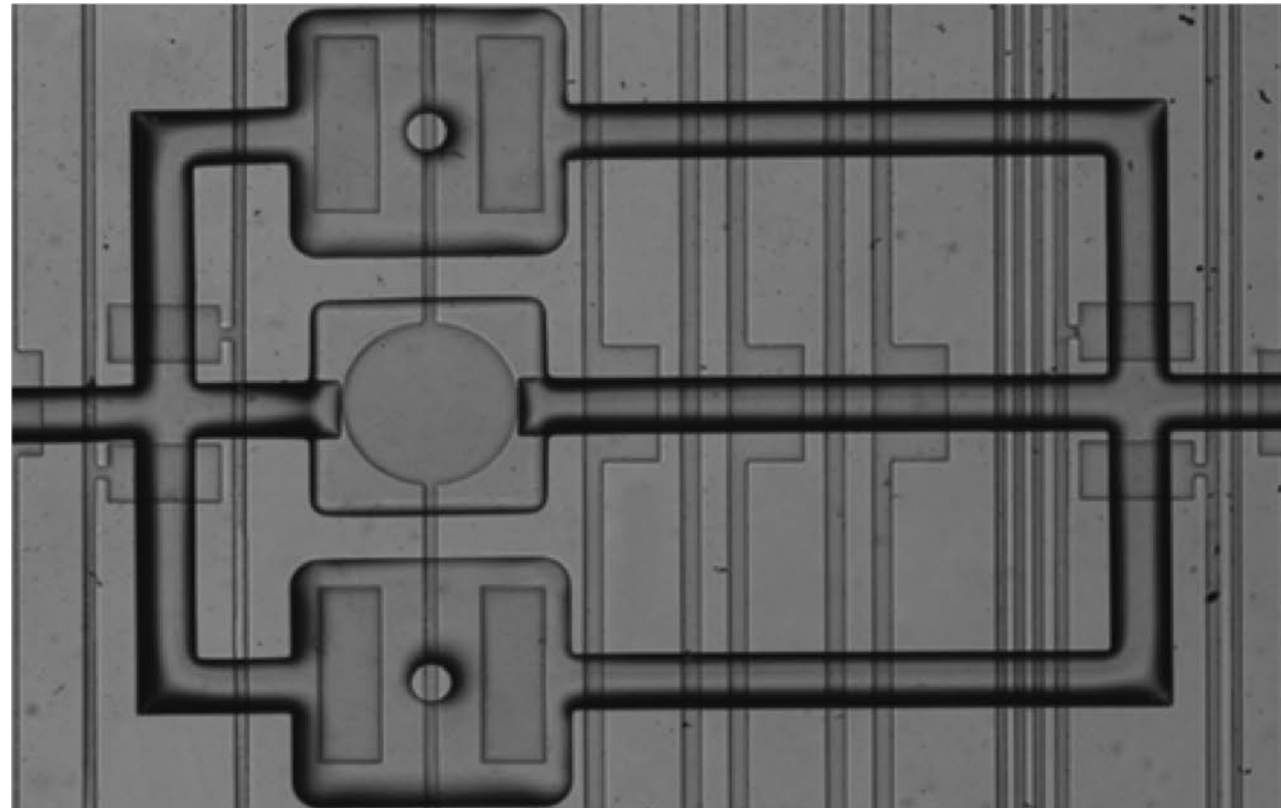
Conventional
peristaltic pump

S-shape delay
peristaltic pump

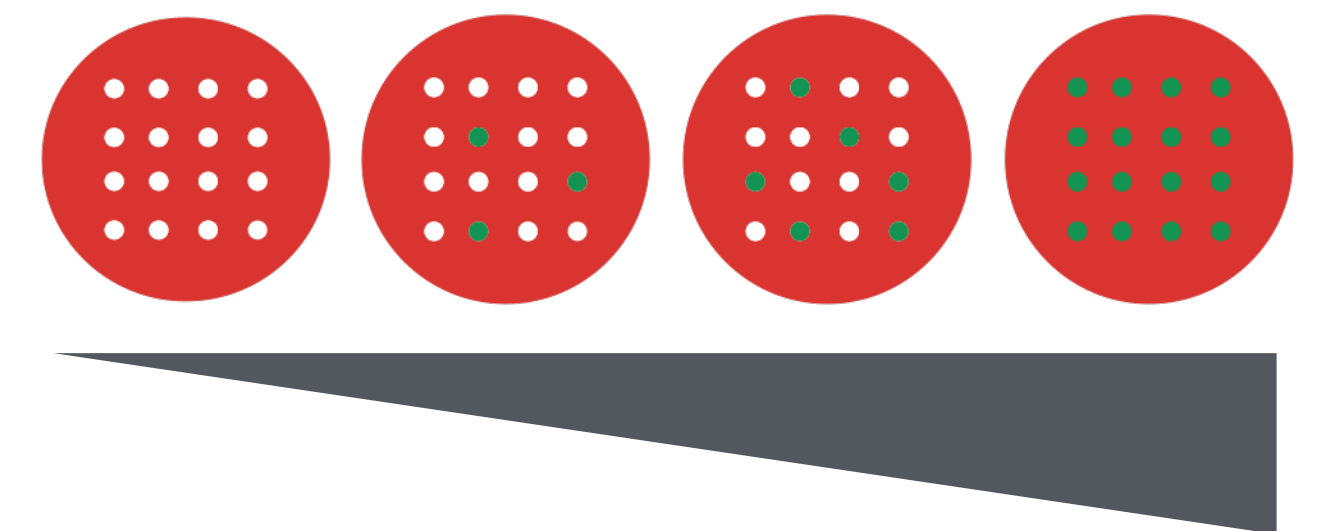
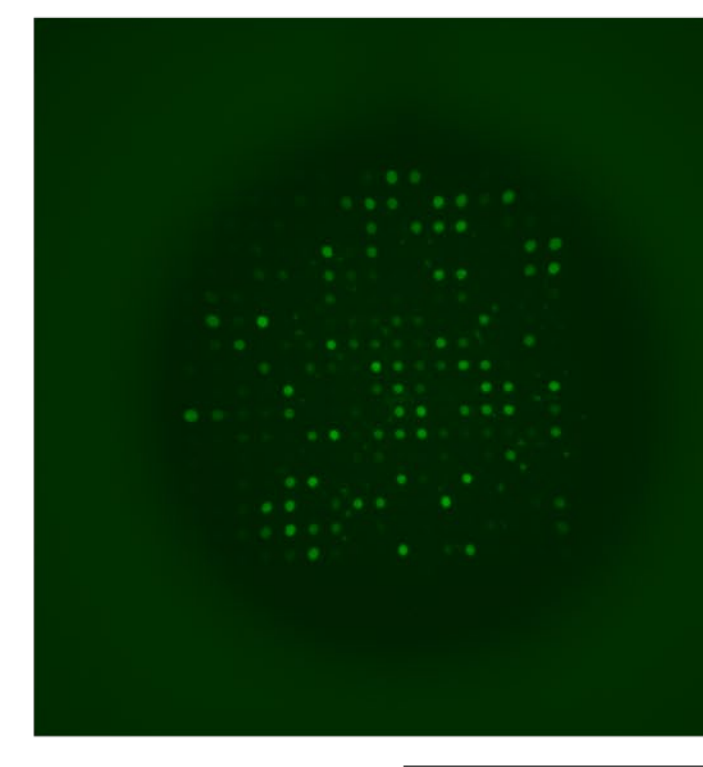
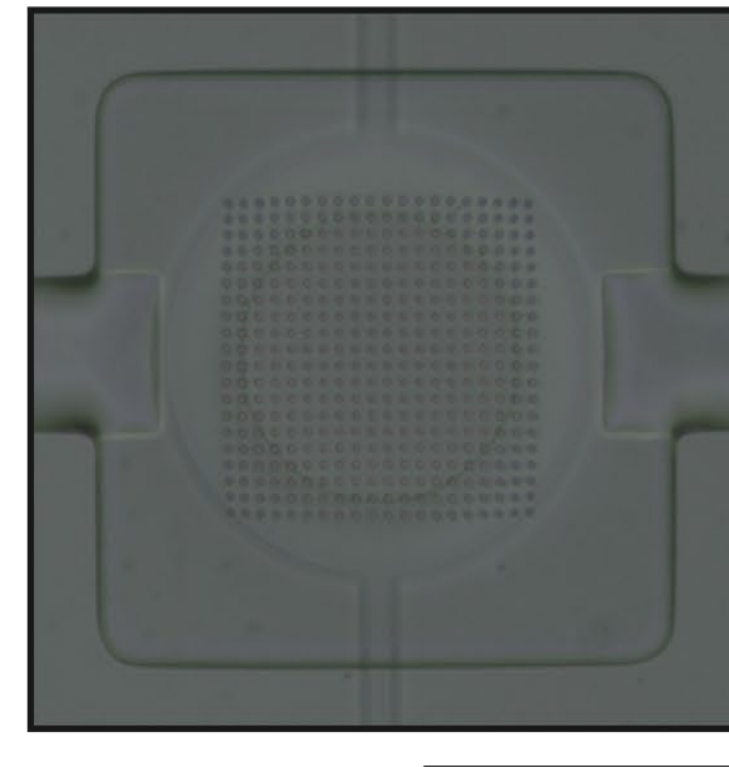
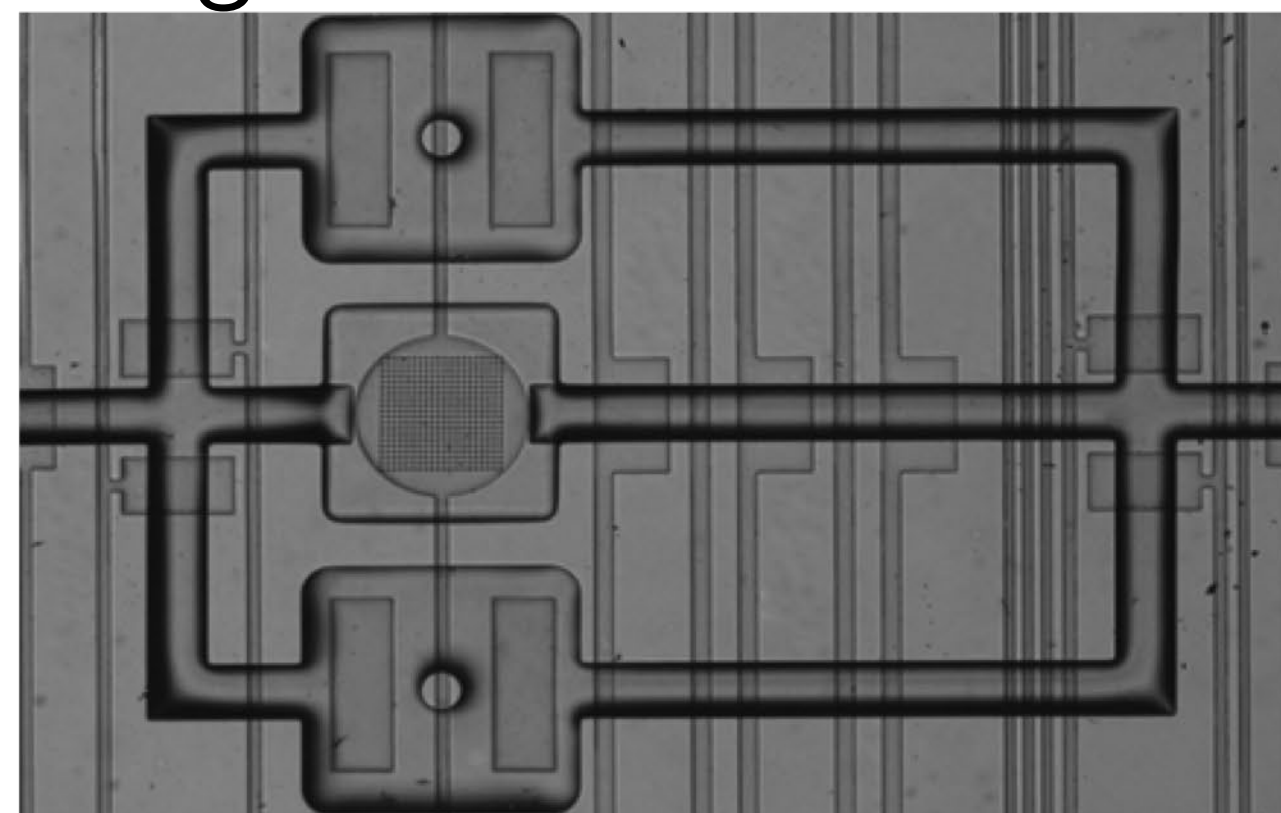


Analog versus Digital Detection

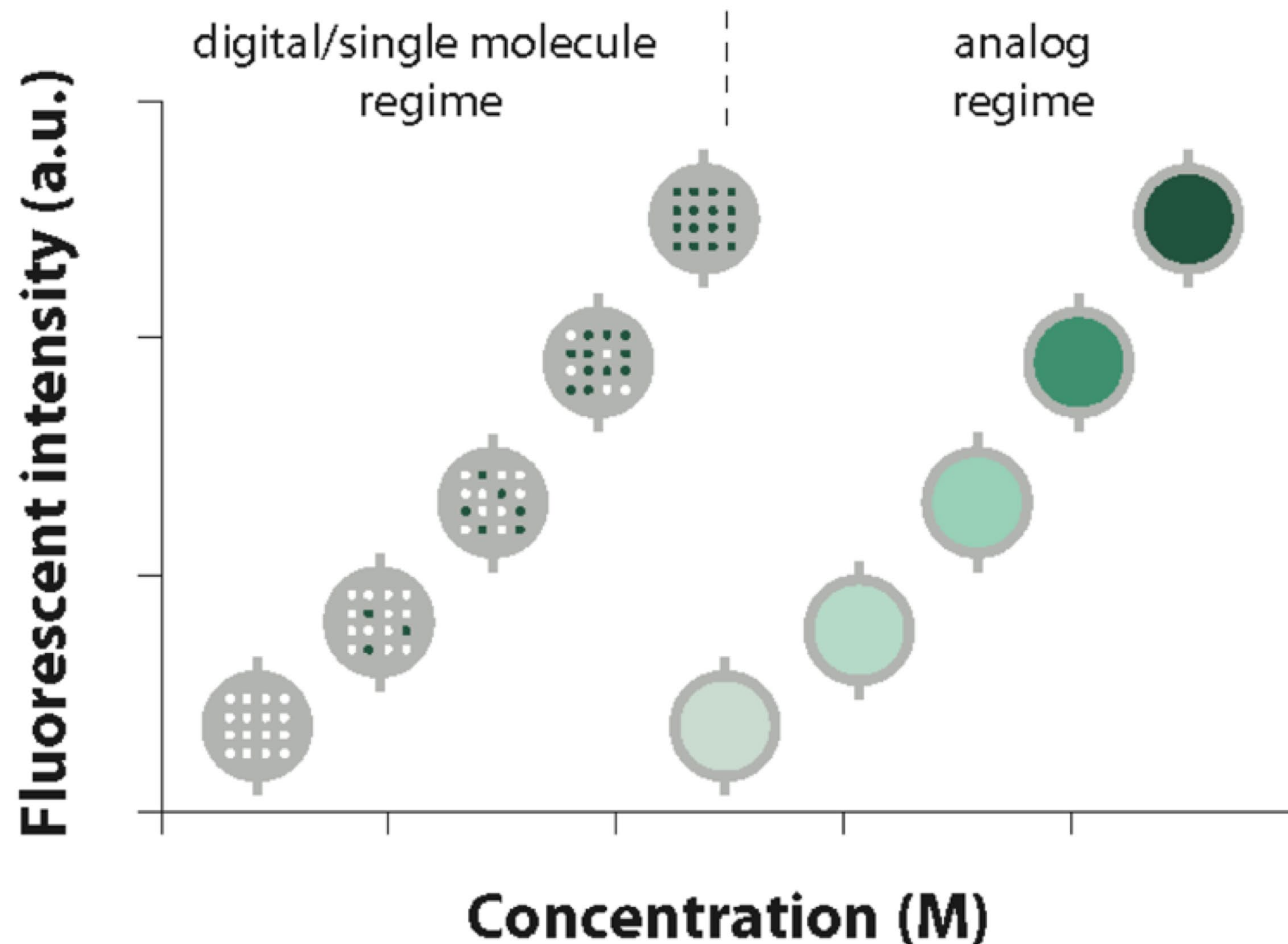
Analog



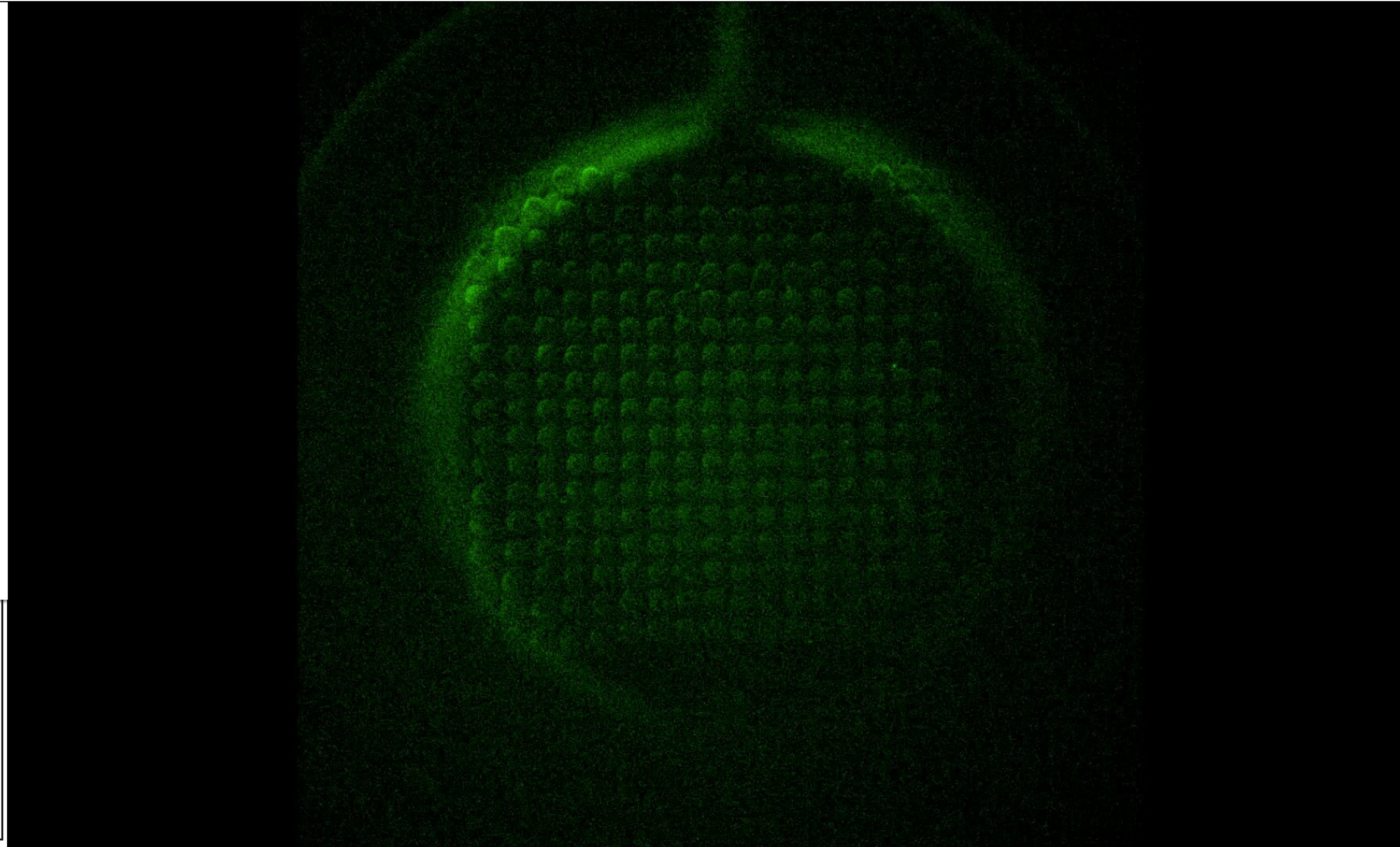
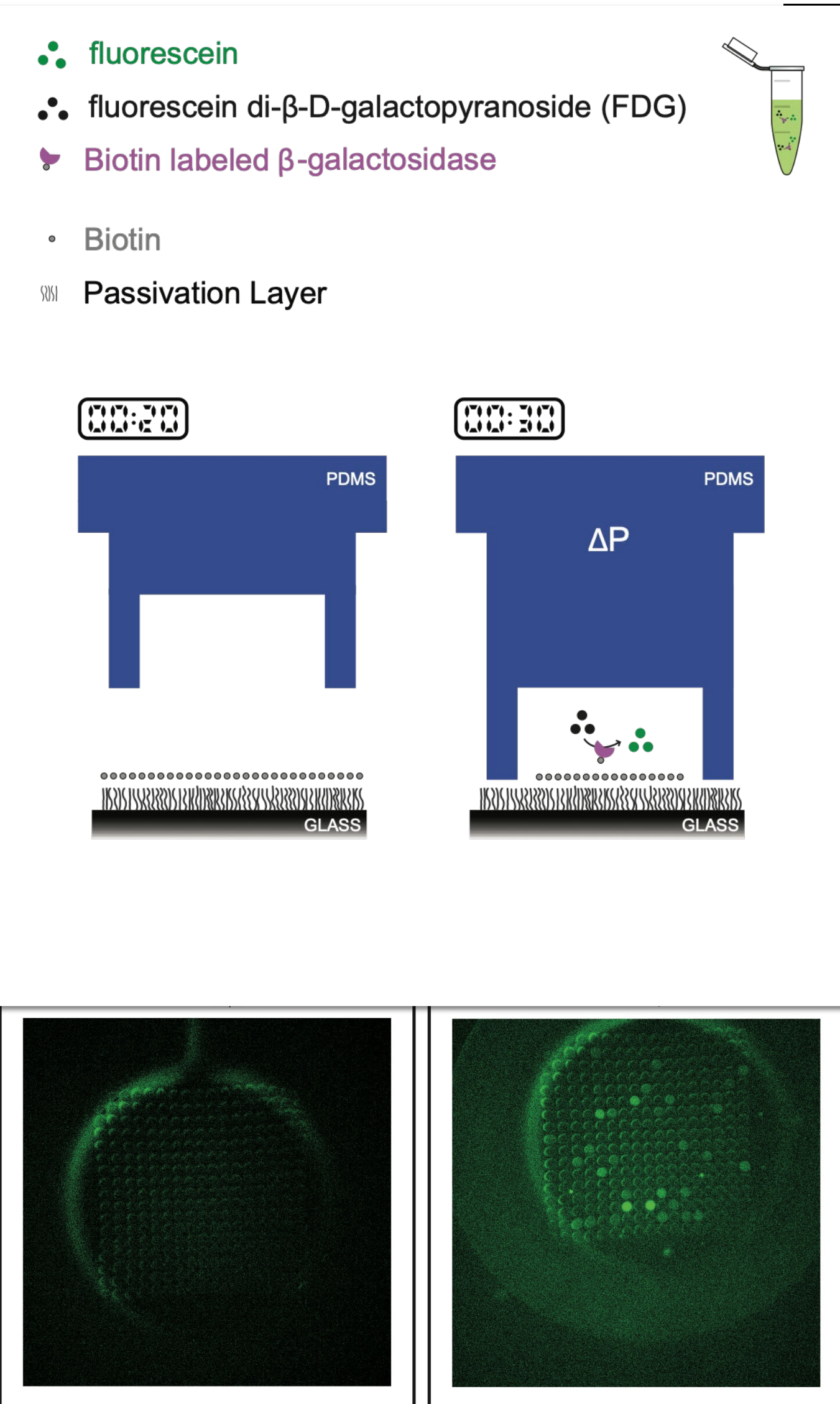
Digital



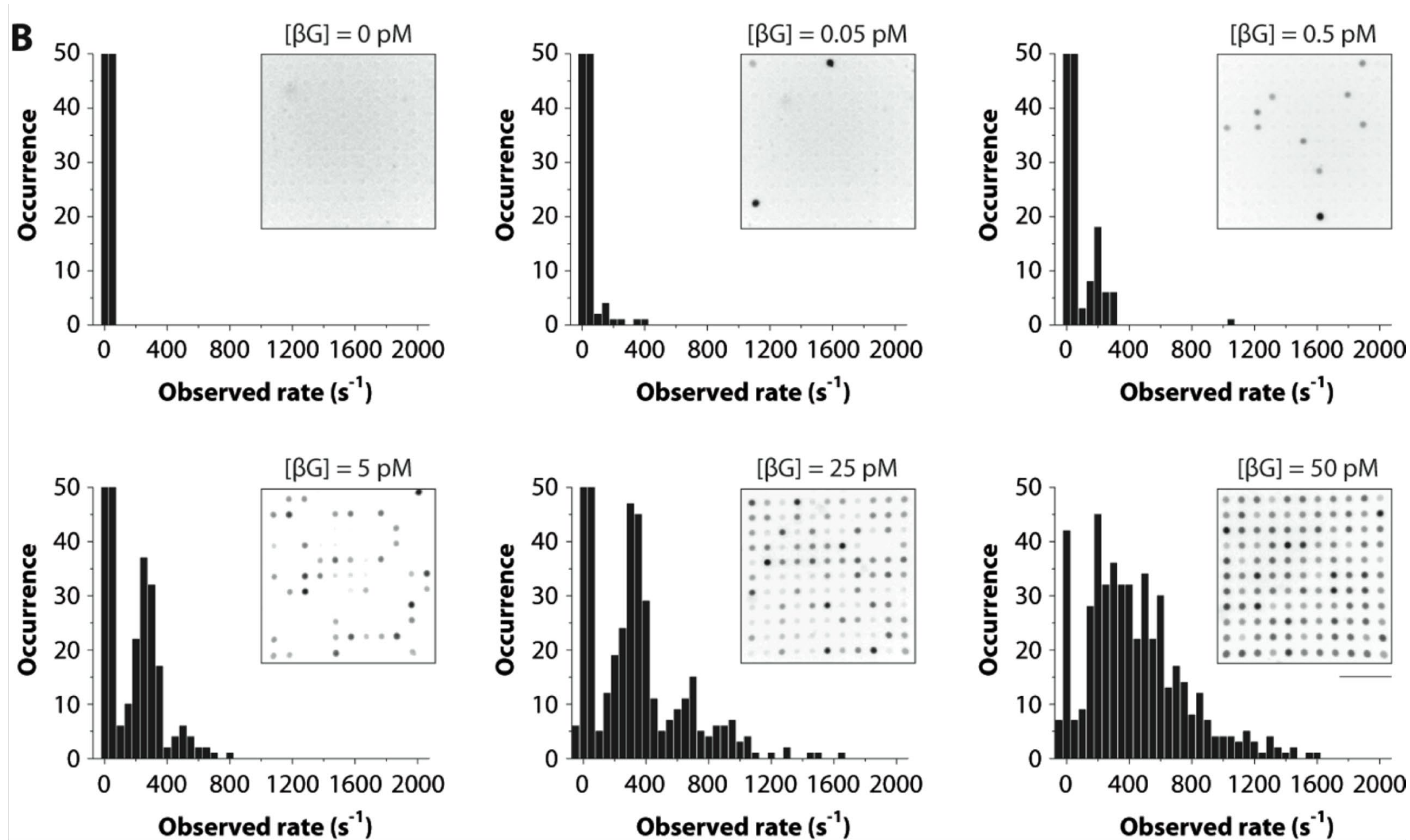
Increased Dynamic Range



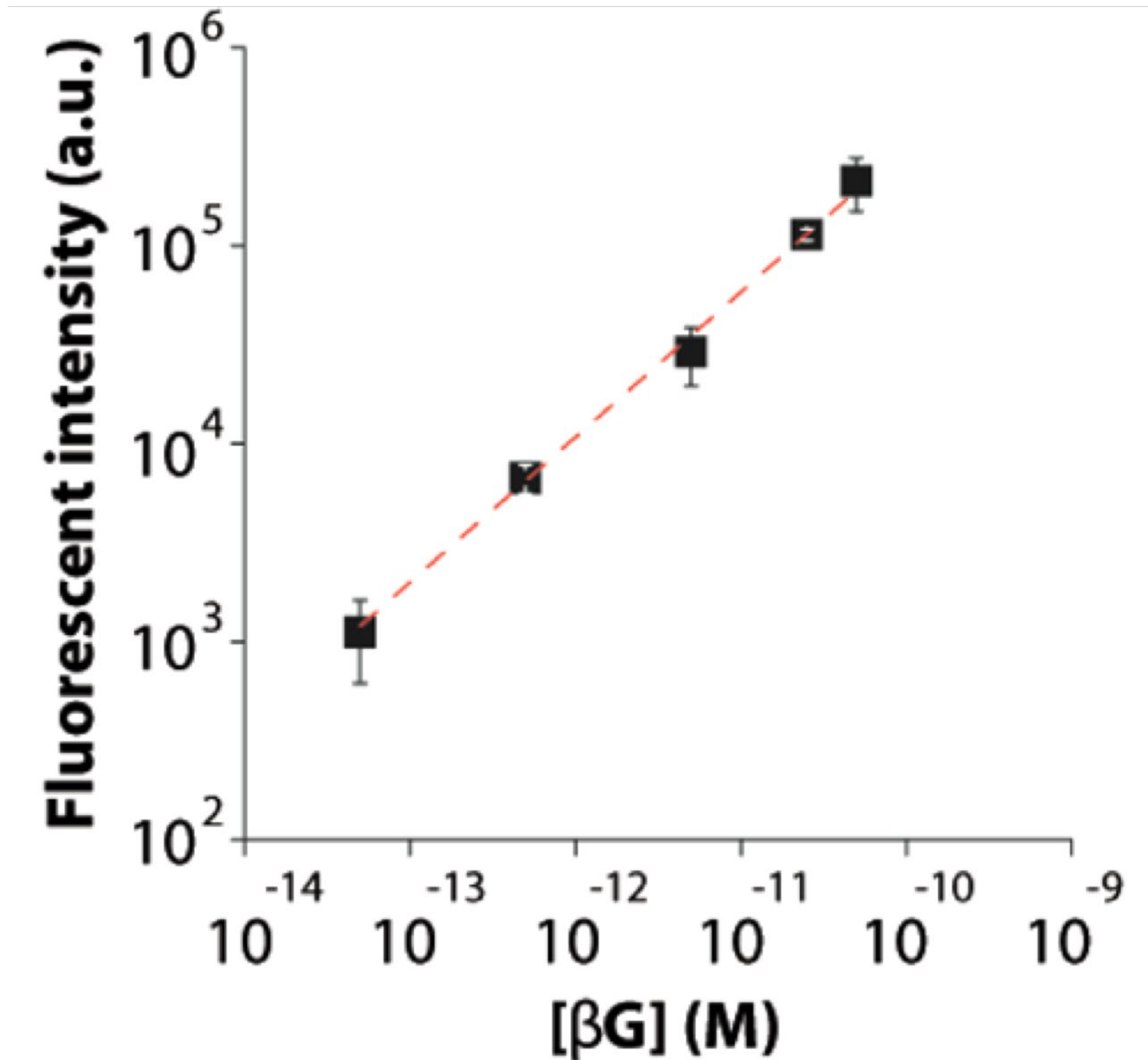
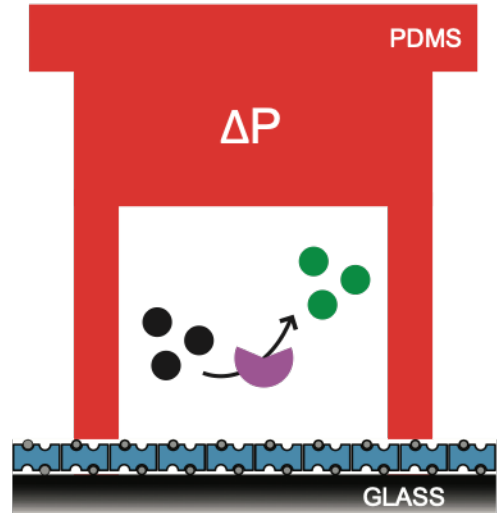
Single Molecule Detection



β -galactosidase in Solution

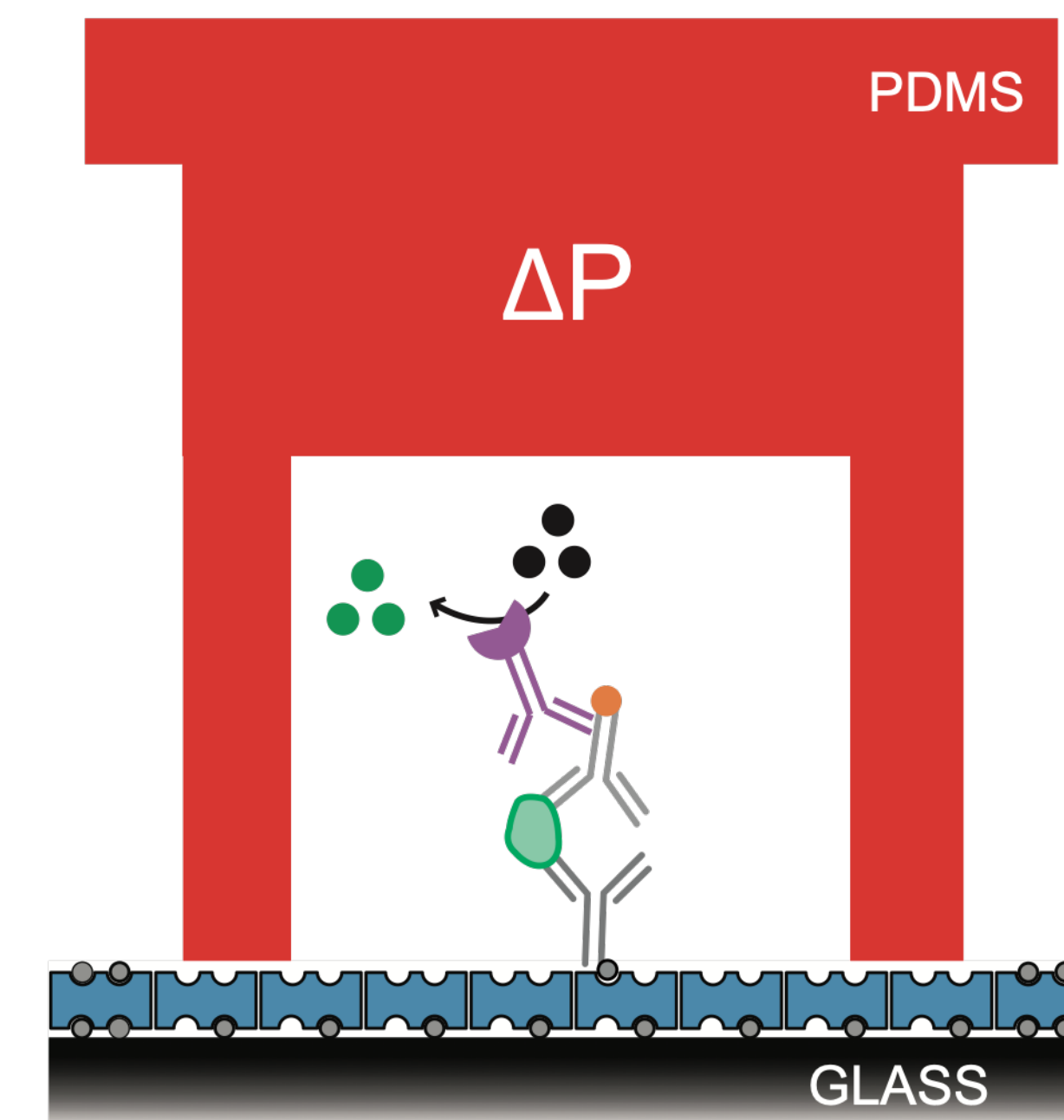


- fluorescein
- fluorescein di- β -D-galactopyranoside (FDG)
- β -galactosidase
- Neutravidin
- Biotin-BSA

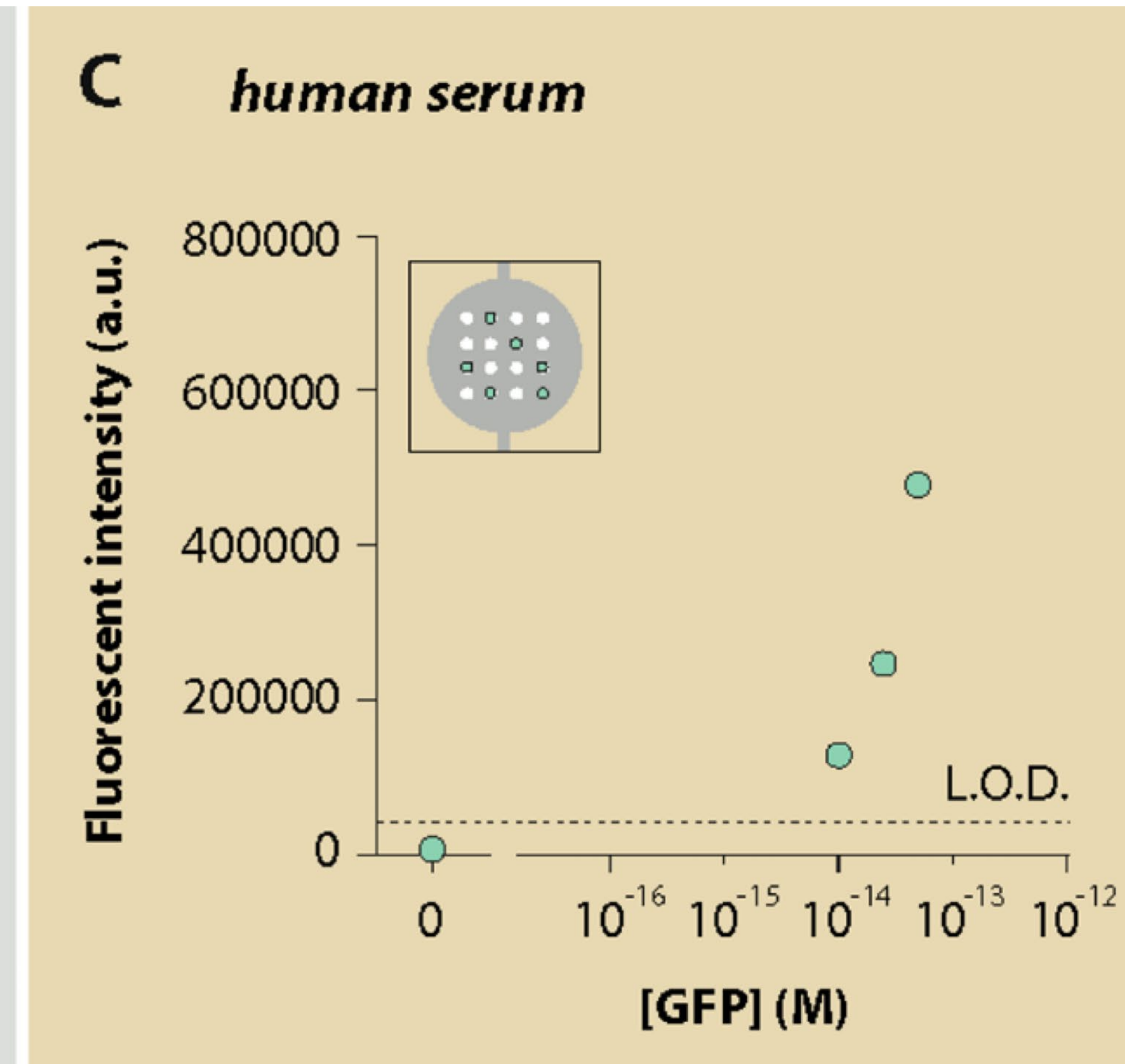
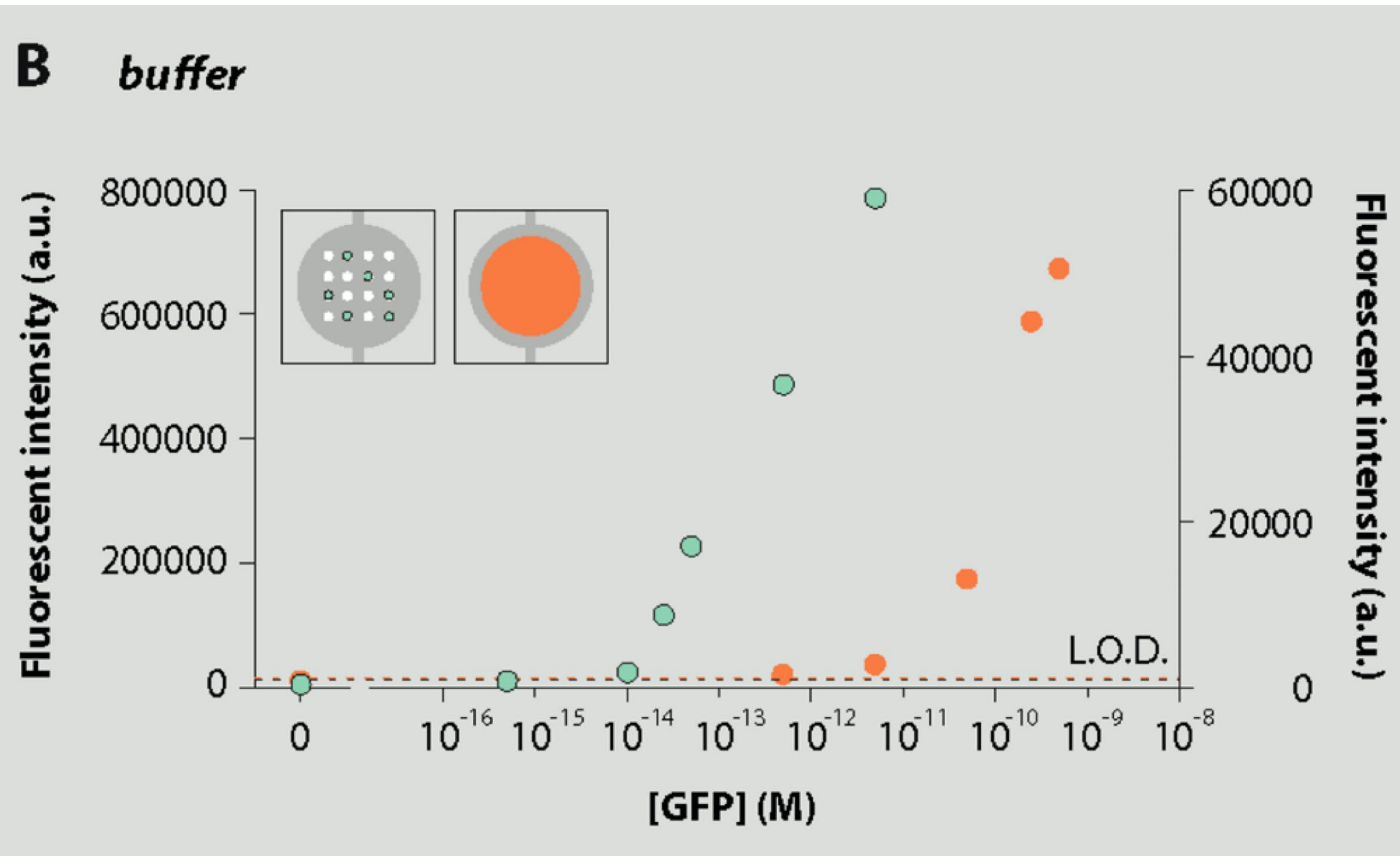


Digital/Analog ELISA

-  fluorescein
-  fluorescein di- β -galactopyranoside
-  Goat Anti-Mouse IgG β -Galactosidase Conjugate
-  Mouse IgG1 Penta-His Alexa Fluor 555 Conjugate
-  EGFP
-  Biotinylated Goat IgG polyclonal to GFP
-  Neutravidin
-  Biotin-BSA



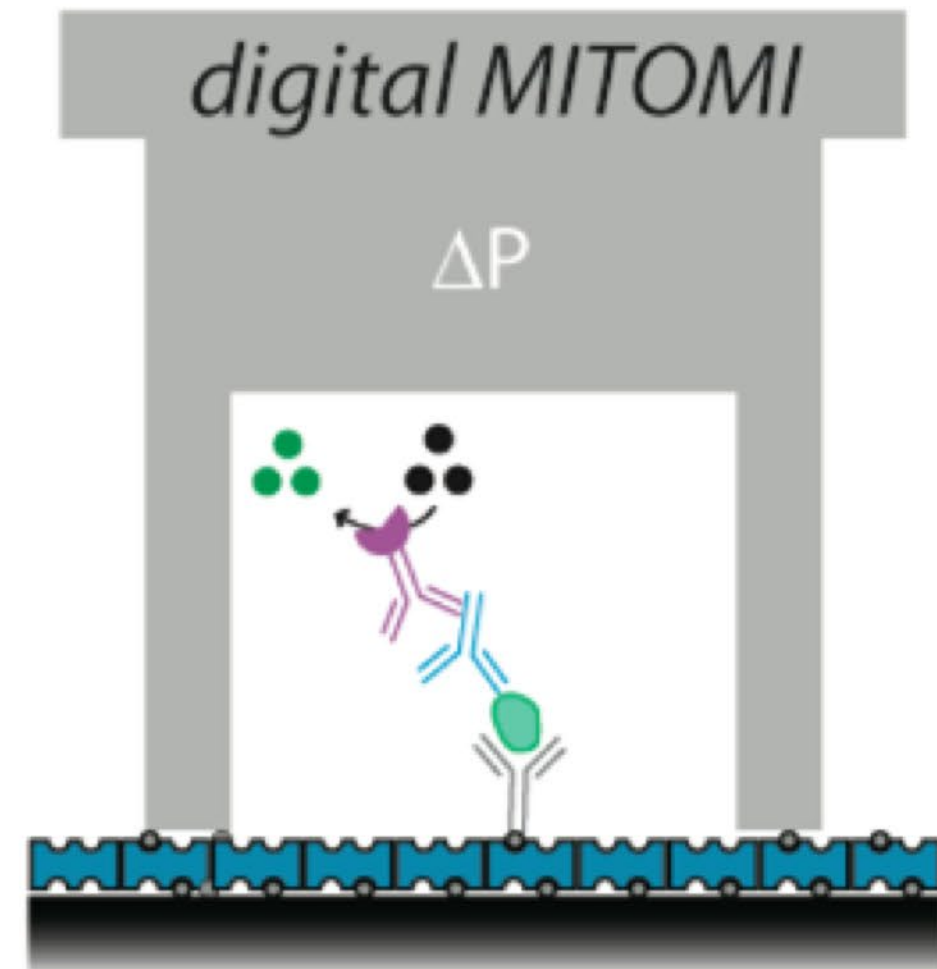
On-chip digital/analog ELISA



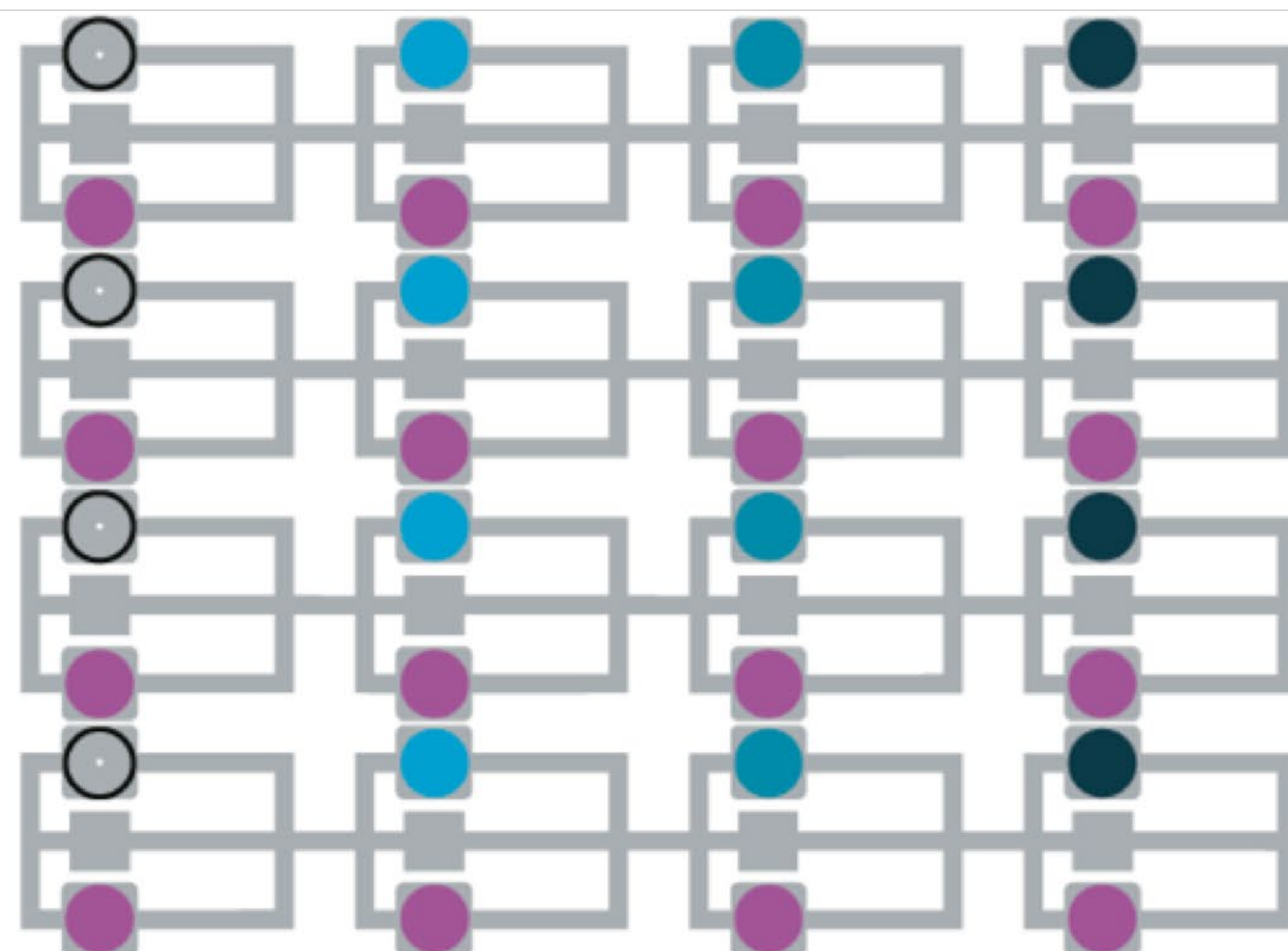
LOD: ~10 fM LOD: ~1 pM

LOD: ~12 fM

Digital Ebola Assay



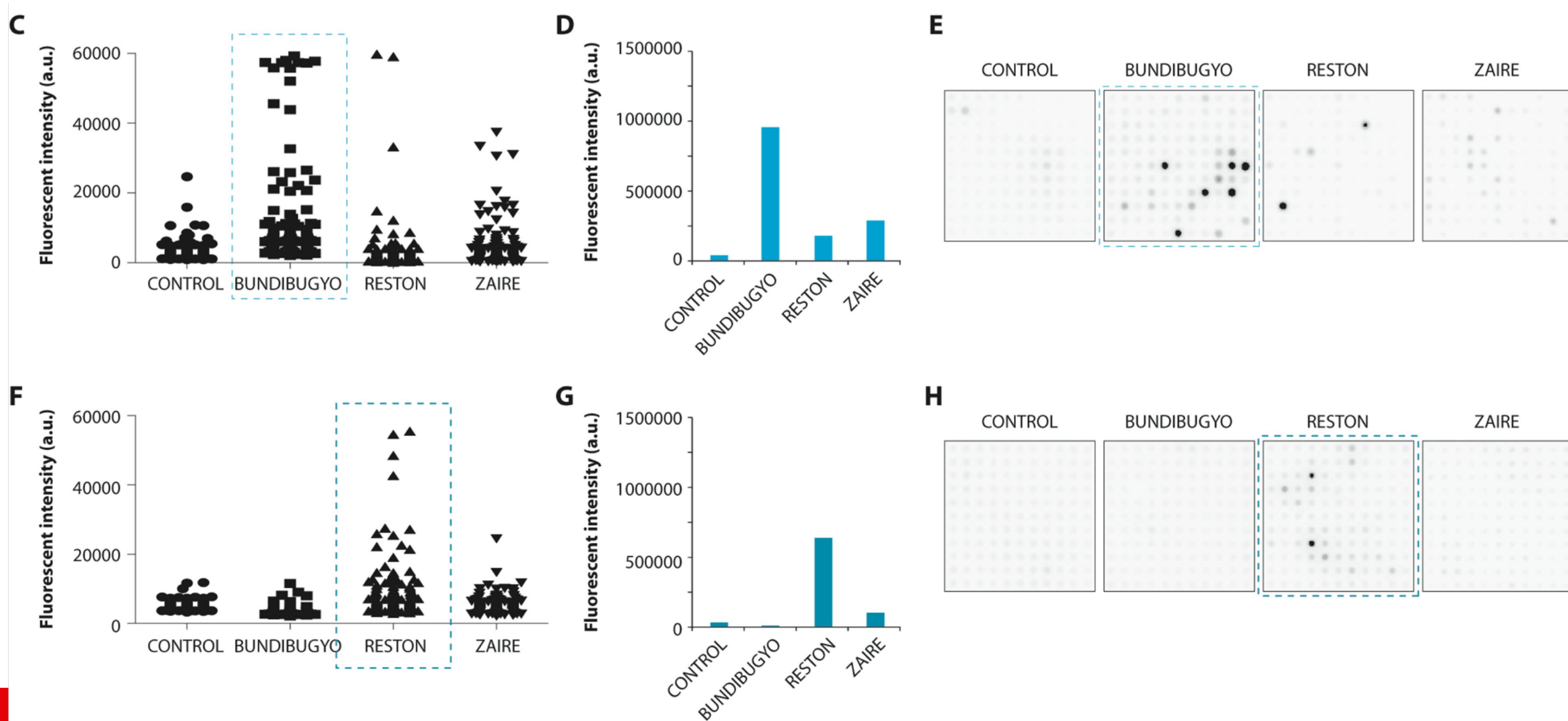
- Fluorescein
- Fluorescein di- β -D-galactopyranoside
- Goat anti-rabbit IgG (β -Galactosidase conjugate)
- Rabbit anti-Ebola virus GP
- Recombinant His-tagged Ebola virus GP
- Mouse anti-Penta-His (Biotinylated)
- Neutravidin
- BSA (Biotinylated)



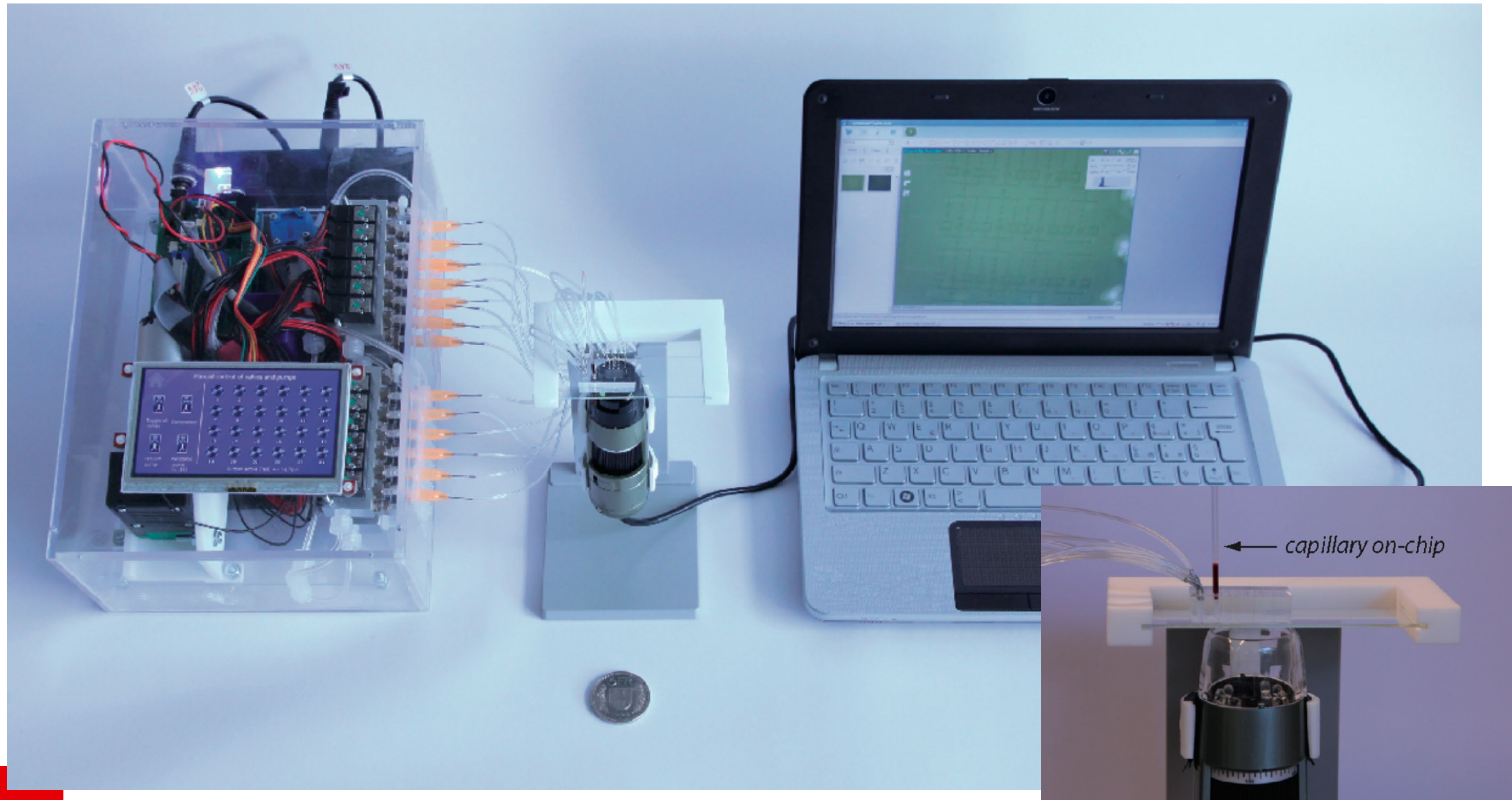
Sample

- Bovine Serum Albumin
- Bundibugyo Ebola virus GP
- Reston Ebola virus GP
- Zaire Ebola virus GP
- Goat anti - rabbit IgG (β -Galactosidase conjugate)

Digital Ebola Assay



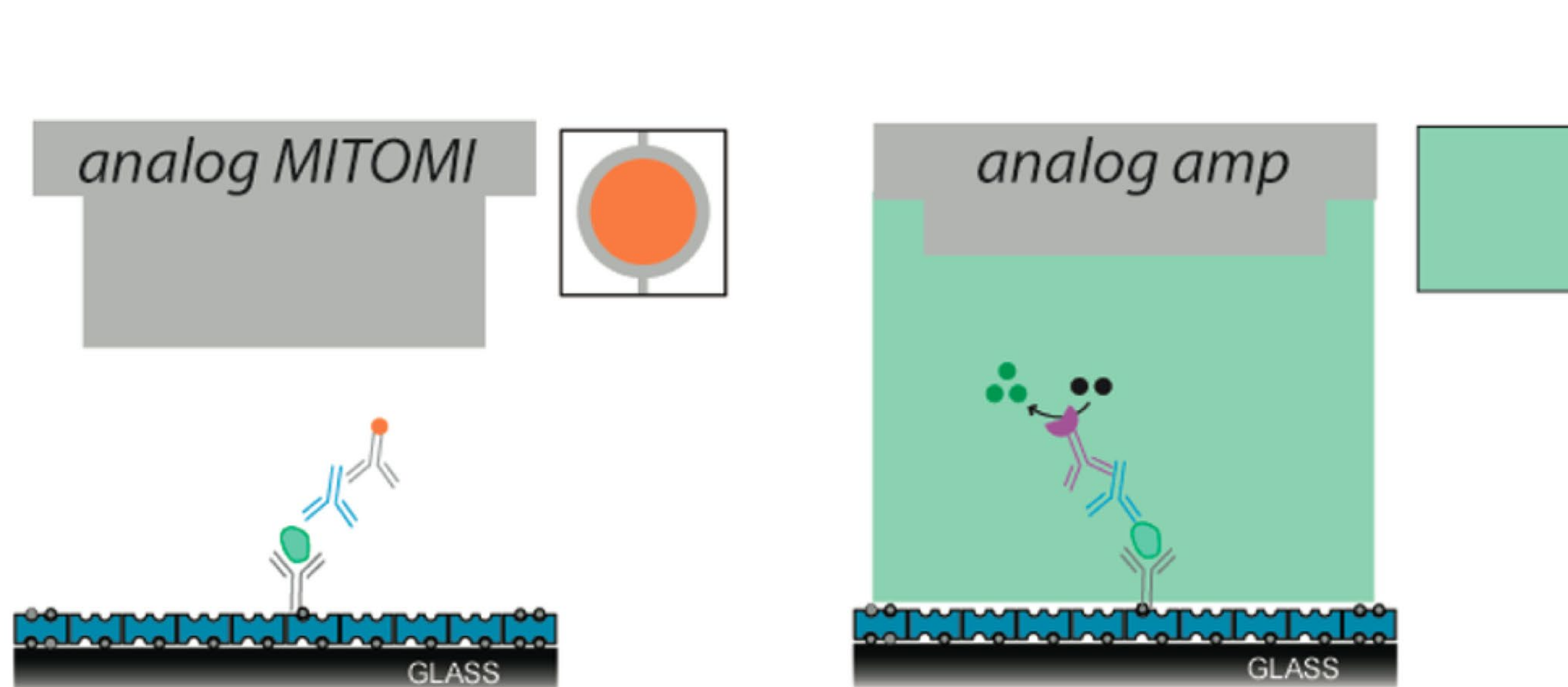
Portable, Low-Cost Diagnostic System



Field-based Ebola Detection

whole blood

A



- Fluorescein
- Fluorescein di- β -D-galactopyranoside
- Goat Anti-rabbit IgG (PE)
- Goat anti-rabbit IgG (β -Galactosidase)
- Rabbit anti-Ebola virus GP
- Recombinant His-tagged Ebola virus GP
- Mouse anti-Penta-His (Biotinylated)
- Neutravidin
- BSA (Biotinylated)

Direct and Amplification Based Detection

