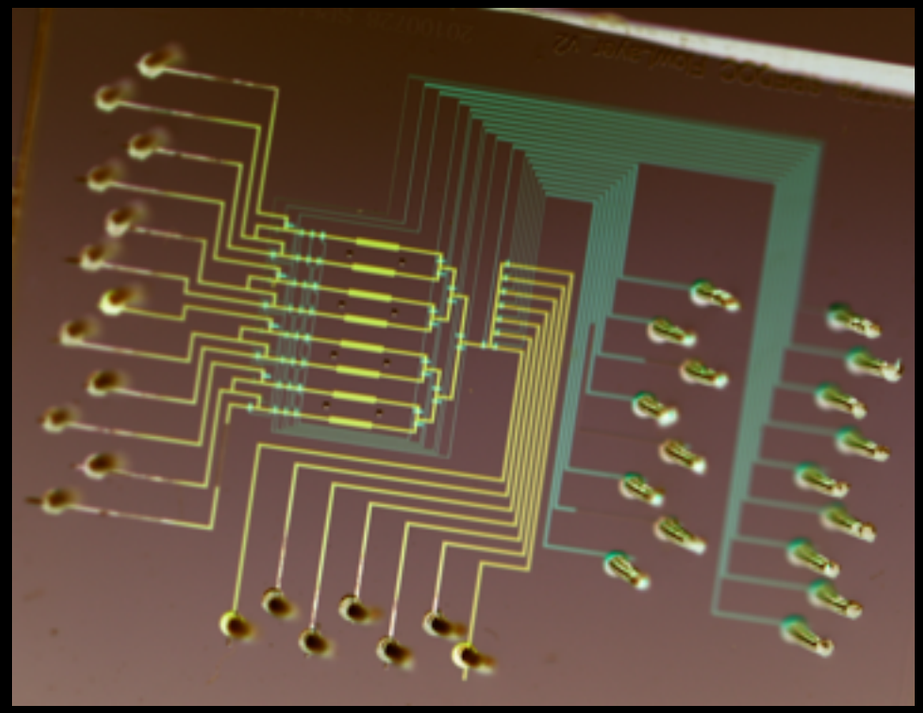
Personalized Molecular Diagnostics

A new healthcare paradigm?

The Future of Diagnostics?



Point of Care Diagnostics
Home-Based Diagnostics

New Diagnostics Paradigm

Towards personal diagnostics and personalized medicine.

<u>Home monitoring of:</u>

std. vital signs

➤ std. blood panel

known and novel biomarkers

disease panel

pathogen panel

allergy panel? (env. monitoring?)

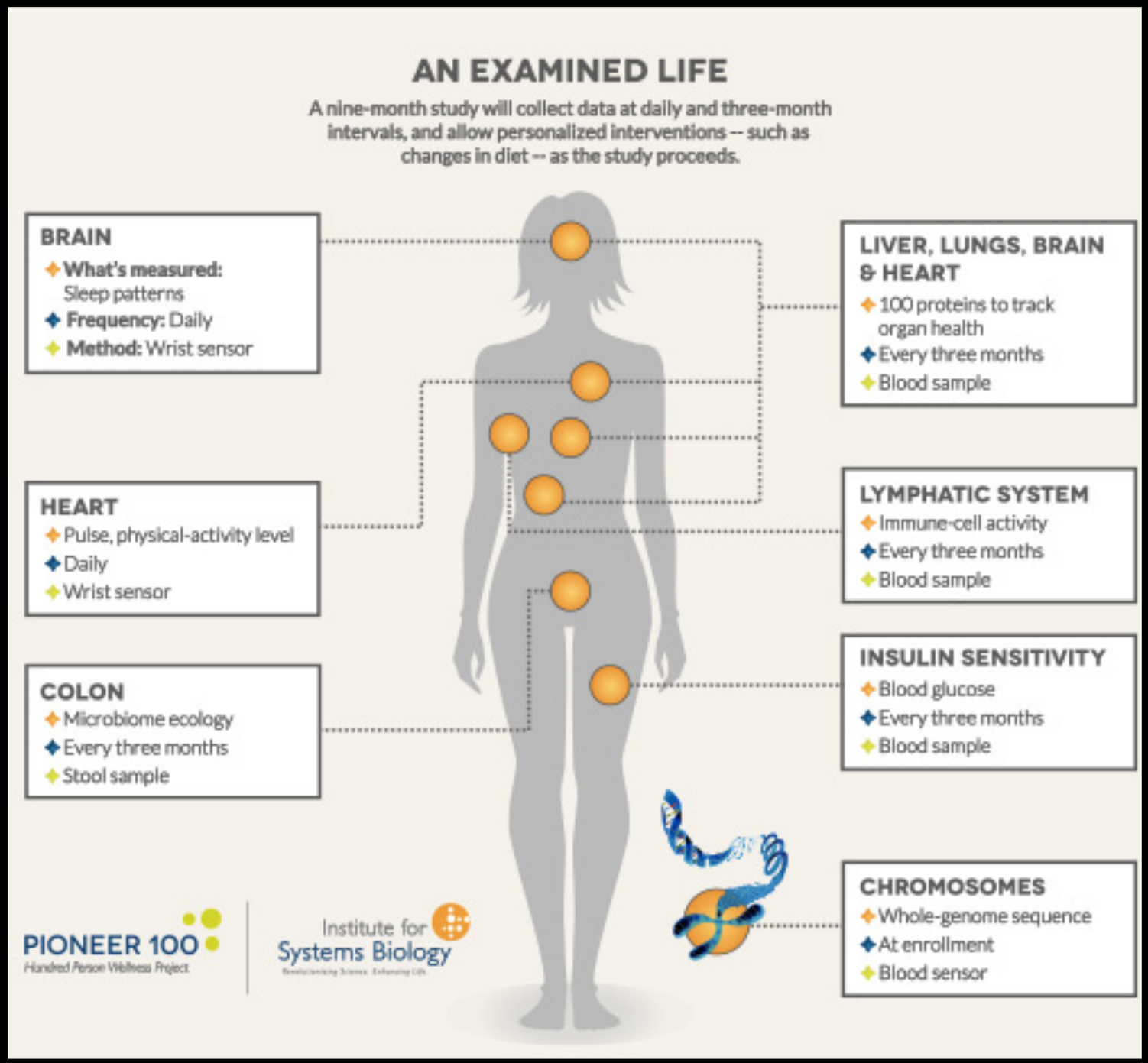
- early medical action
- preventative behavioral changes
- state level actions (prevent epidemics)

Health Care Professional

INFORMATION

Academia Industry Health Insurance

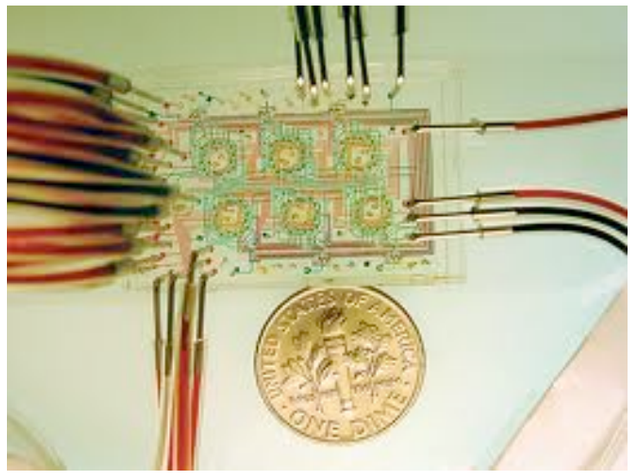
100k Wellness Project

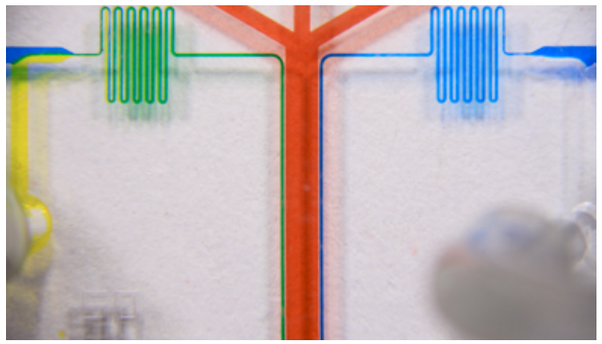


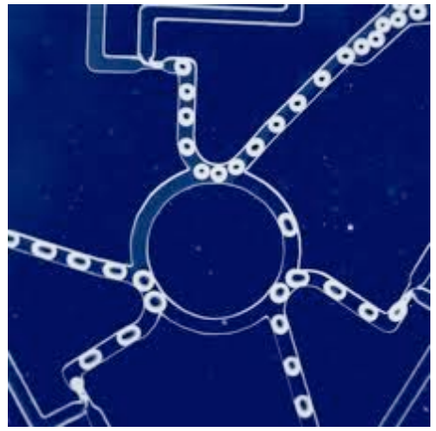
Predictive, Preventive, Personalized & Participatory: P4 Medicine

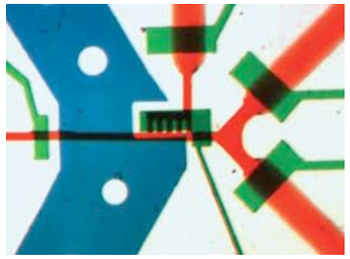
- P4 Medicine is proactive.
- P4 Medicine focuses on the individual patient.
- P4 Medicine places major emphasis on maintaining and enhancing wellness.
- P4 Medicine generates patient data clouds of billions of data points.
- P4 Medicine realizes that each patient is genetically and environmentally unique.
- Consumer activated social networks will be a major driving force for gaining acceptance of P4 Medicine.

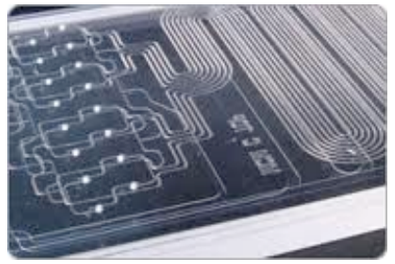
http://research.systemsbiology.net/100k/

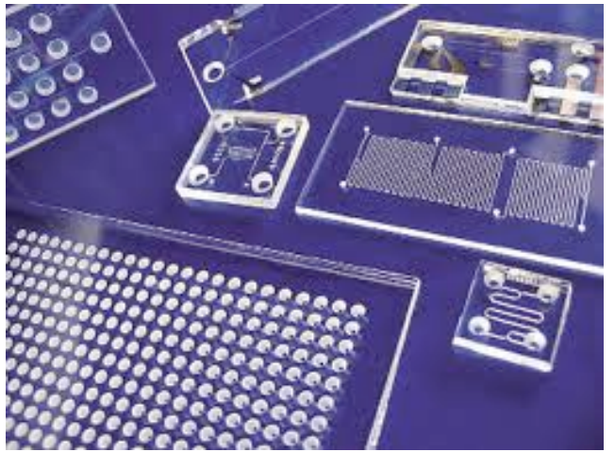

UNITED STATES OF AMERICA
ONE DIME

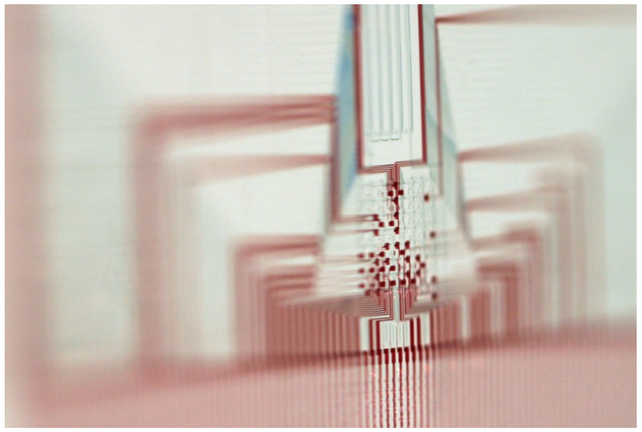


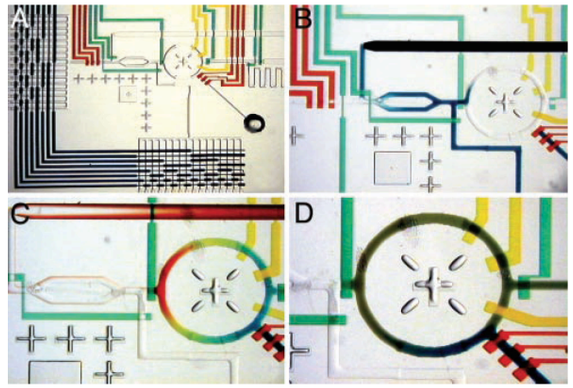


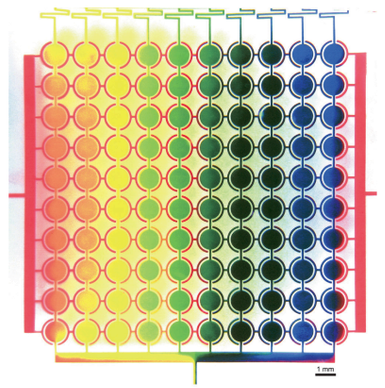


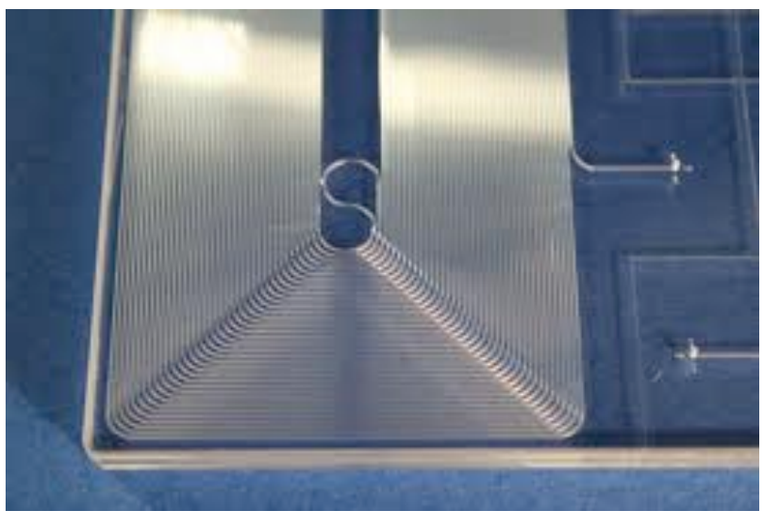


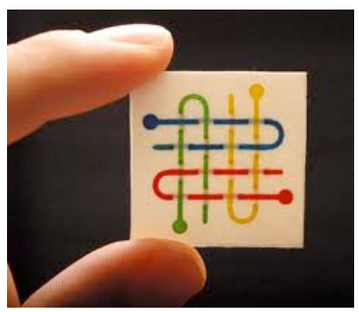


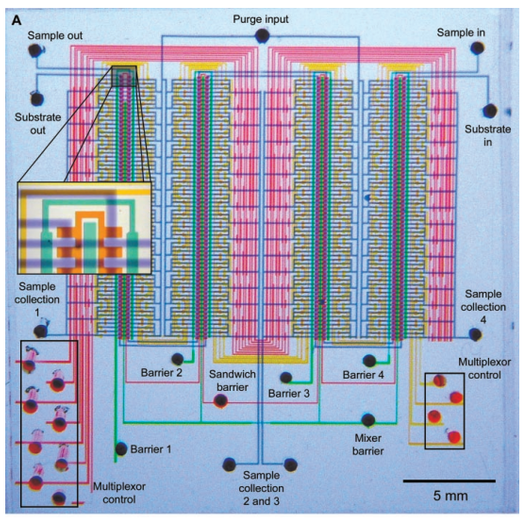


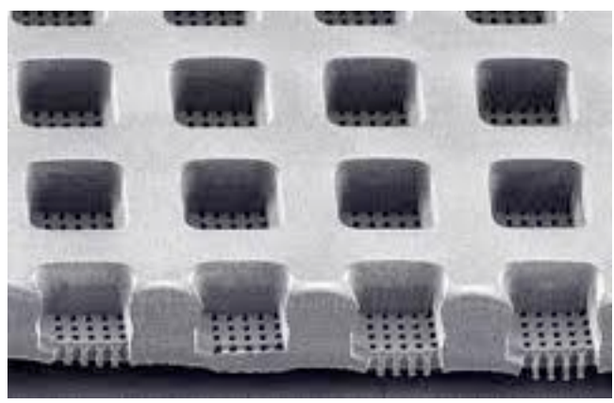

A
B
C
D


1 mm






A
Sample out
Purge input
Sample in
Substrate out
Substrate in
Sample collection 1
Sample collection 4
Barrier 2
Sandwich barrier
Barrier 3
Barrier 4
Multiplexor control
Mixer barrier
Barrier 1
Sample collection 2 and 3
Multiplexor control
5 mm

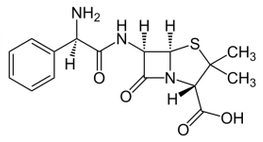


Benefits of Microfluidics

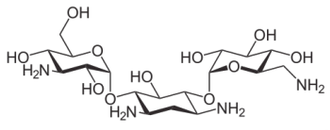
- Economy of Scales
  - Volume reductions by several orders of magnitude over bench-top experiments
  - Extreme cost reduction for biological experiments
    - ITT costs **25 CHF/rxn**, on a fluidic device the cost is **0.005 CHF/rxn**
  - Rare samples (stem cells) can be studied in more detail
- Integration
  - Thousands of complex experiments can be performed in parallel
  - Next generation multiwell plates
  - Integration with solid state optics, MEMS, and NEMS detectors
- Automation
  - All steps can be fully automated, reducing labor costs
- Cheap Mass-production

Cost of biologics

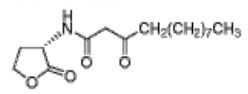
Ampicillin



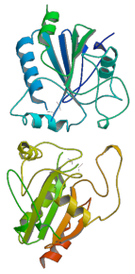
Kanamycin



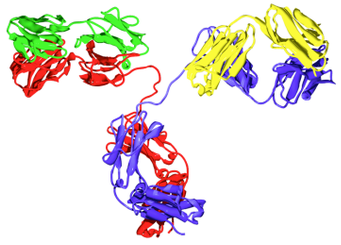
AHL



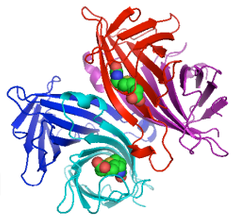
HRP



Antibodies

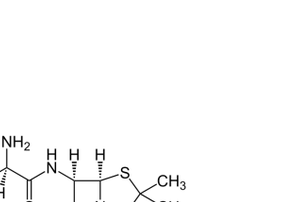


Streptavidin

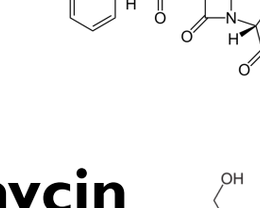


Pt

Au



Ag



Cu

Diamond

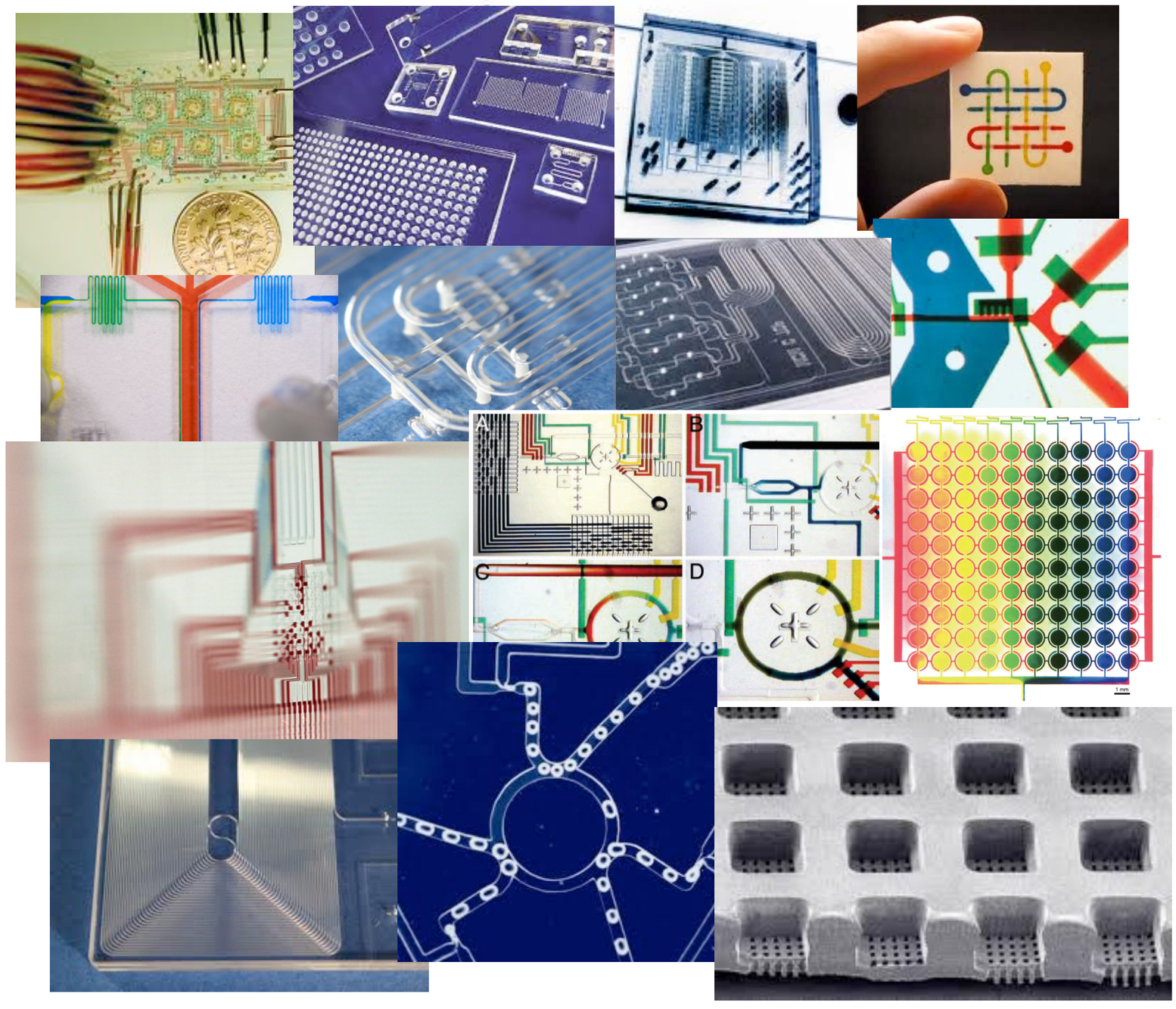
Sapphire

1.E-02 1.E-01 1.E+00 1.E+01 1.E+02 1.E+03 1.E+04 1.E+05 1.E+06 1.E+07

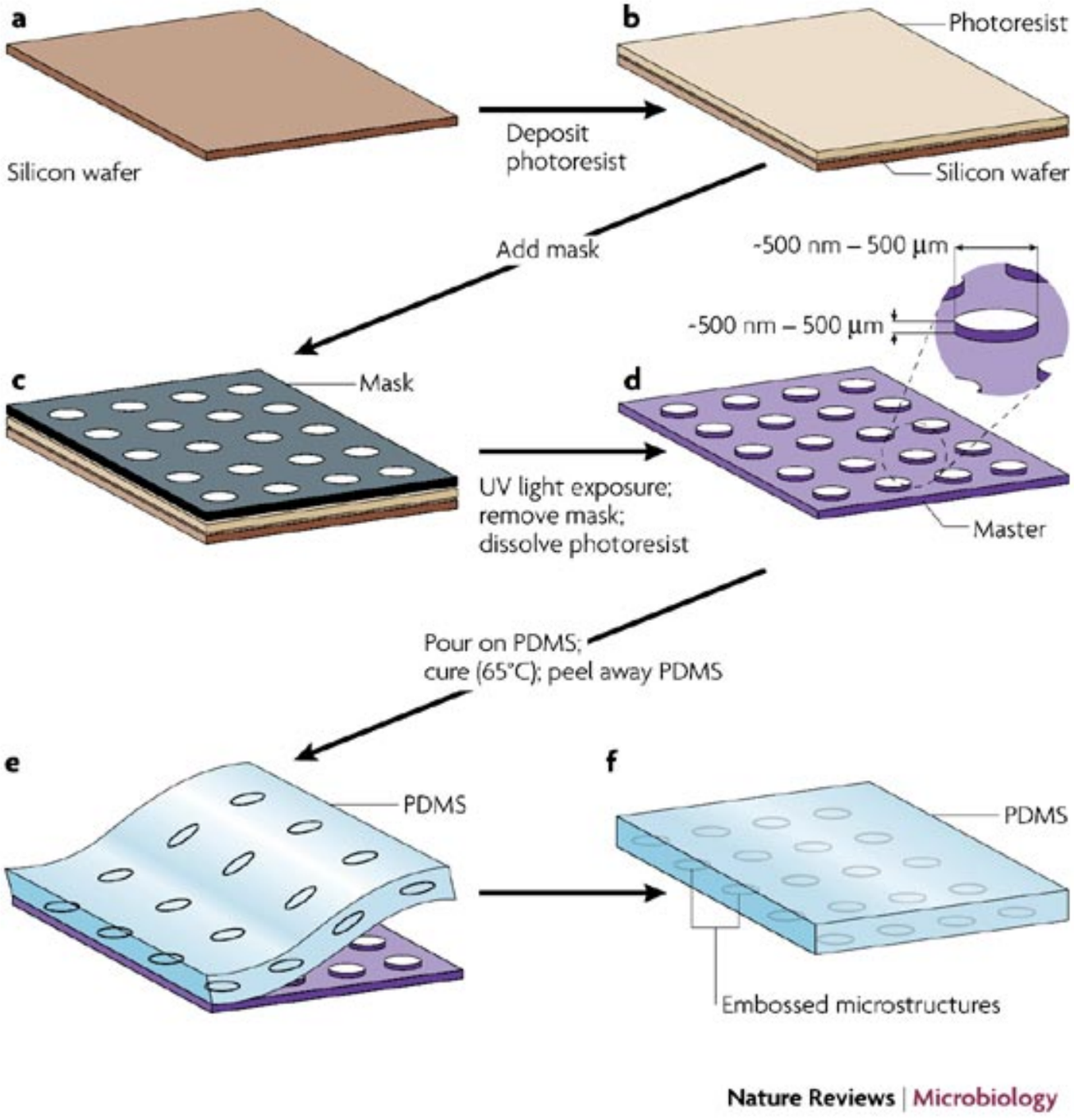
Cost in CHF/gram

Application Areas

- Basic Science
  - Synthesis
    - Protein synthesis
    - DNA synthesis
  - Screening
    - Molecular interaction screens
    - Crystallization screens
  - Cell based methods
- Health
  - Drug development
  - Drug screening
  - Diagnostics
  - Point of care devices
- Environment
- Chemistry



Device Fabrication: Soft Lithography



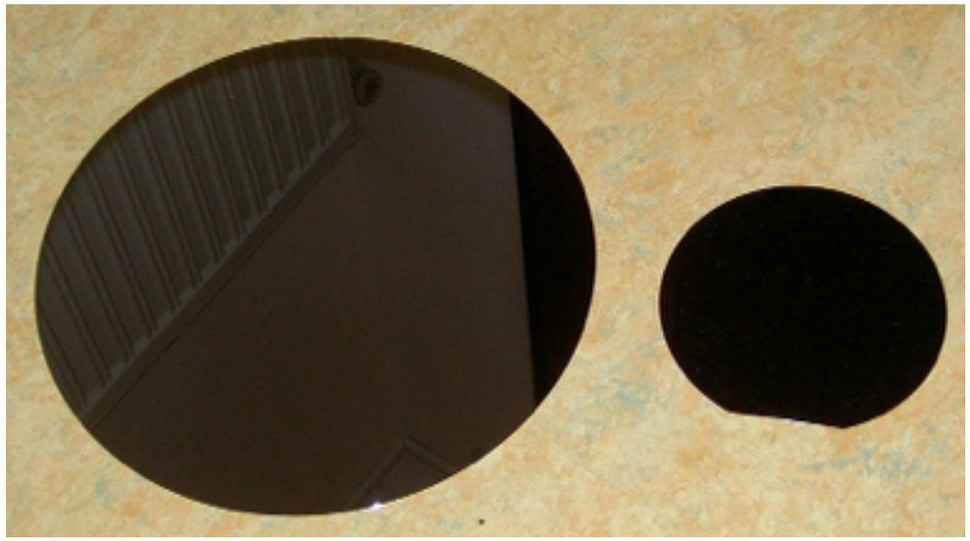
Rapid Prototyping using PDMS
D.C. Duffy, J.C. McDonald, O.J.A. Schueller, G.M. Whitesides, Anal. Chem., 70 (1998).

Process Overview

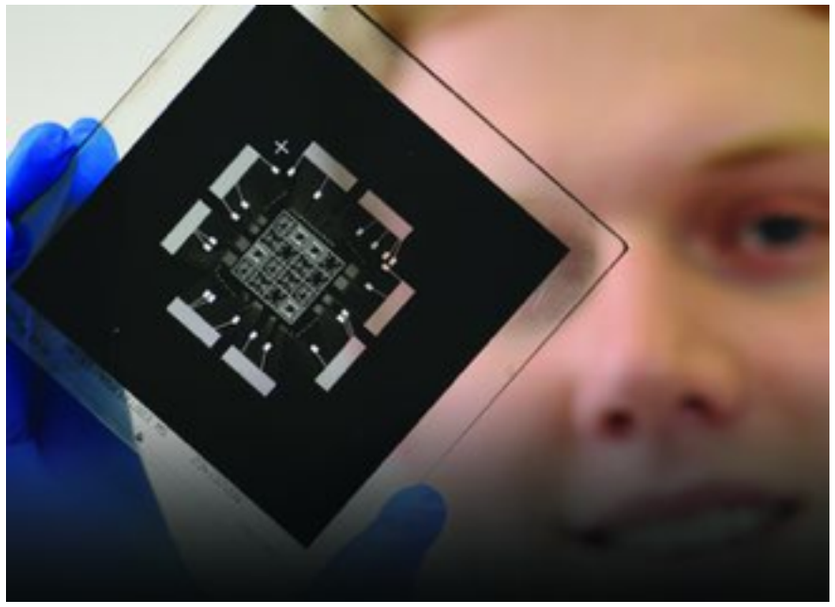
- Design device in a CAD program
- Write masks (DWL200, Laser printer)
- Coat wafer with photoresist
- Place mask on wafer and expose to light
- Develop wafer (now called a mold)
- Fabricate PDMS devices from mold

Photolithography

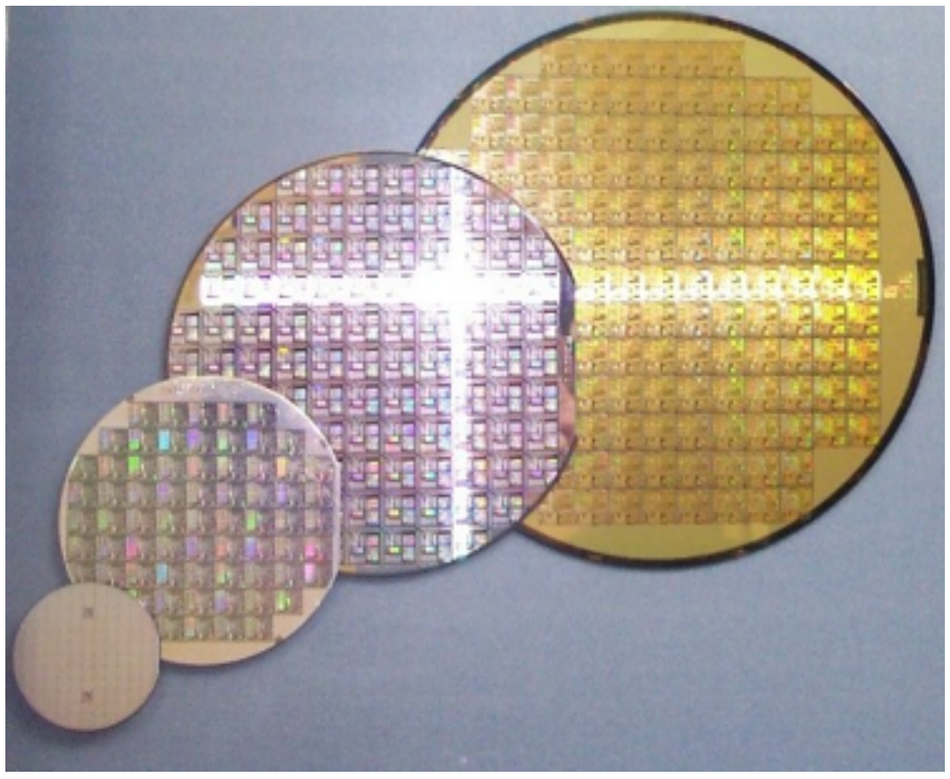
Silicon Wafer

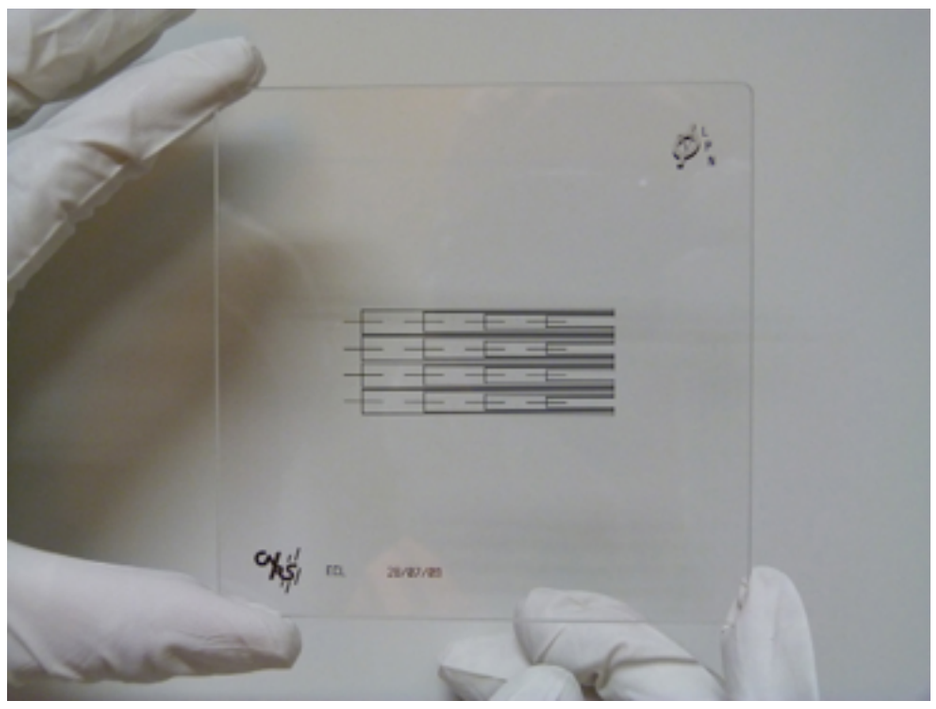


Photolithography Masks

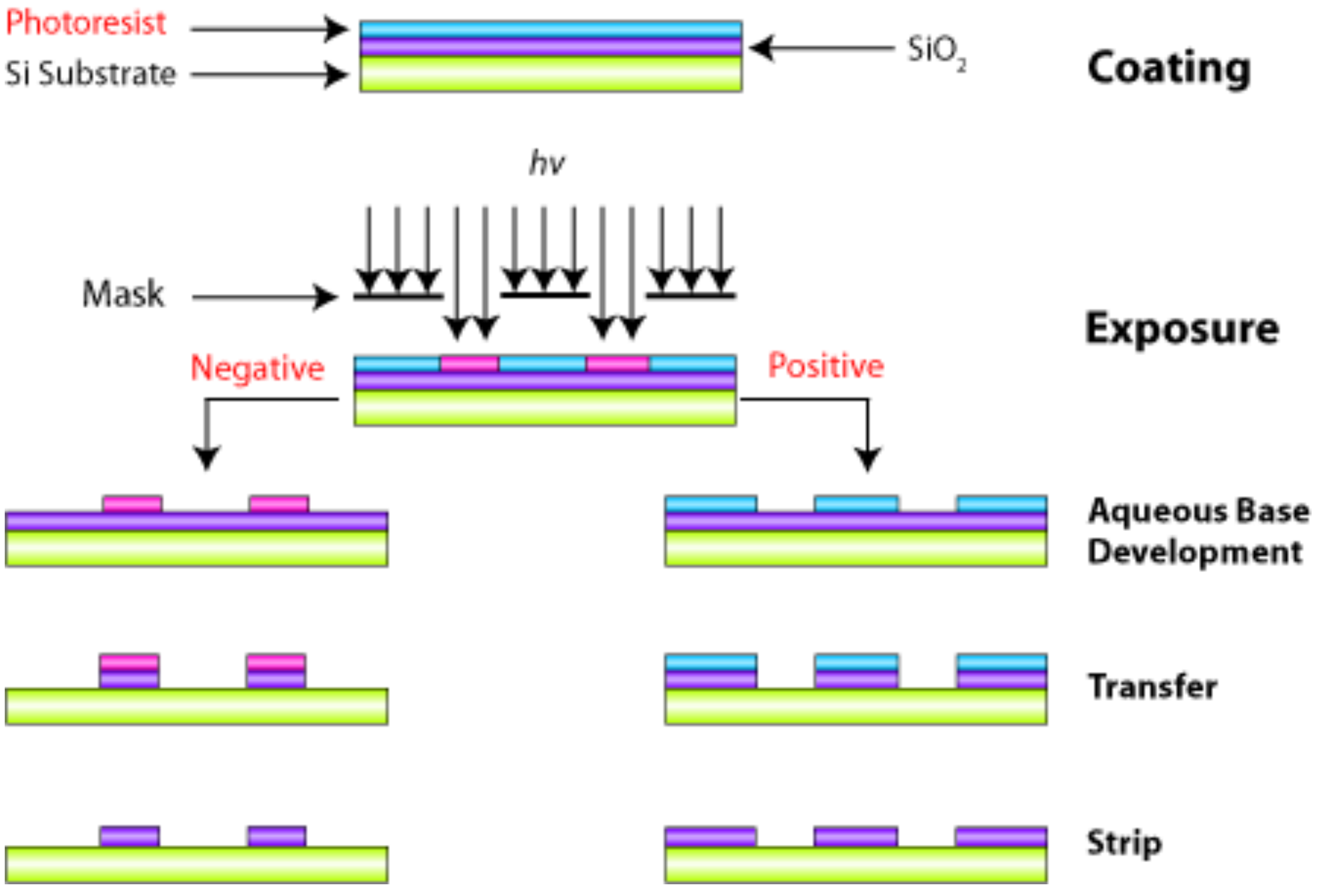


Patterned Wafers





Photolithographic Process



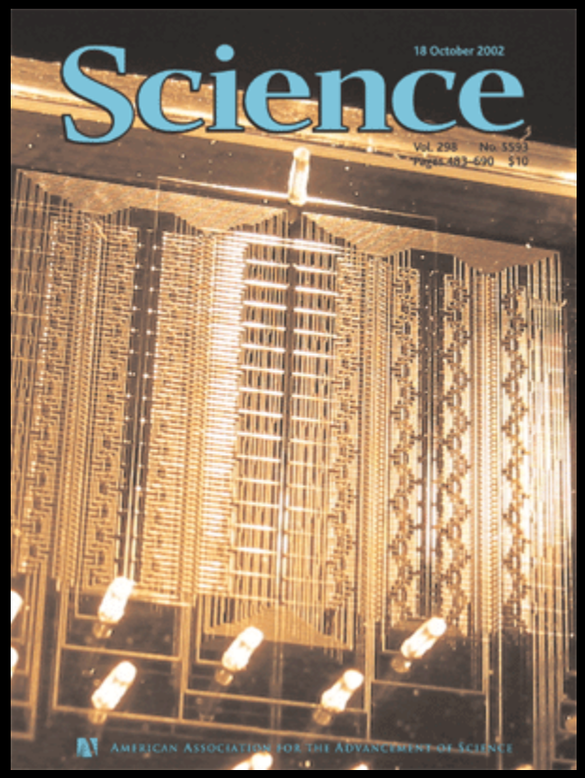
Photolithography Equipment

Spin Coater

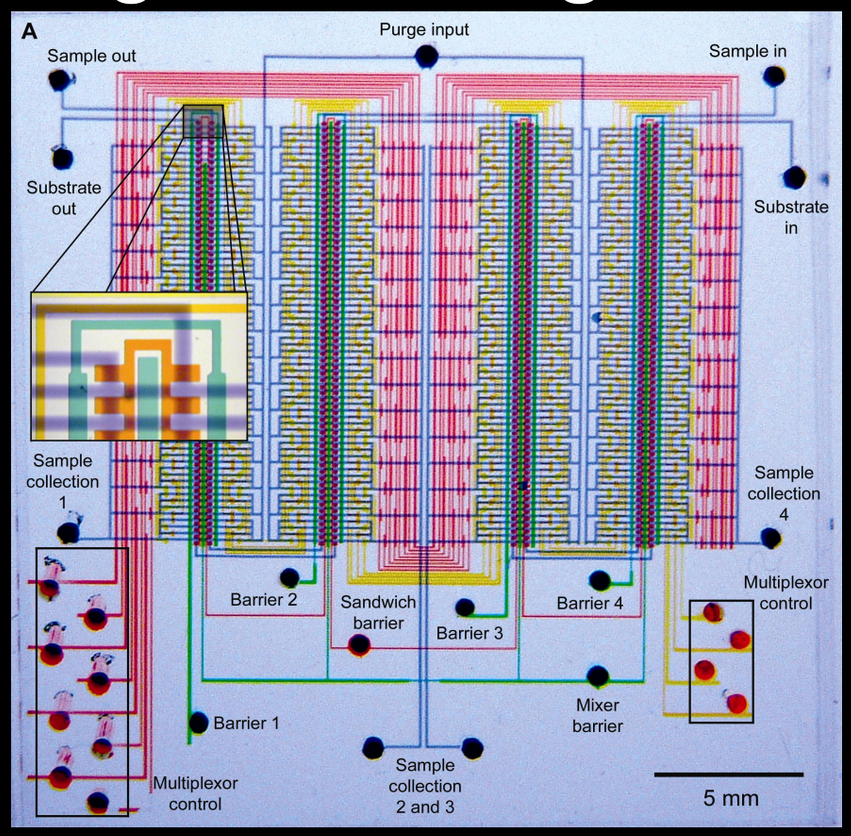
Some EPFL Labs Using Microfluidics

- John McKinney (SV)
- Melody Swartz (SV)
- Matthias Lutolf (SV)
- Yann Barrandon (SV)
- Bart Deplancke (SV)
- Martin Gijs (STI)
- Olivier Martin (STI)
- Henry Markram (SV)
- Jeffrey Hubbell (SV)
- Joerg Huelsken (SV)
- Carlotta Guiducci (STI)
- Demetri Psaltis (STI)
- Sebastian Maerkl (STI)
- Philippe Renaud (STI)

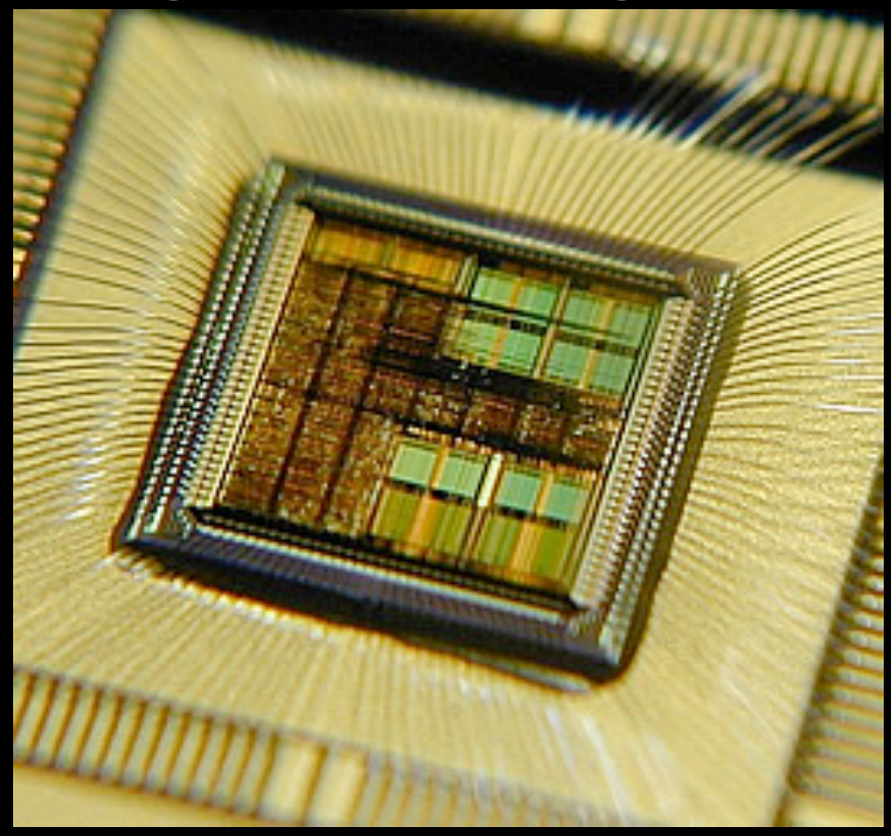
Microfluidics



Microfluidic Large-Scale Integration

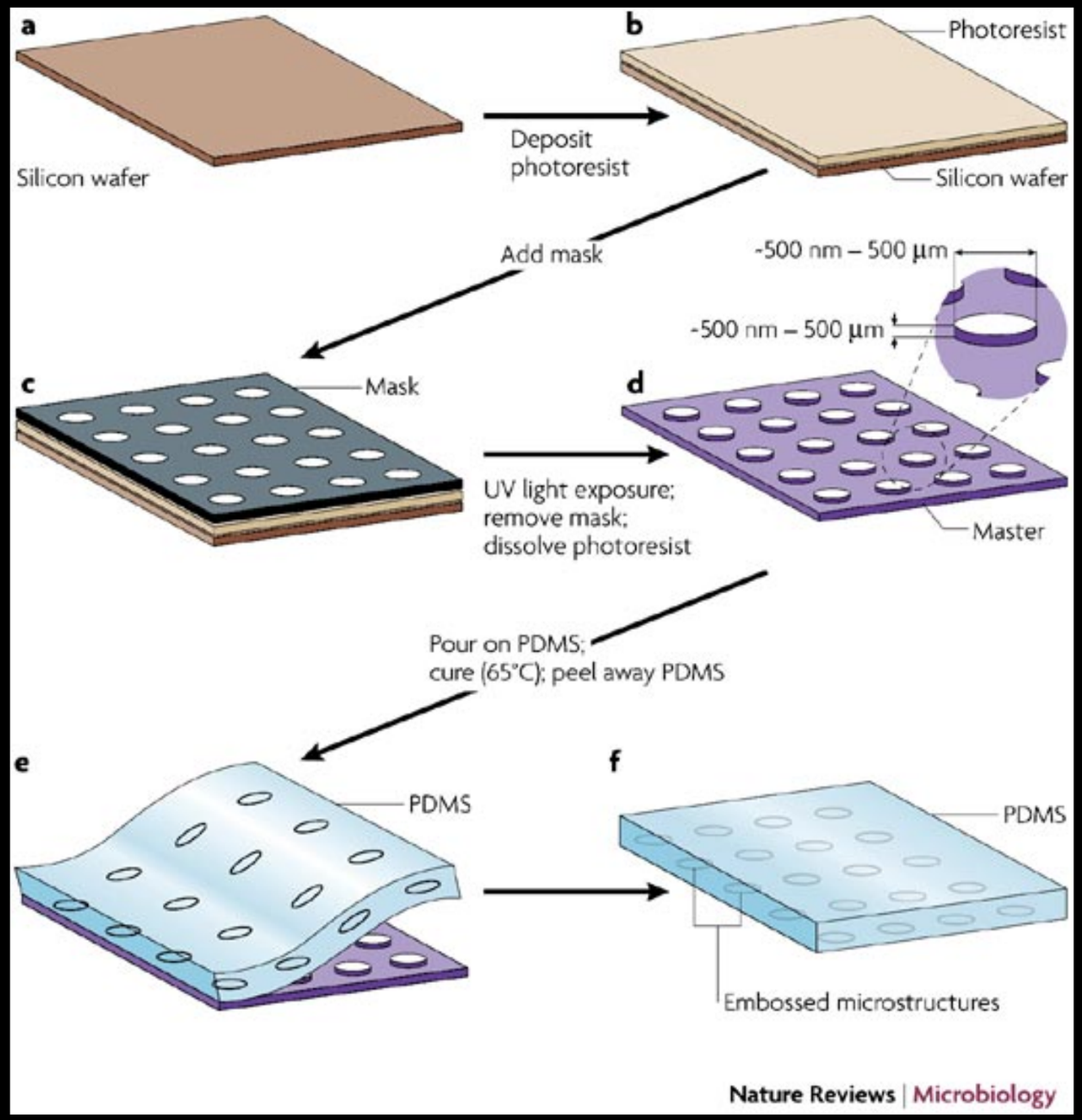


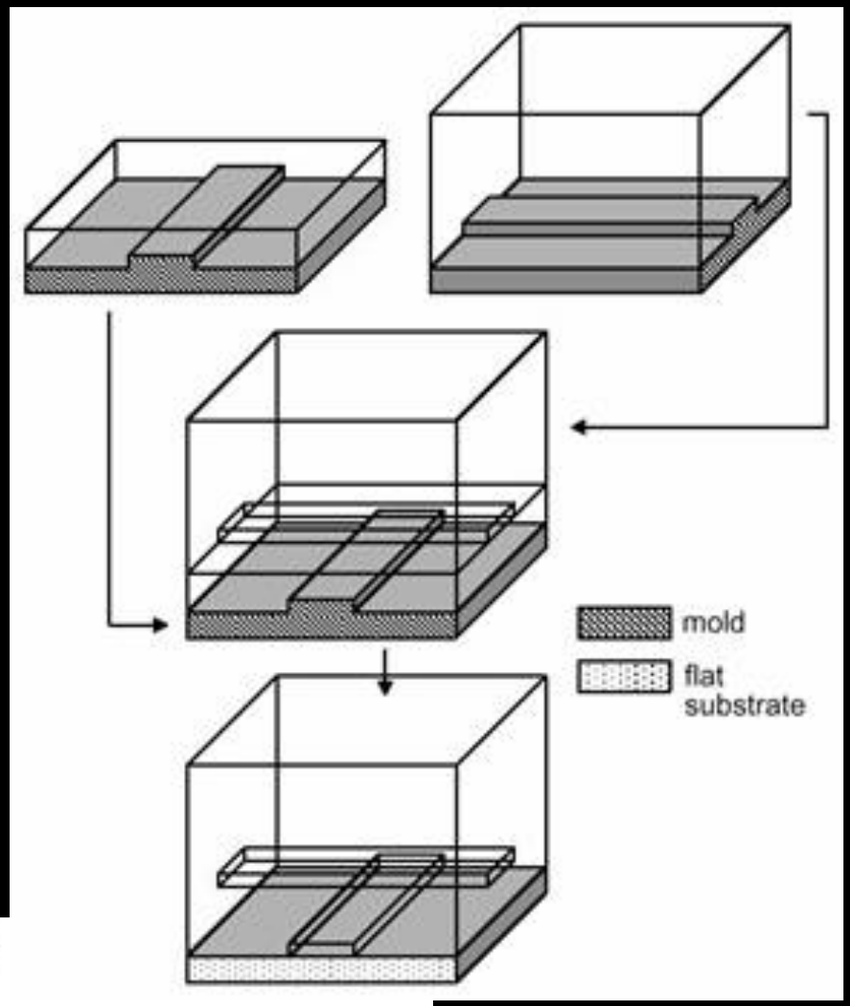
Electronic Large-Scale Integration

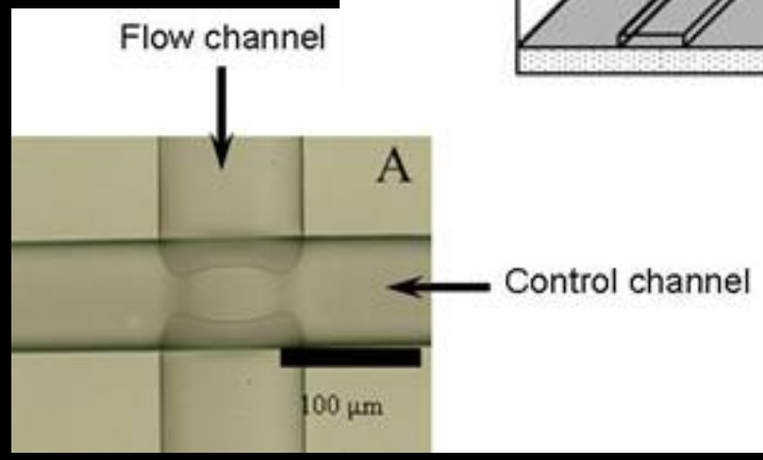


Highly-integrated devices containing thousands of micro-mechanical valves (the microfluidic analog to the transistor)

Soft Lithography

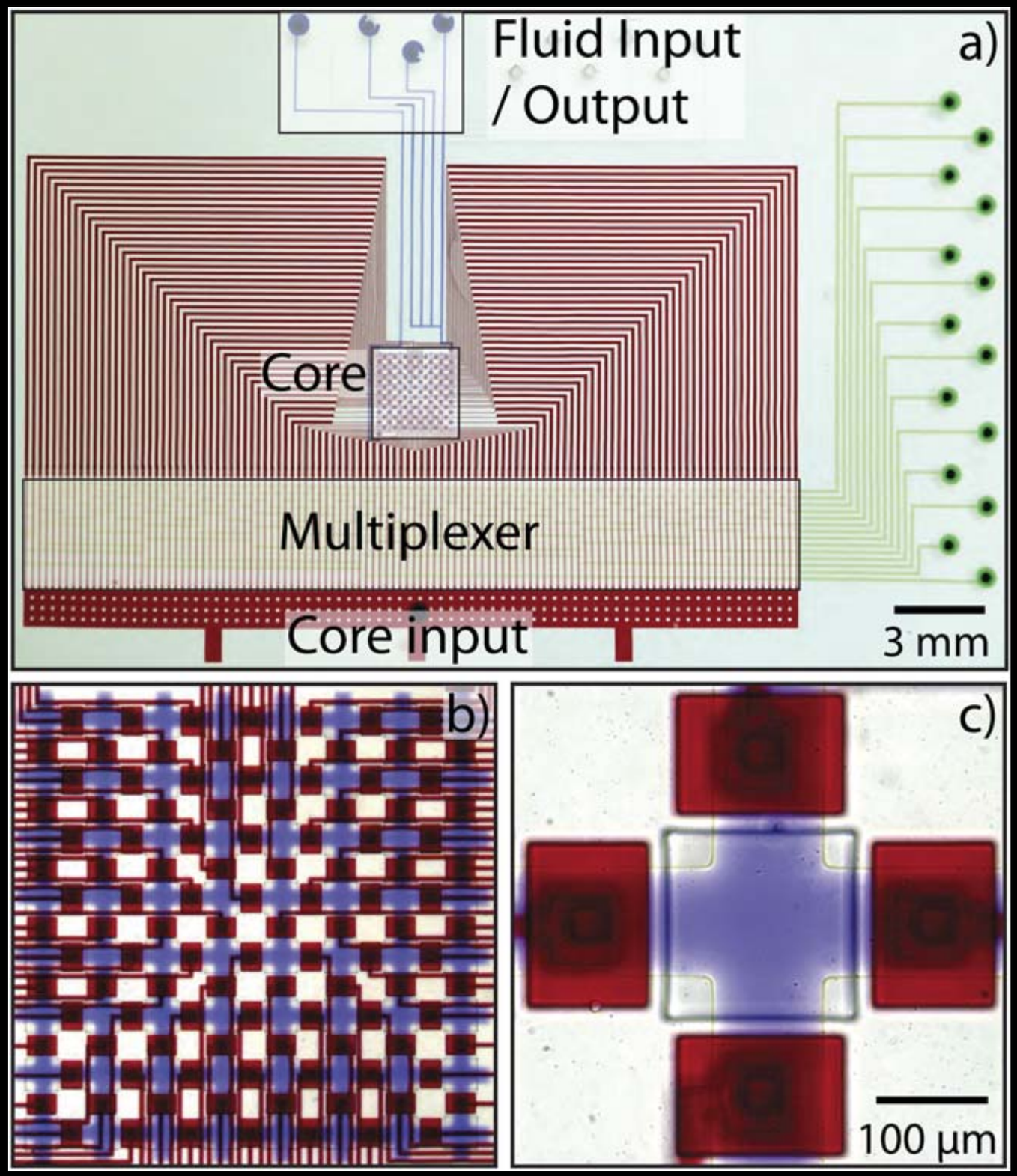




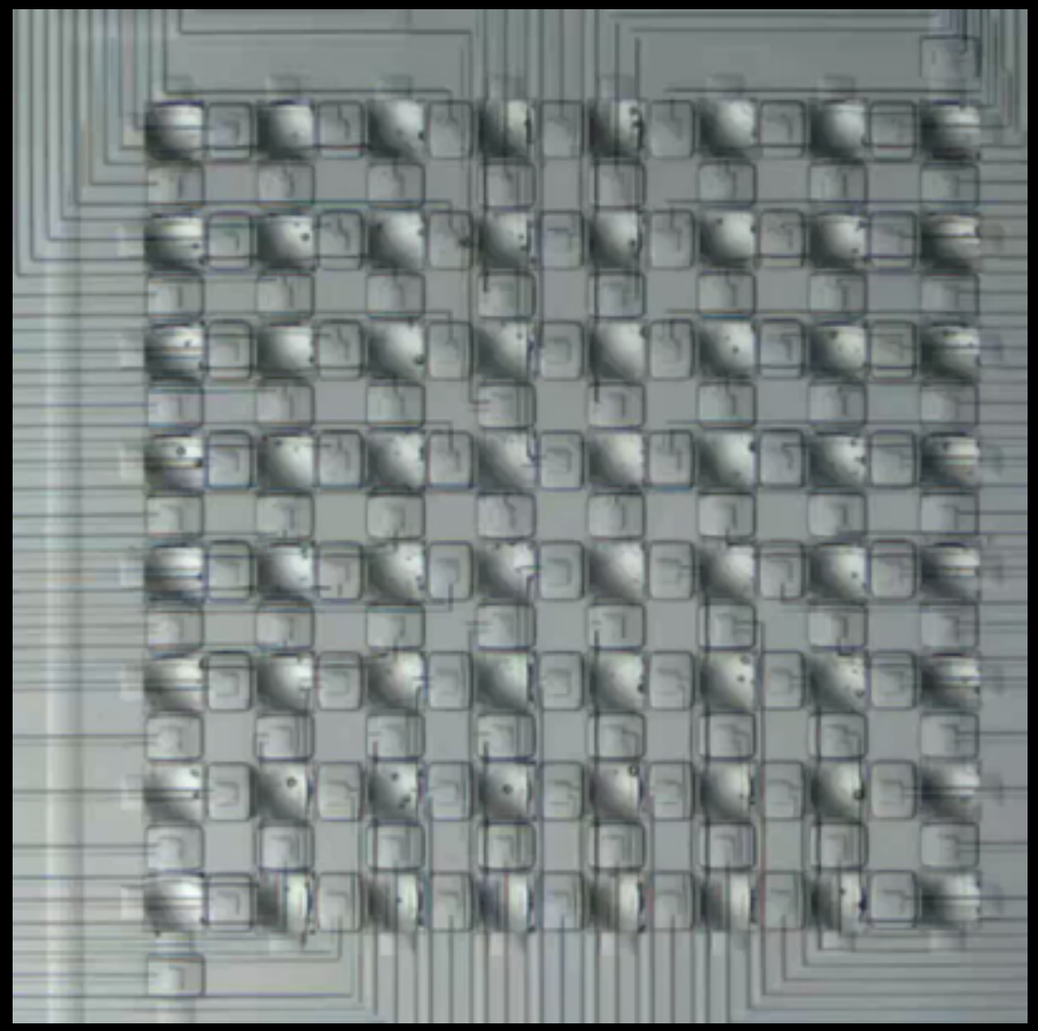


1st Micromechanical Valve
M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, S. R. Quake
Science 288, 113 (2000).
Rapid Prototyping using PDMS
D.C. Duffy, J.C. McDonald, O.J.A. Schueller, G.M. Whitesides, Anal. Chem., 70 (1998).

Complex Microfluidics



The Core contains 114 individually addressable valves.
Completely software reconfigurable architecture!



What is measured and how?

Protein / Small Molecule

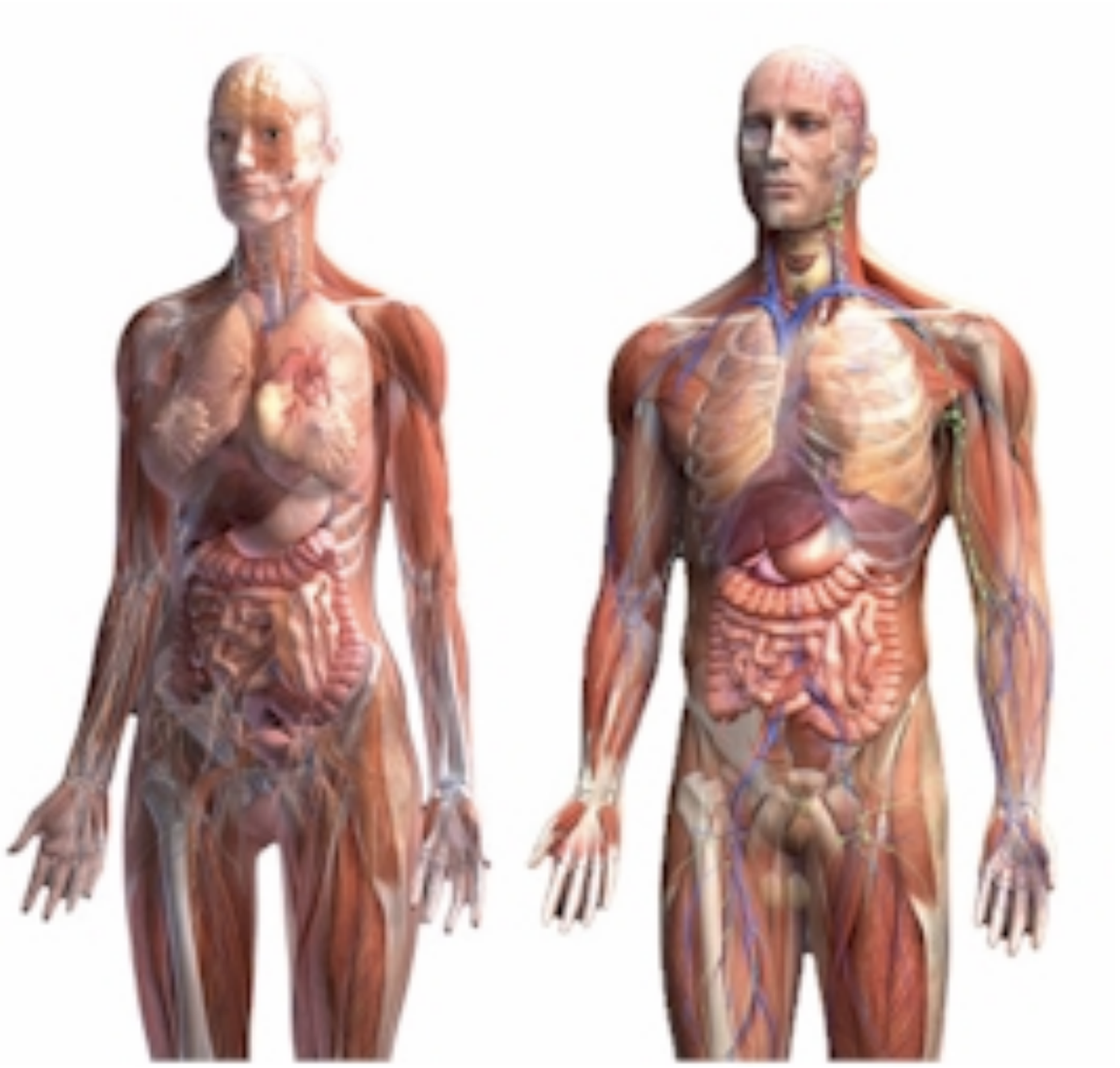
Immunoassay
Enzymatic
Mass Spectrometry
Chemical

DNA / RNA

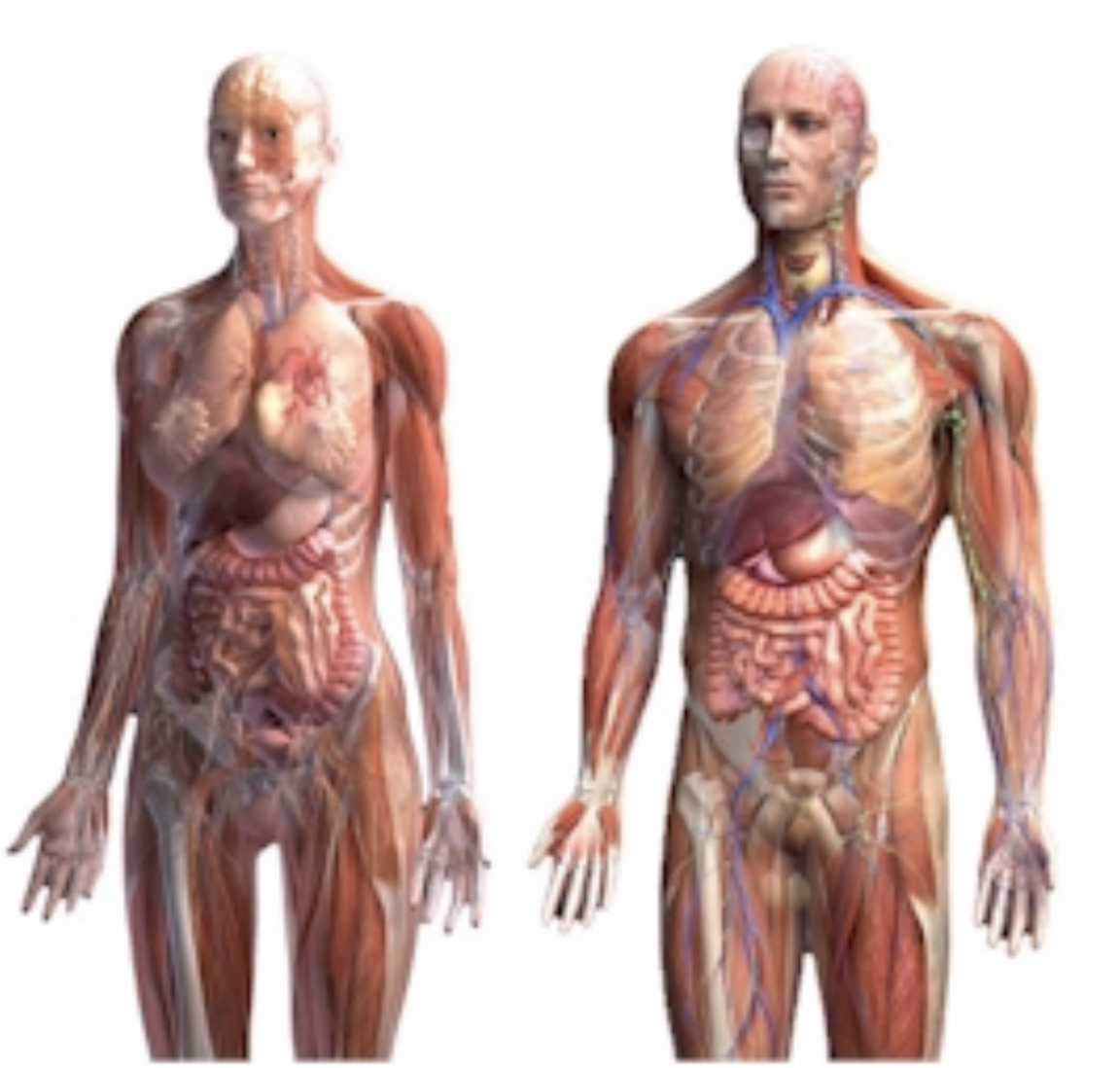
PCR
Sequencing
Hybridization

Optical
Electrical
Mechanical

Samples…



Samples…



Tears
Nasal Swab
Saliva
Sputum
Mouth Swab

Cerebrospinal Fluid

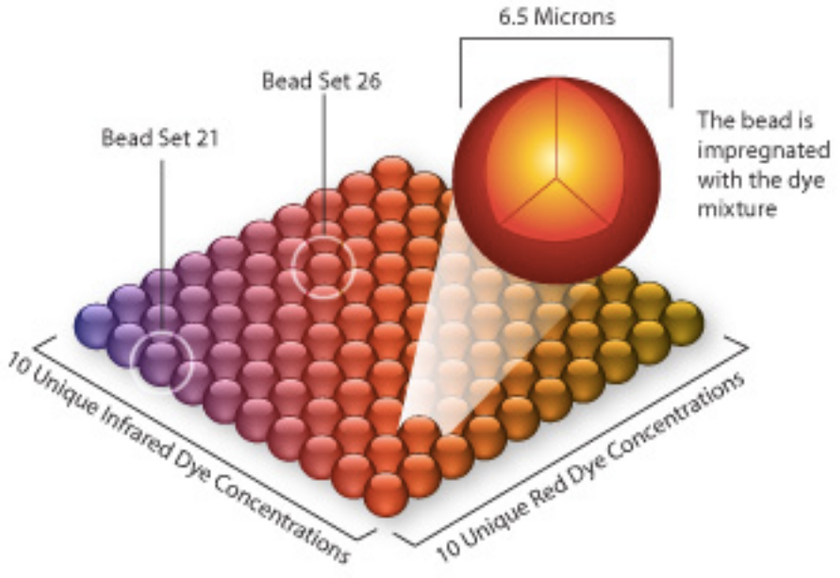
Breast Milk
Amniotic Fluid

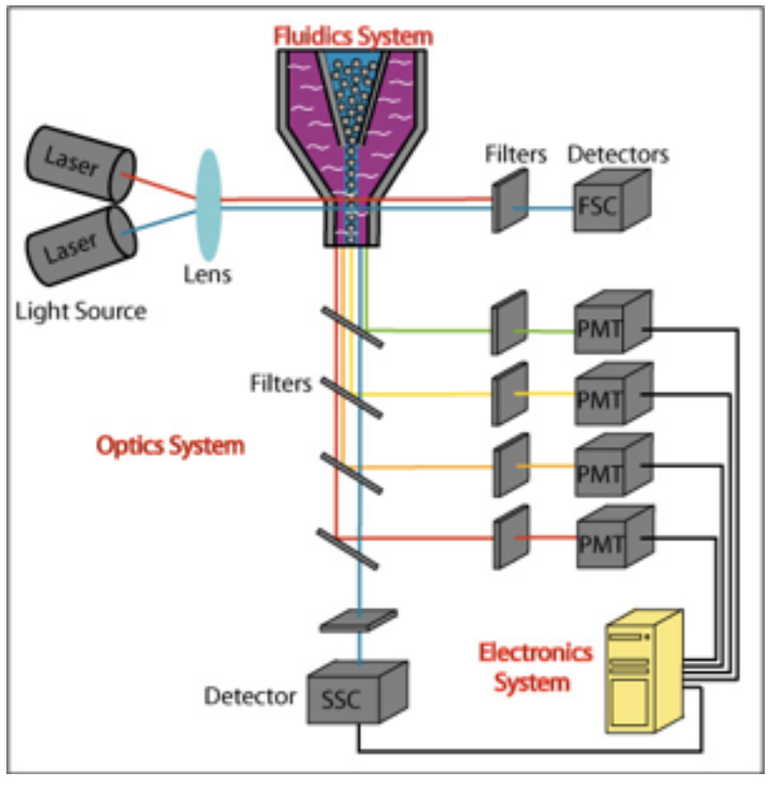
Blood
Lymph

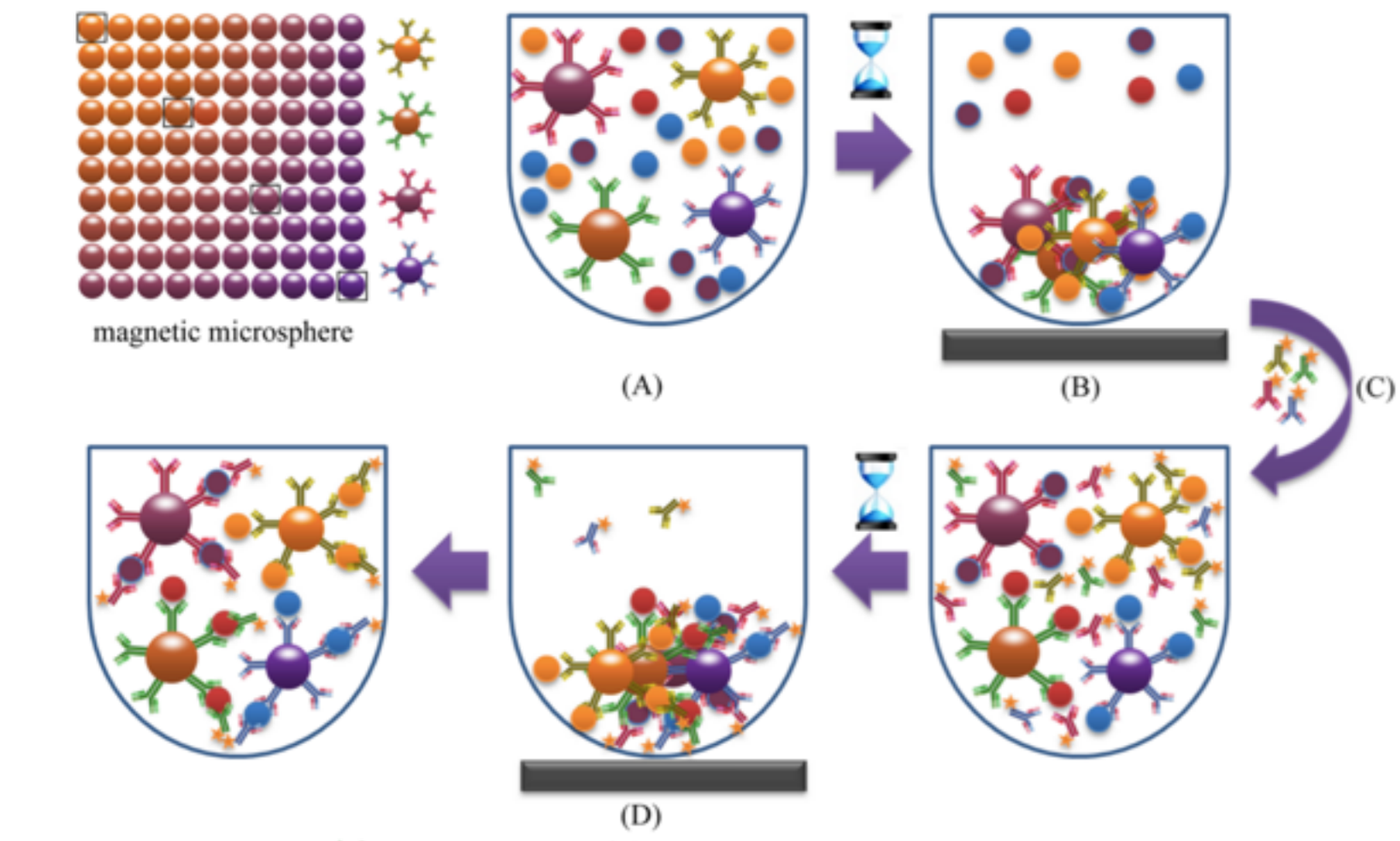
Semen

Urine
Feces

Luminex Technology







Multiplexed Detection

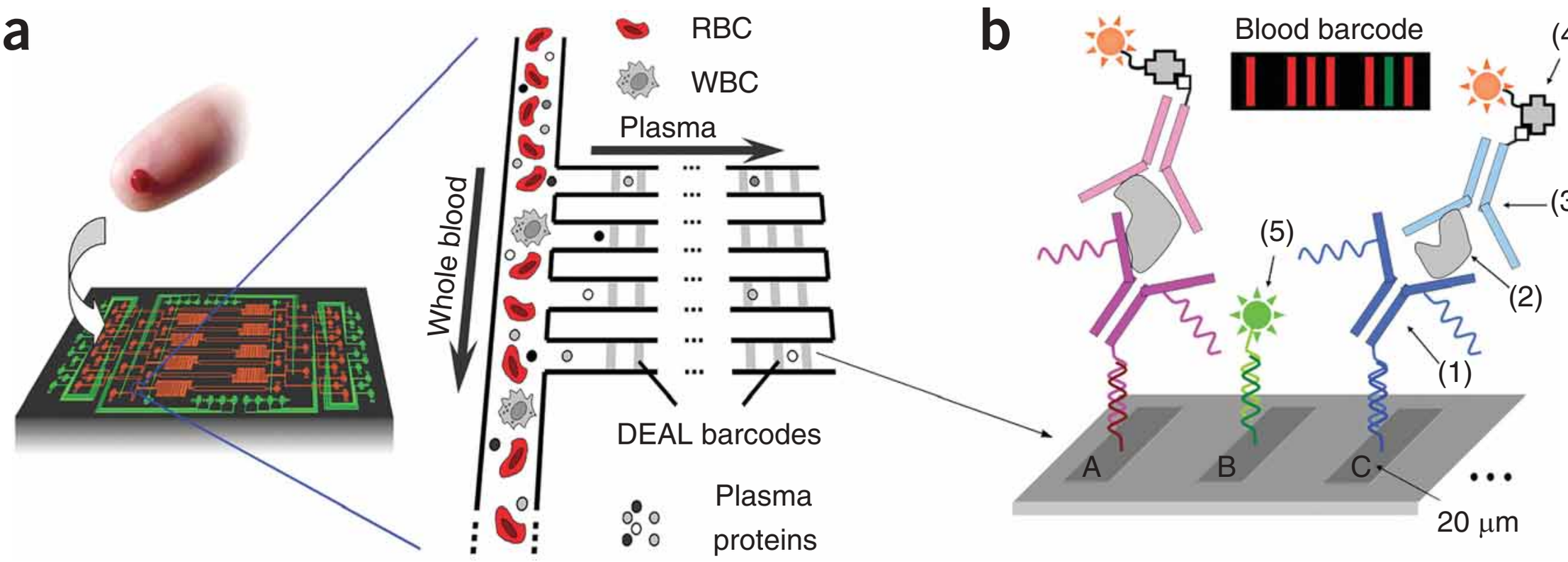


Figure 1 Design of an integrated blood barcode chip (IBBC). (**a**) Scheme depicting plasma separation from a finger prick of blood by harnessing the Zweifach-Fung effect. Multiple DNA-encoded antibody barcode arrays are patterned within the plasma-skimming channels for *in situ* protein measurements. (**b**) DEAL barcode arrays patterned in plasma channels for *in situ* protein measurement. A, B, C indicate different DNA codes. (1)–(5) denote DNA-antibody conjugate, plasma protein, biotin-labeled detection antibody, streptavidin-Cy5 fluorescence probe and complementary DNA-Cy3 reference probe, respectively. The inset represents a barcode of protein biomarkers, which is read out using fluorescence detection. The green bar represents an alignment marker.

Fan, R. et al. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. Nat Biotechnol 26, 1373–1378 (2008).

Multiplexed Detection

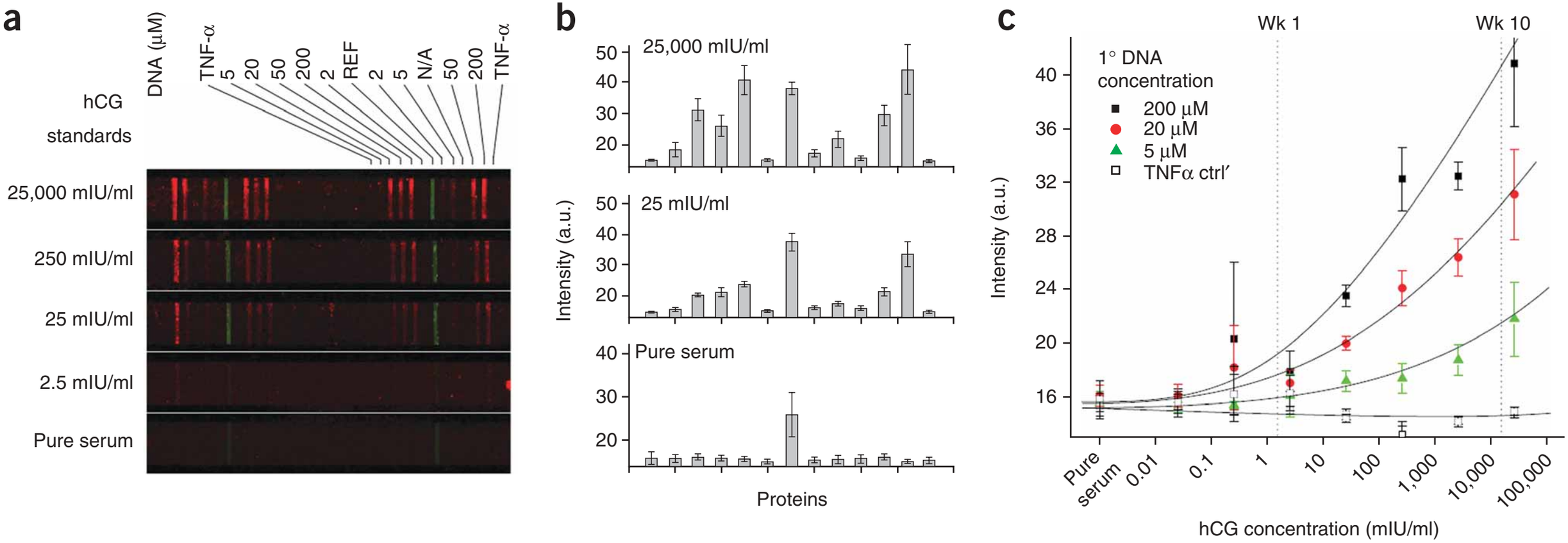
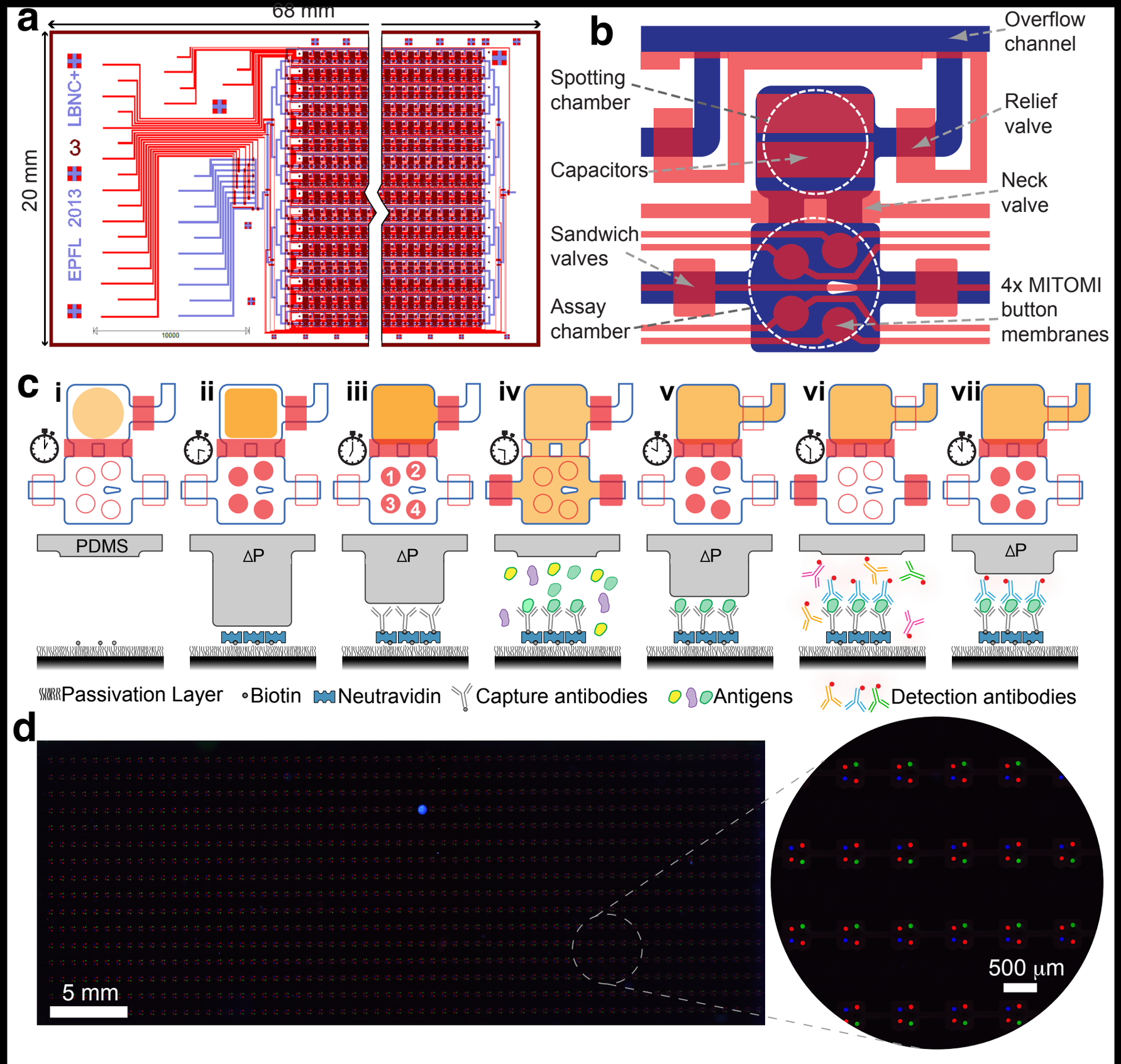


Figure 2 Measurement of human chorionic gonadotropin (hCG) in sera. (**a**) Fluorescence images of DEAL barcodes showing the measurement of a series of standard serum samples spiked with hCG. The bars used to measure hCG were patterned with DNA strand A at different concentrations. TNF-α encoded by strand B was employed as a negative control. The green bars (strand M) serve as references. (**b**) Quantification of the full barcodes for three selected samples. (**c**) Mean values of fluorescence signals corresponding to three sets of bars with different DNA loadings. Broken lines indicate the typical physiological levels of hCG in sera after 1 or 10 weeks of pregnancy. Error bars, 1 s.d.

Fan, R. et al. Integrated barcode chips for rapid, multiplexed analysis of proteins in microliter quantities of blood. Nat Biotechnol 26, 1373–1378 (2008).

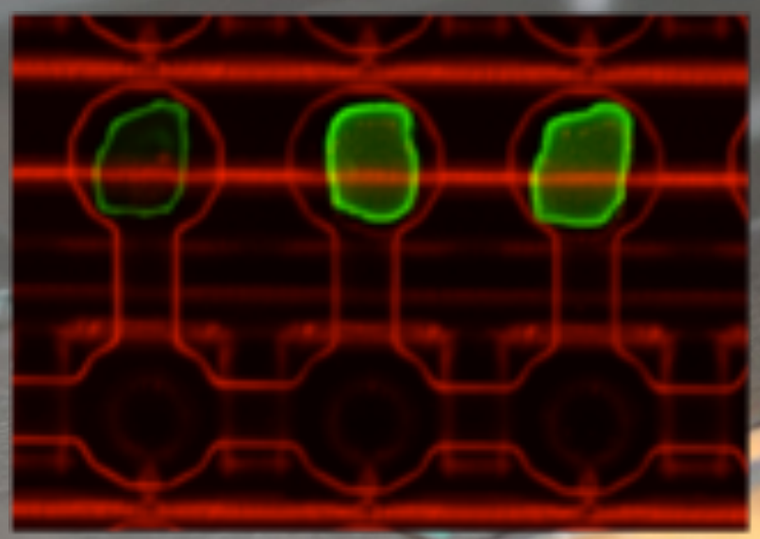
High-throughput Biomarker Quantitation

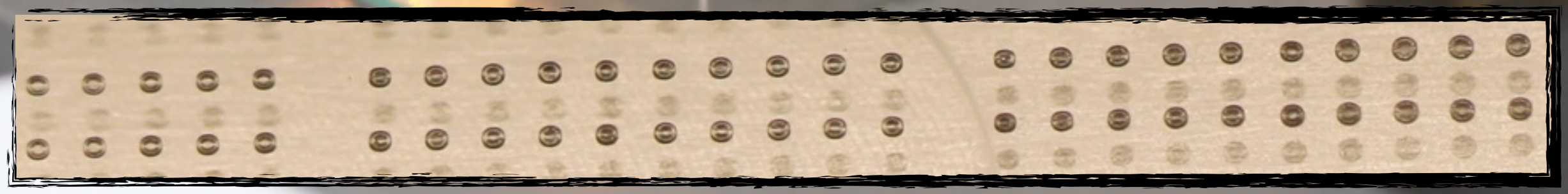
4,096 Immunoassays on a Single Device

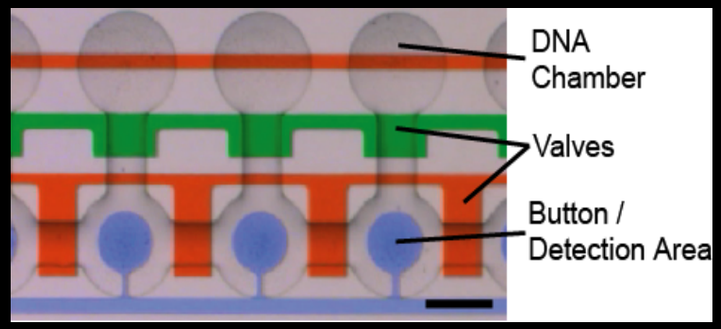


Programming Microfluidic Devices

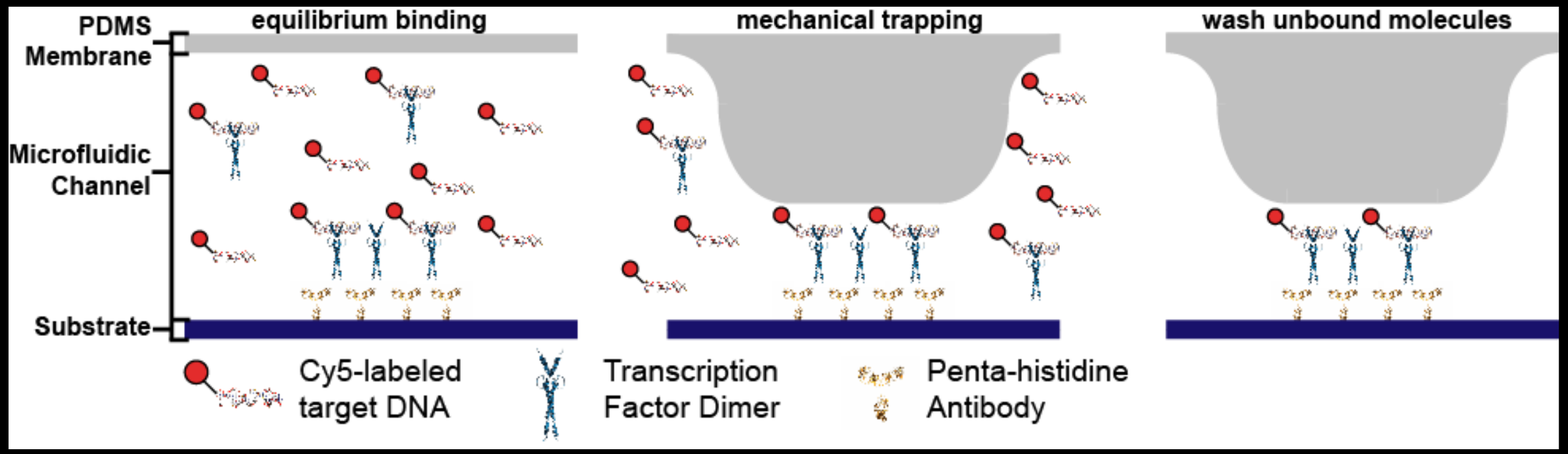
Use a std. DNA microarrayer to solve the world-to-chip interface problem!



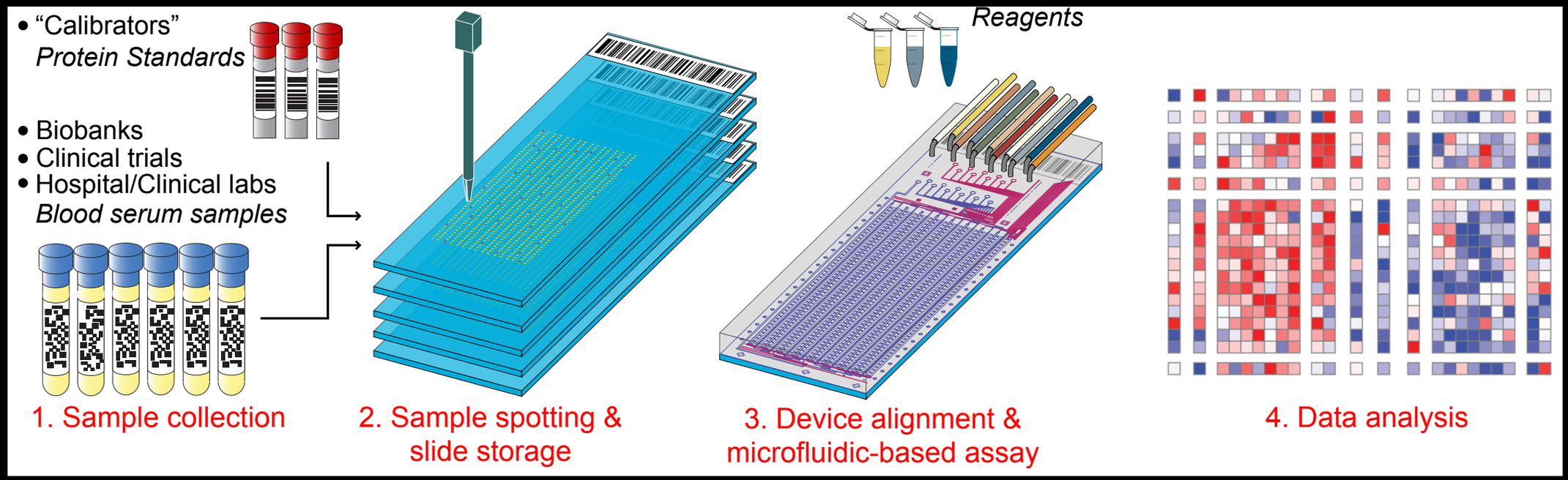



DNA
Chamber
Valves
Button /
Detection Area

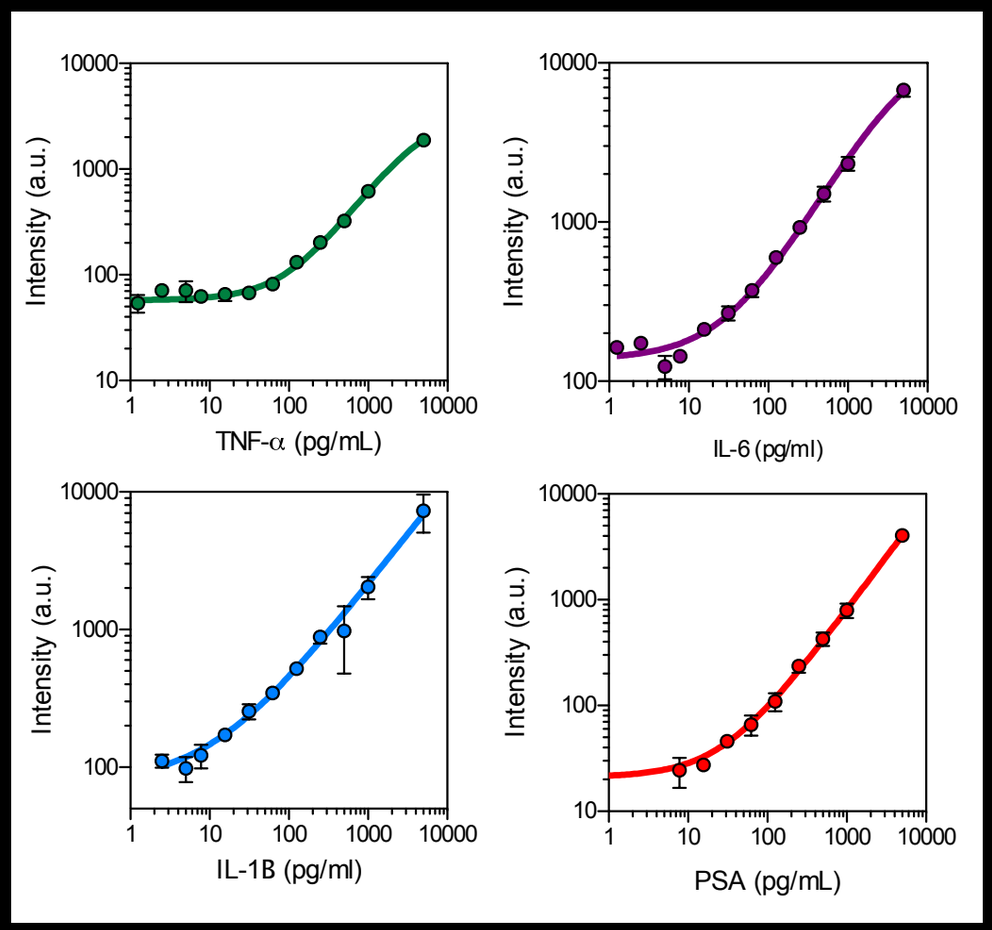
MITOMI


PDMS
Membrane
Microfluidic
Channel
Substrate
equilibrium binding
mechanical trapping
wash unbound molecules
Cy5-labeled
target DNA
Transcription
Factor Dimer
Penta-histidine
Antibody

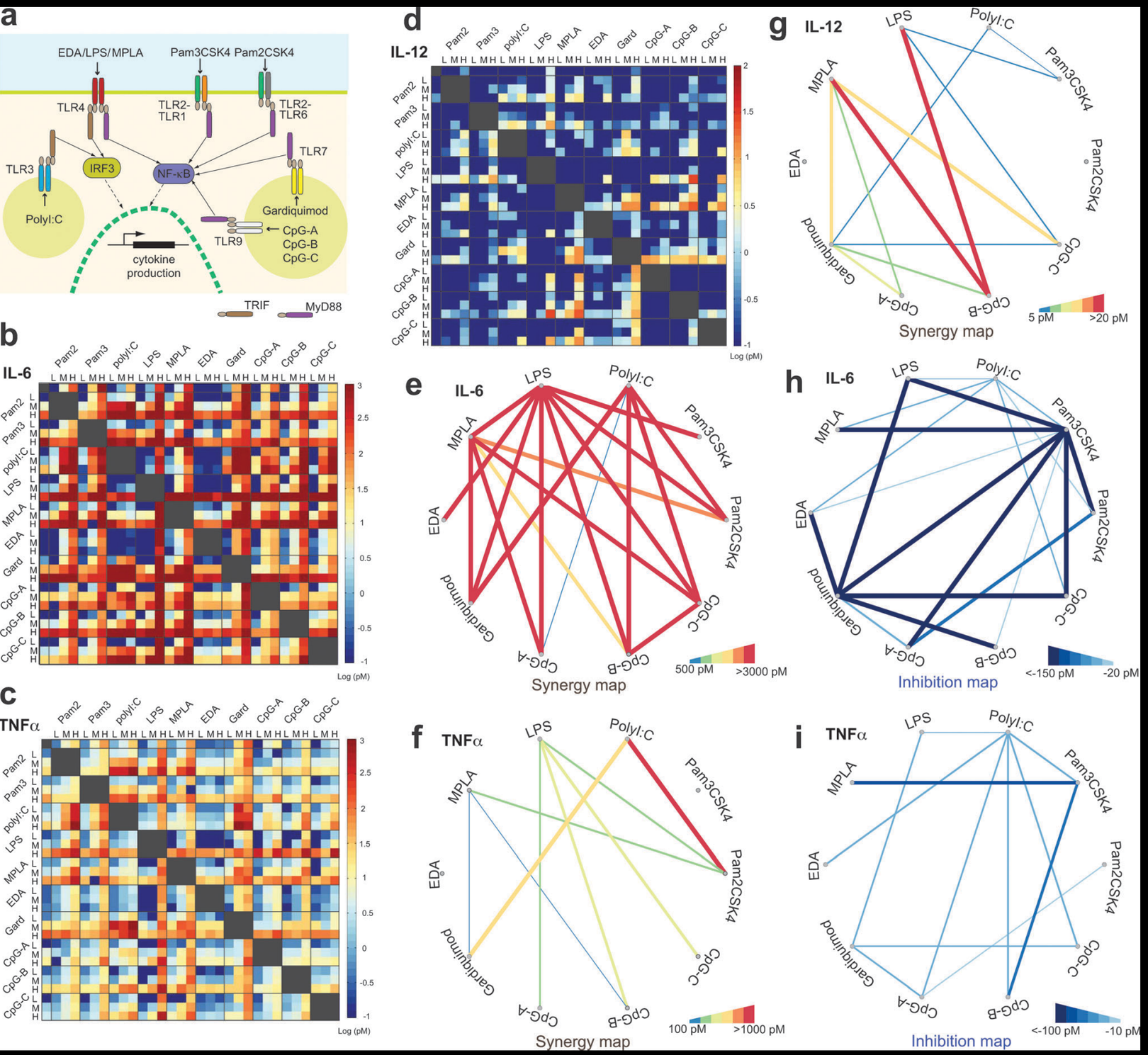
High-Content Diagnostics



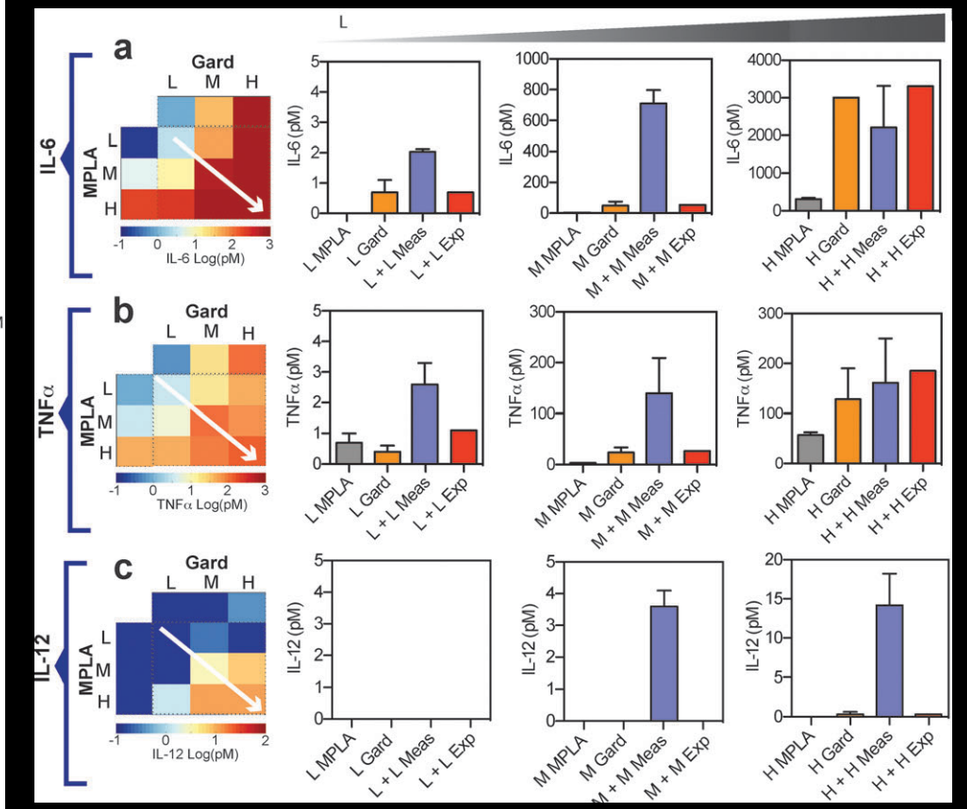
- 1,024 samples on 10 cm^2
- Multiplexed - 4 biomarkers per sample
- 4,096 assays per device
- Sensitivity - 100's fM from nL-volume samples (zmol)
- Matrix insensitive - human serum, cell culture media, buffers, etc.
- Total cost of reagents per assay = *€ 0.0001*
- Total cost per chip (reagents) = € 0.1



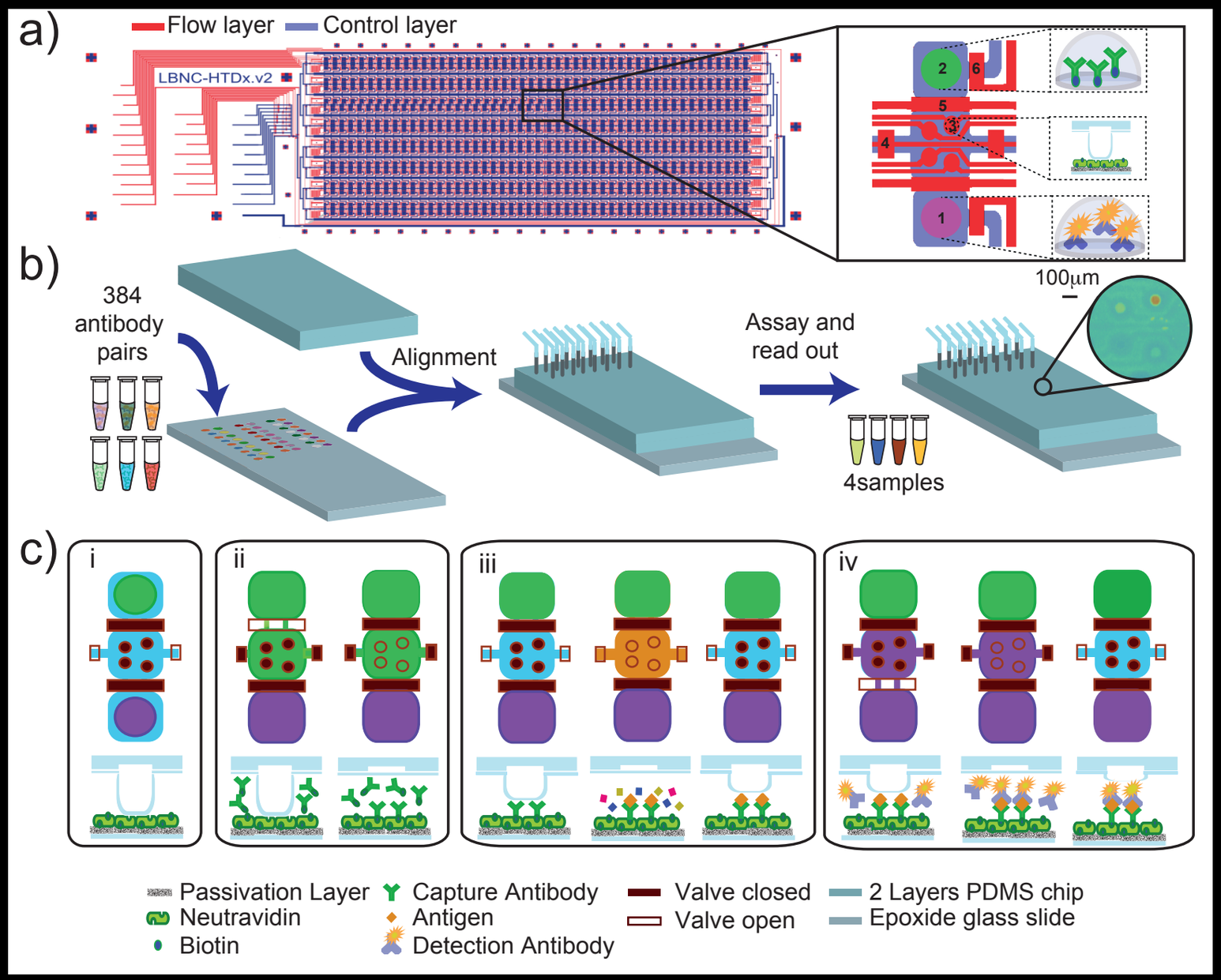
Large-Scale Adjuvant Screening

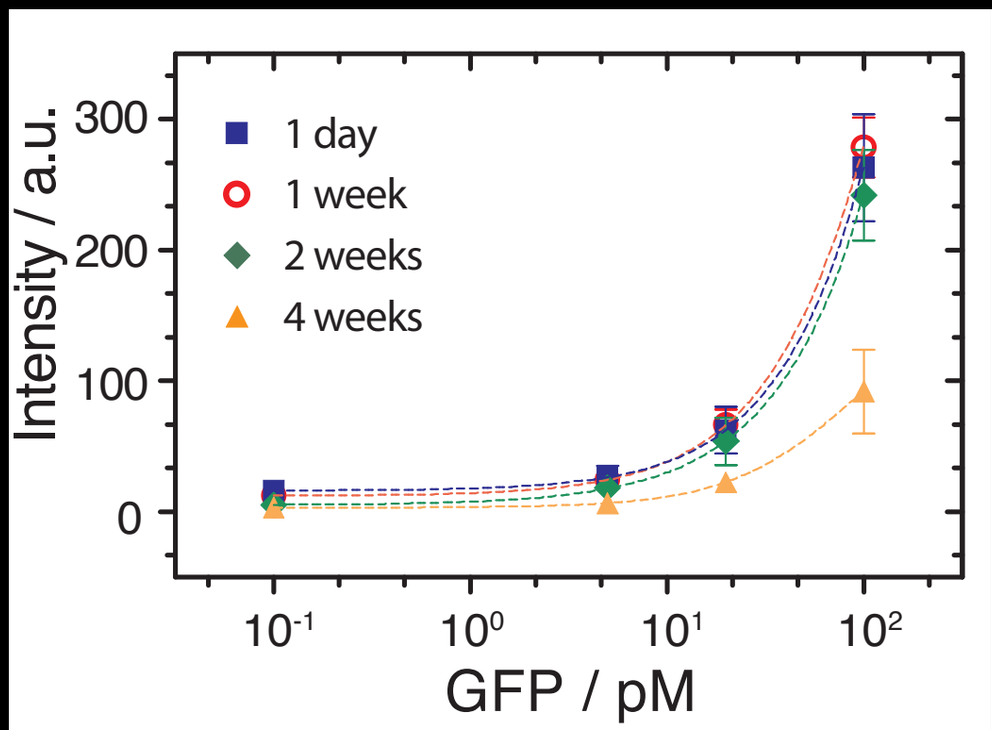


- **10,000+** measurements
- **405** binary adjuvant combinations
- **4 cytokines** measured for each combination



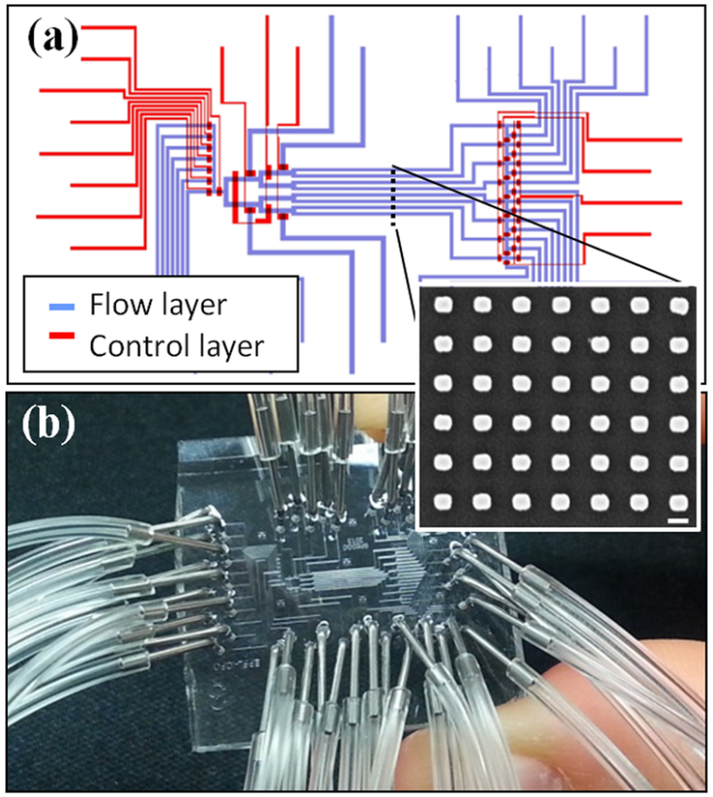
Multiplexed Biomarker Quantitation

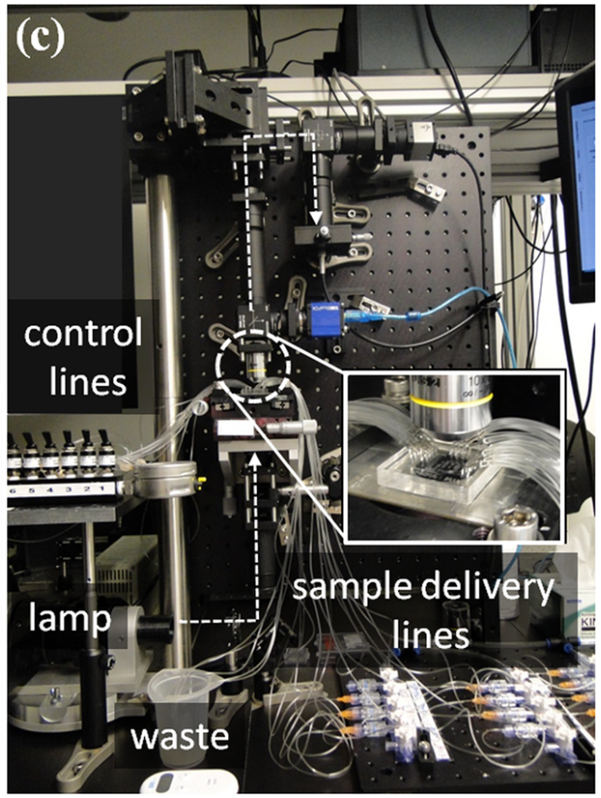


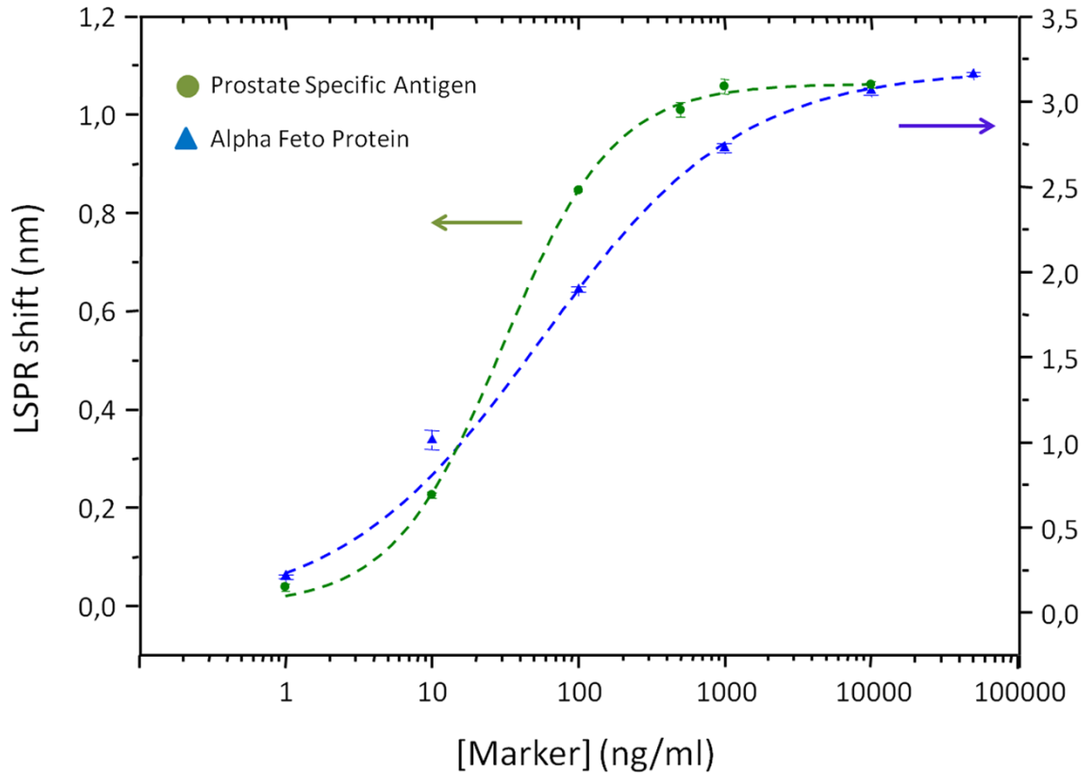


- **384** biomarkers per device
- **4** samples
- **1,536** assays per device
- fully automated
- device assembly and usage are decoupled
- device can be stored at ambient conditions for weeks prior to use
- academic, research, and point of care applications

Localized Surface Plasmon Resonance

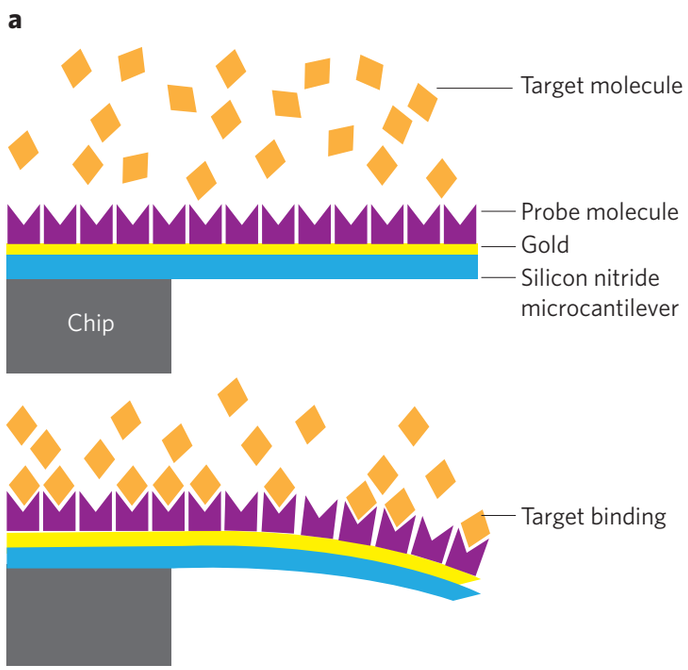


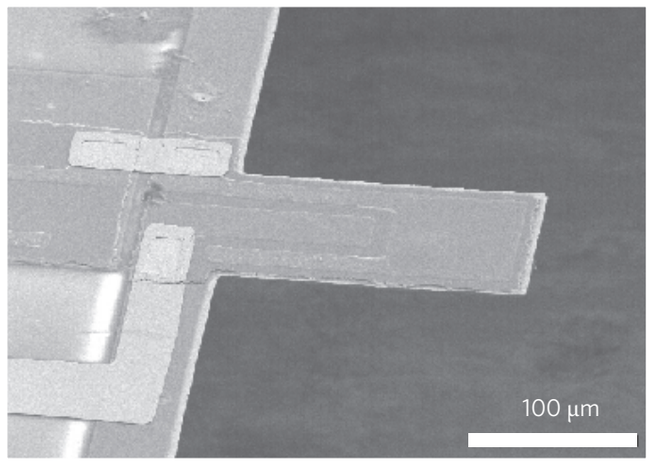


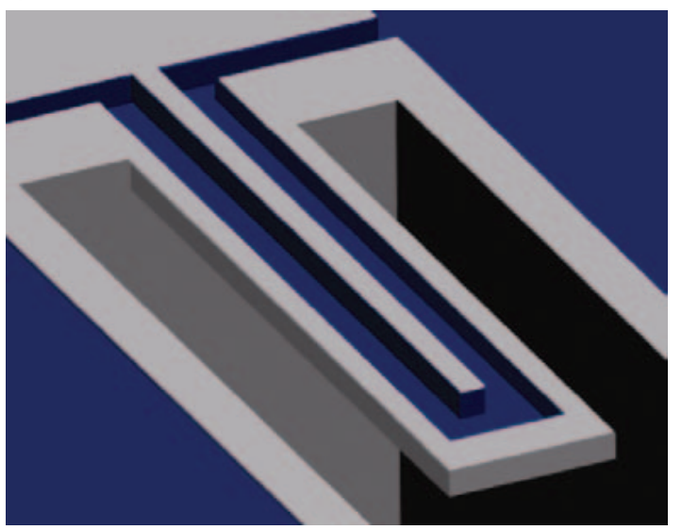


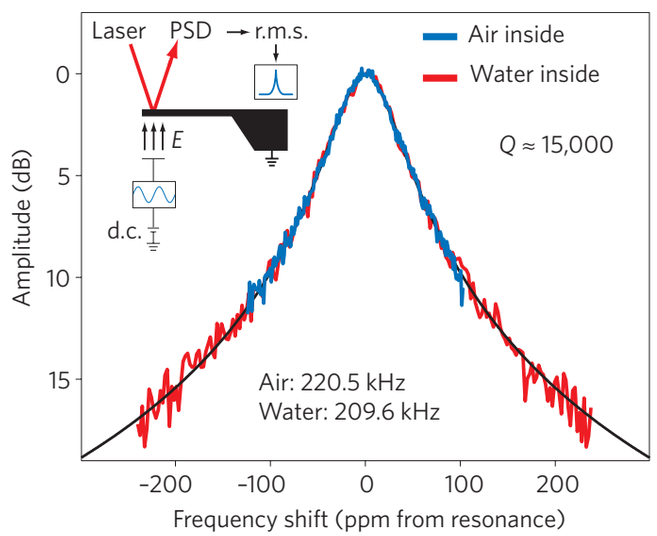
Aćimović, S. S. et al. LSPR Chip for Parallel, Rapid, and Sensitive Detection of Cancer Markers in Serum. Nano Lett 140422102938004 (2014). doi:10.1021/nl500574n

Mechanical Based Detection (optical/electrical)









Arlett, J. L., Myers, E. B. & Roukes, M. L. Comparative advantages of mechanical biosensors. Nature Nanotechnology 6, 203–215 (2011).

Real-Time Monitoring

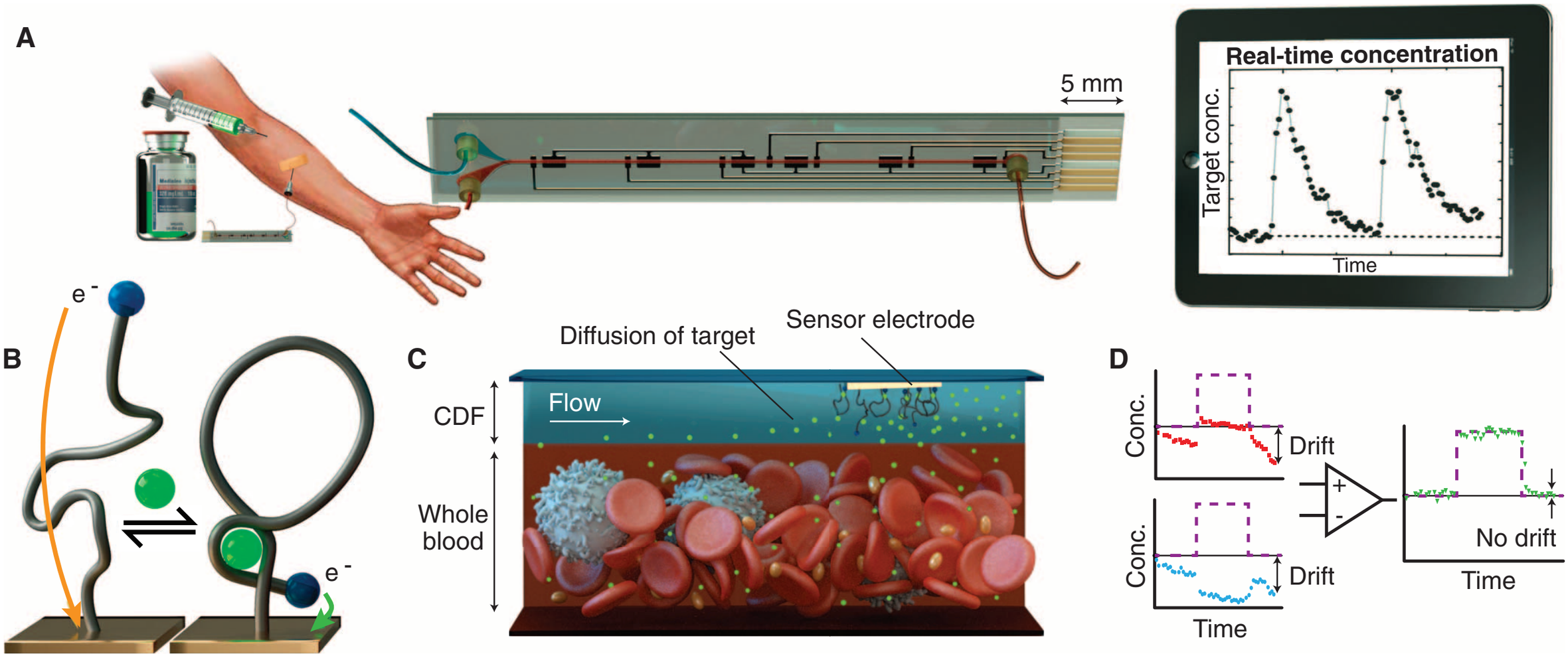
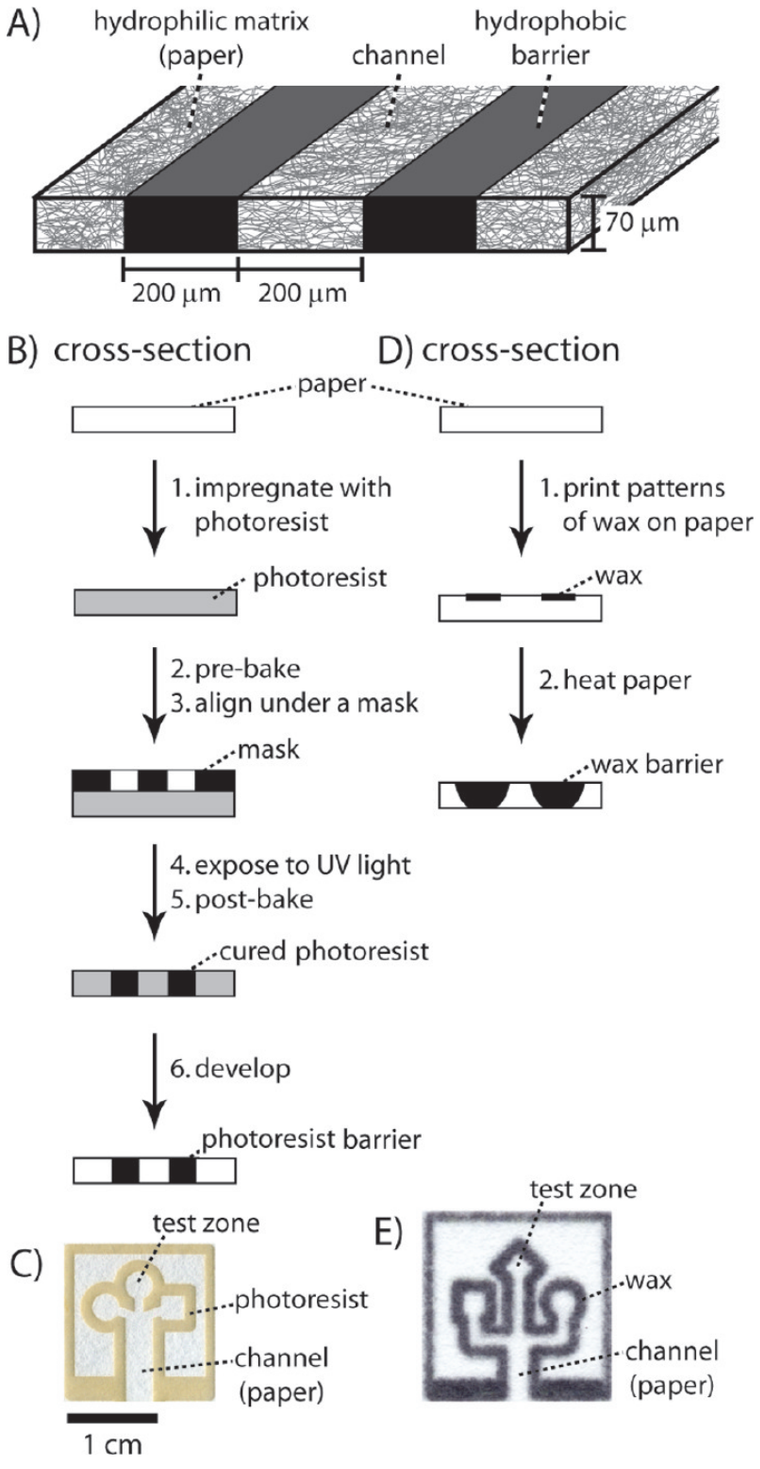
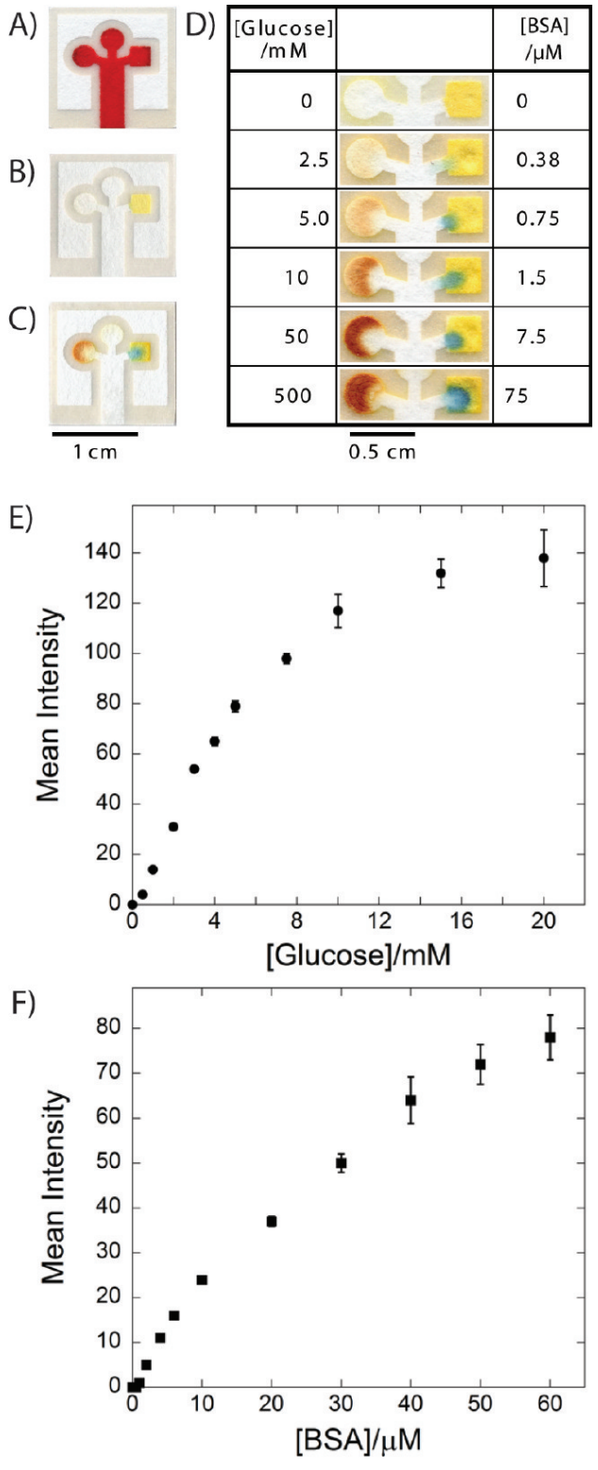


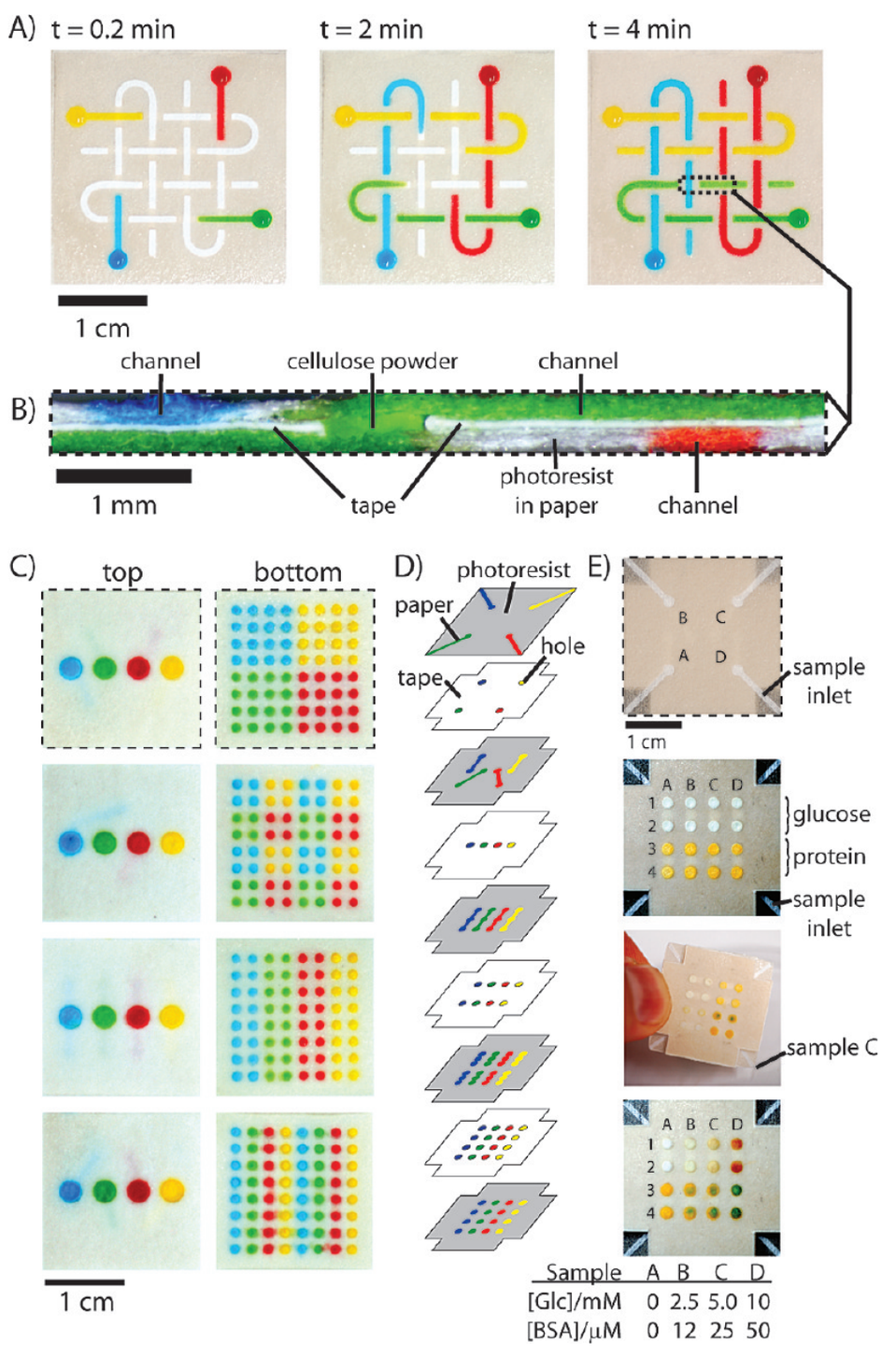
Fig. 1. MEDIC overview. MEDIC achieves real-time quantitative measurement of specific molecules in the blood of living animals. (**A**) Envisioned setup, where the MEDIC chip is connected to the patient's bloodstream to measure drug pharmacokinetics. (**B**) The aptamer probe is tethered to the gold electrode. Binding of target (green) induces a reversible conformational change in the probe, increasing the rate of electron transfer between an electrochemical redox reporter (blue) and a microfabricated electrode, yielding a measurable current change, shown in (A) as a function of time. (**C**) The continuos-flow diffusion filter (CDF), formed by vertically stacked laminar flow of buffer (blue) and blood (red), as shown in the microfluidic device in (A), permits access to the target molecules while selectively excluding blood-borne interferents. (**D**) Signal-on (red) and signal-off (blue) both exhibit significant drift in response to a pulse of target (purple). Kinetic differential measurement (KDM; green) improves accuracy of real-time current measurements by minimizing drift and enhancing SNR.

Ferguson, B. S. et al. Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. Science Translational Medicine 5, 213ra165–213ra165 (2013).

Paper Diagnostics

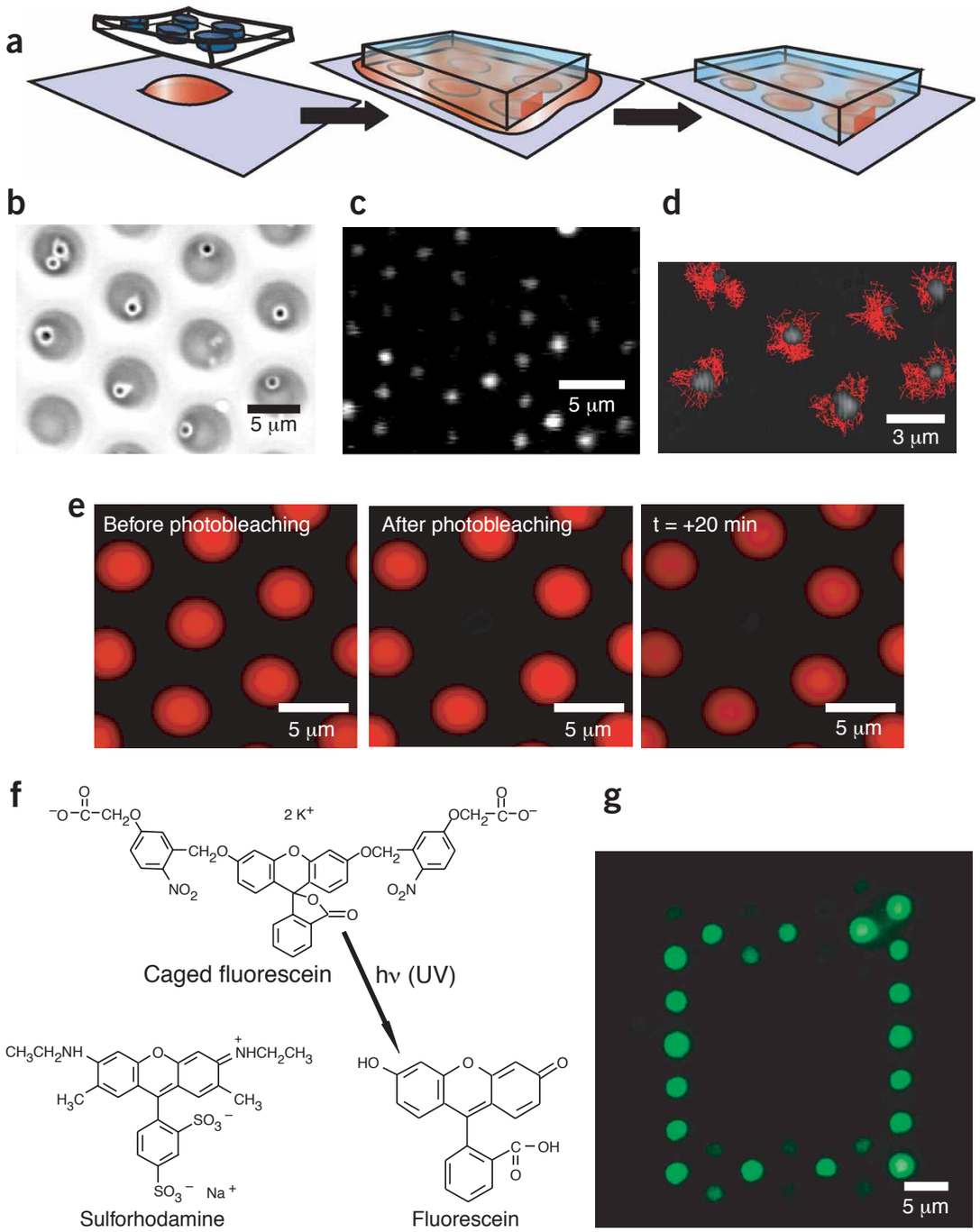


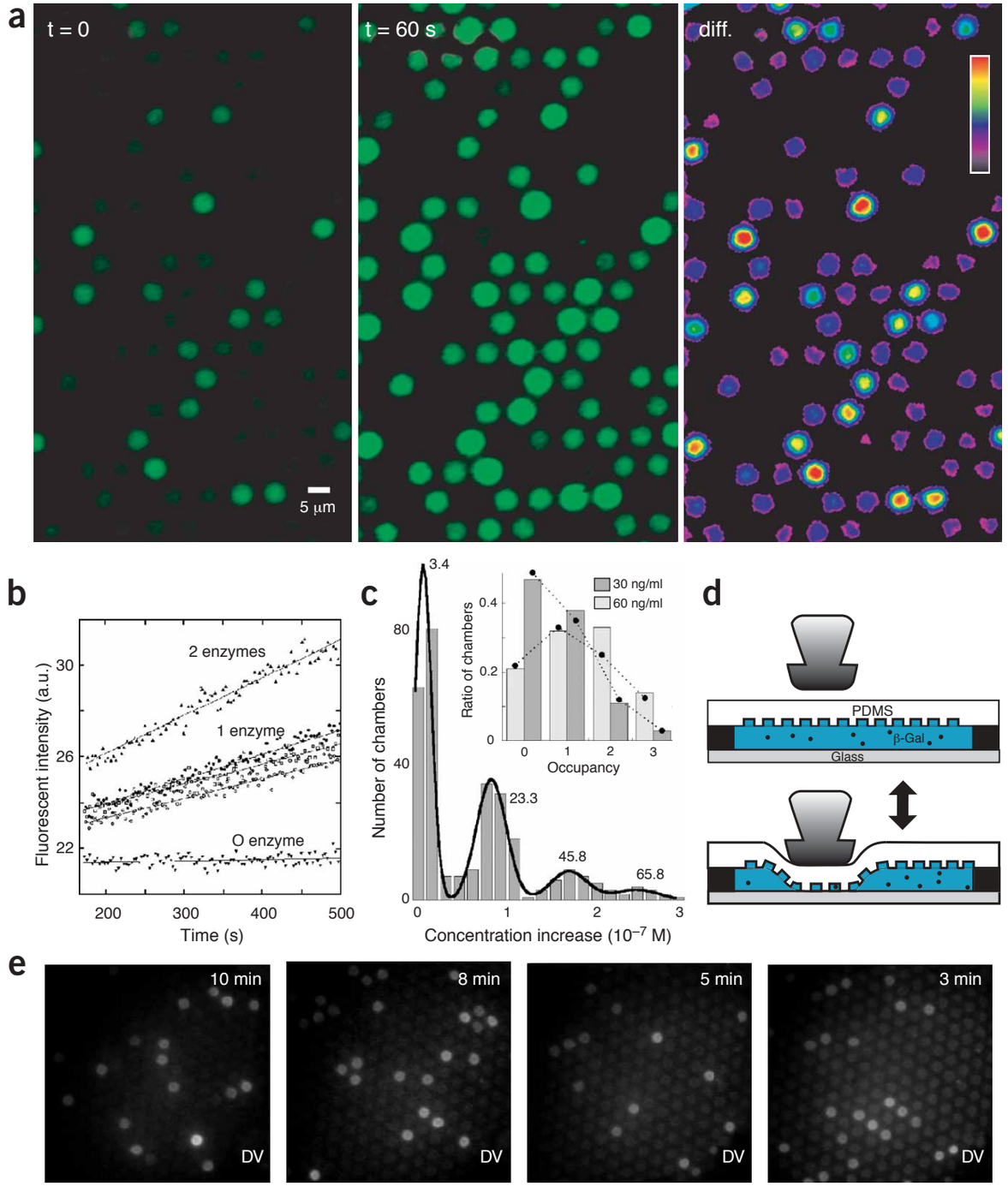




Martinez, A. W., Phillips, S. T., Whitesides, G. M. & Carrilho, E. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. Anal Chem 82, 3–10 (2010).

Single Molecule Detection

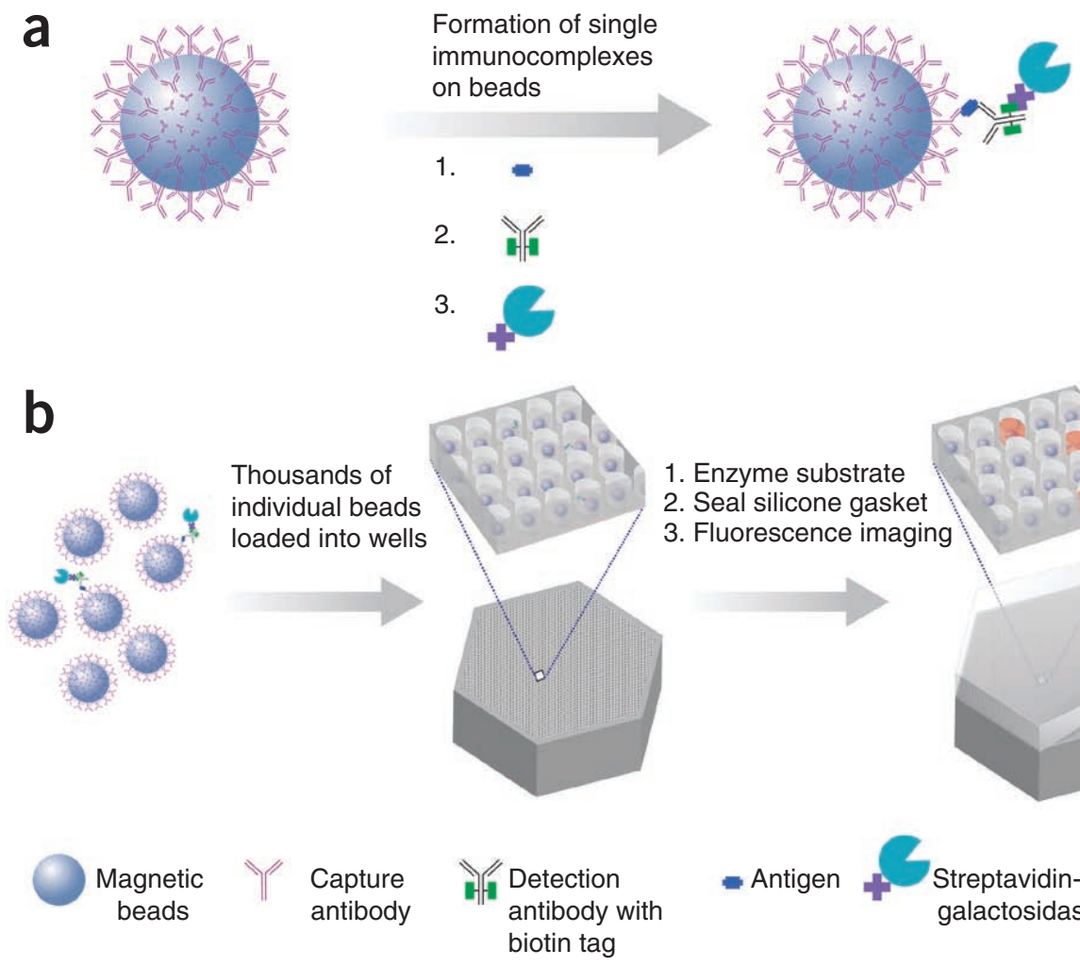


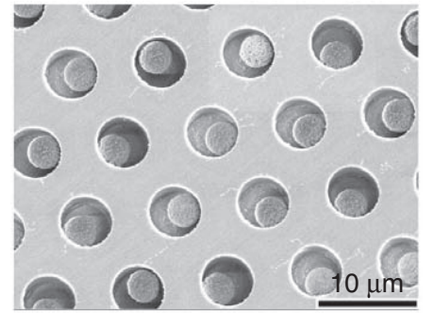


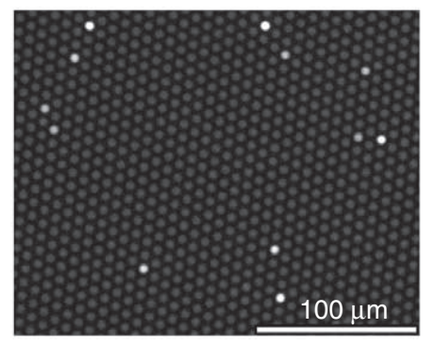
Rondelez, Y. et al. Microfabricated arrays of femtoliter chambers allow single molecule enzymology. Nat Biotechnol 23, 361–365 (2005).

Digital ELISA

Figure 1 Digital ELISA based on arrays of femtoliter-sized wells. (**a**,**b**) Single protein molecules are captured and labeled on beads using standard ELISA reagents (**a**), and beads with or without a labeled immunoconjugate are loaded into femtoliter-volume well arrays for isolation and detection of single molecules by fluorescence imaging (**b**). (**c**) Scanning electron micrograph of a small section of a femtoliter-volume well array after bead loading. Beads (2.7 μm diameter) were loaded into an array of wells with diameters of 4.5 μm and depths of 3.25 μm. (**d**) Fluorescence image of a small section of the femtoliter-volume well array after signals from single enzymes are generated. Whereas the majority of femtoliter-volume chambers contain a bead from the assay, only a fraction of those beads possess catalytic enzyme activity, indicating a single, bound protein molecule. The concentration of protein in bulk solution is correlated to the percentage of beads that carry a protein molecule.







Rissin, D. M. et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 28, 595–599 (2010).

Digital ELISA

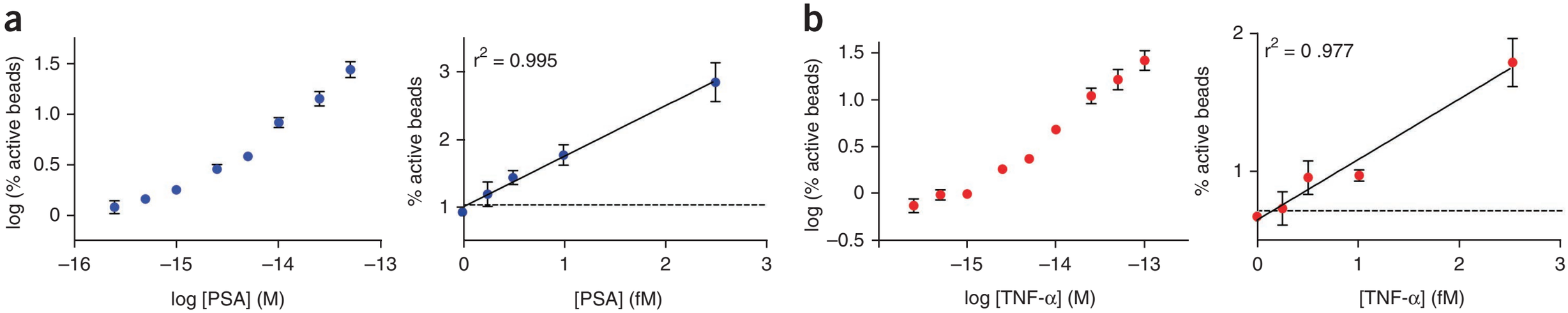
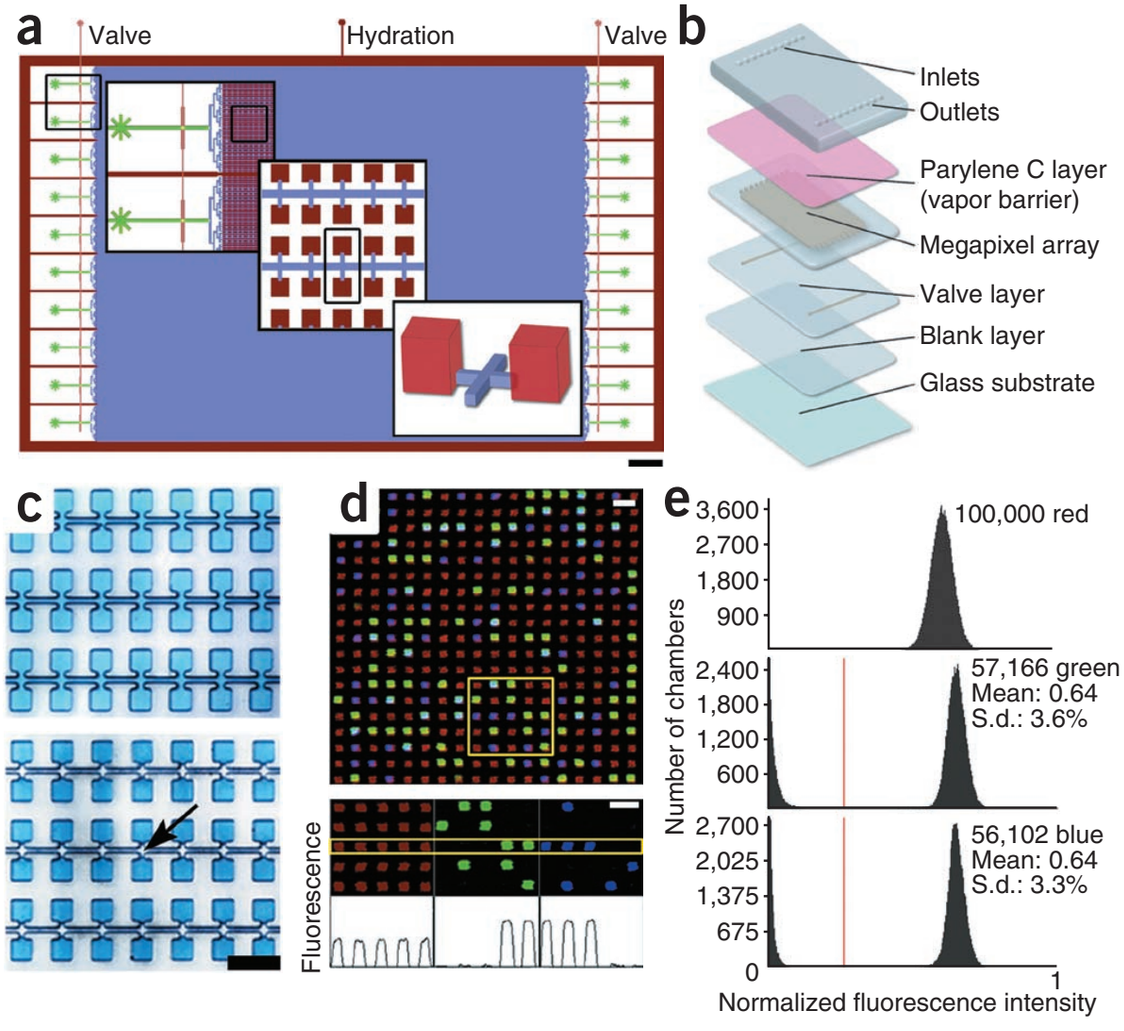
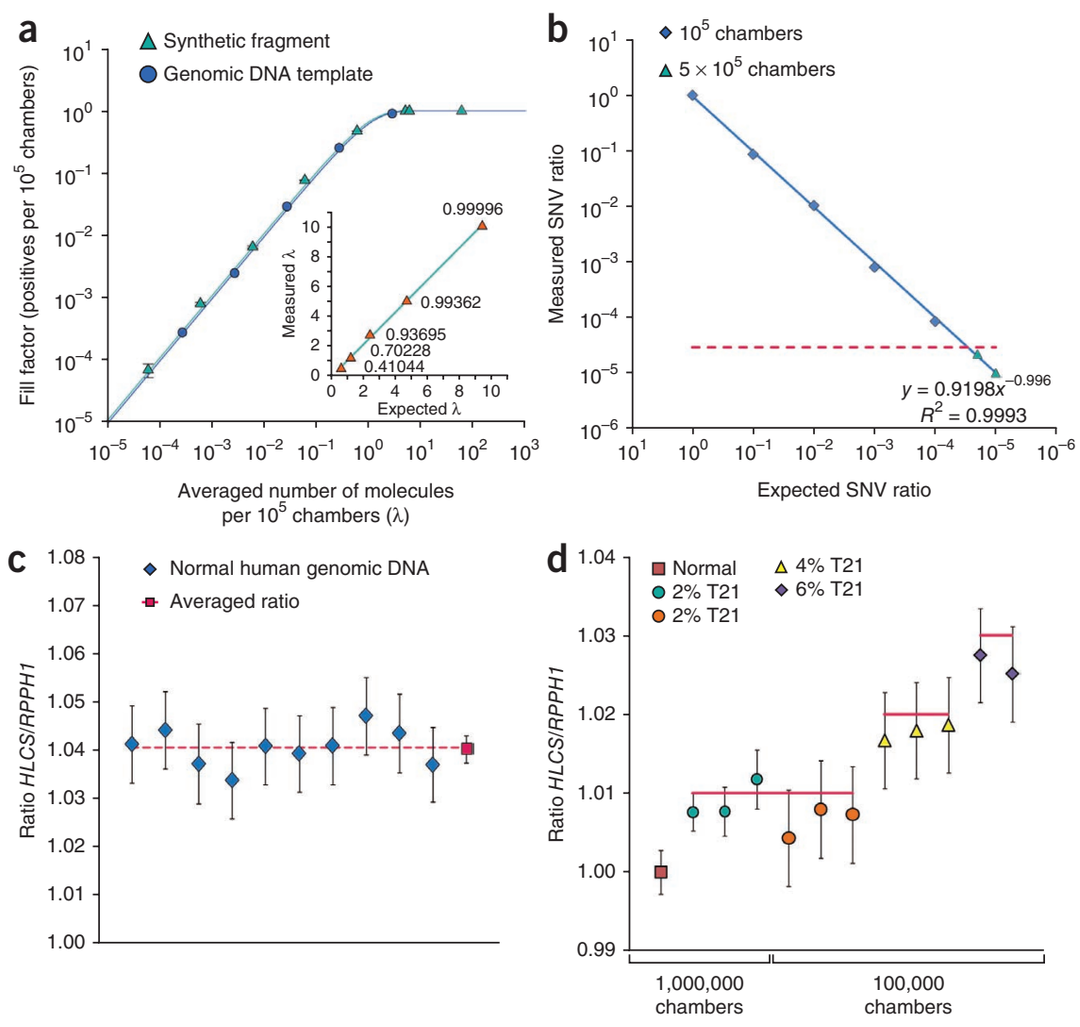


Figure 3 Subfemtomolar detection of proteins in serum using digital ELISA. (**a**,**b**) Changes in the percentage of active beads with changes in analyte concentration for human prostate-specific antigen (PSA) spiked into 25% serum (**a**) and human tumor necrosis factor-α (TNF-α) spiked into 25% serum (**b**). The concentrations plotted on the *x* axes refer to the final concentration of spiked protein in the diluted sample. The plots on the left-hand side show the assay response over the concentration range tested in log-log space. The plots on the right-hand side show the assay response in the femtomolar range in linear-linear space to illustrate the limit of detection (LODs) and linearity of response. LODs were determined by extrapolating the concentration from the signal equal to background signal plus 3 s.d. of the background signal. Broken lines, signal at the LOD. Error bars, s.d. over three replicates.

Rissin, D. M. et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 28, 595–599 (2010).

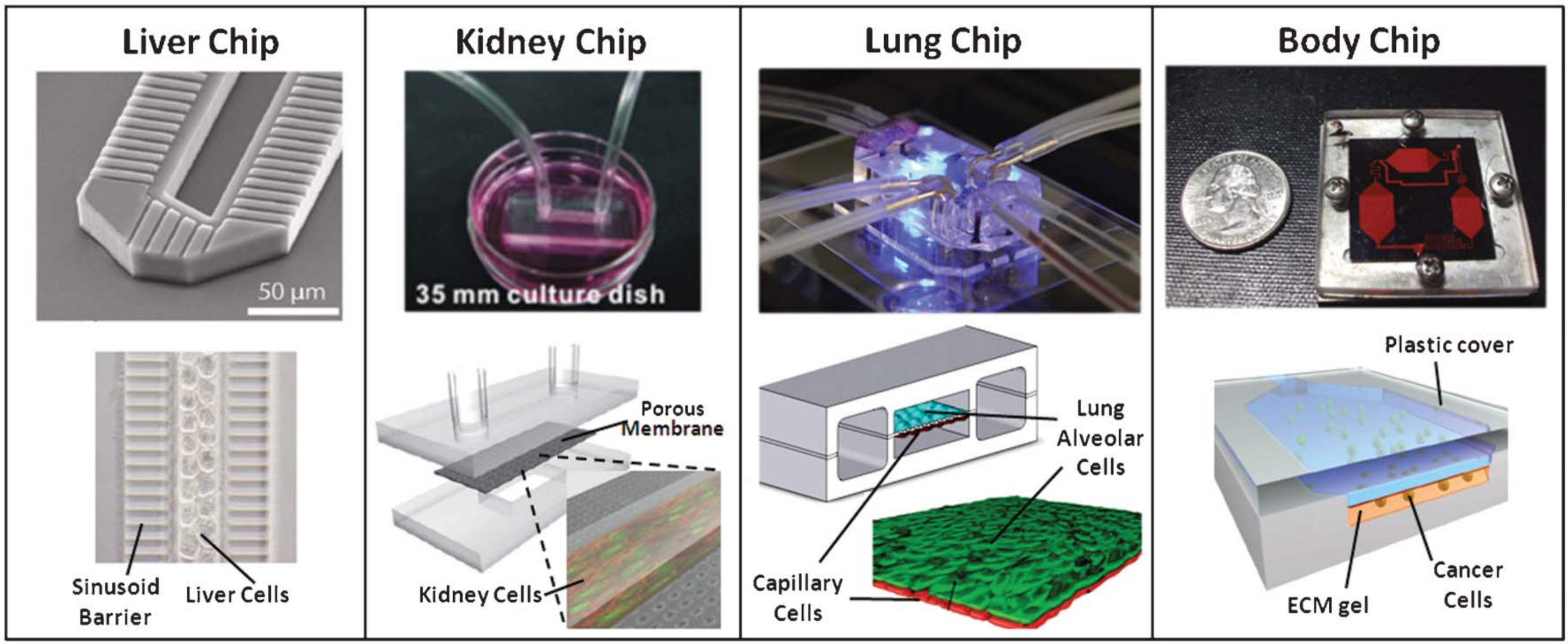
Digital PCR





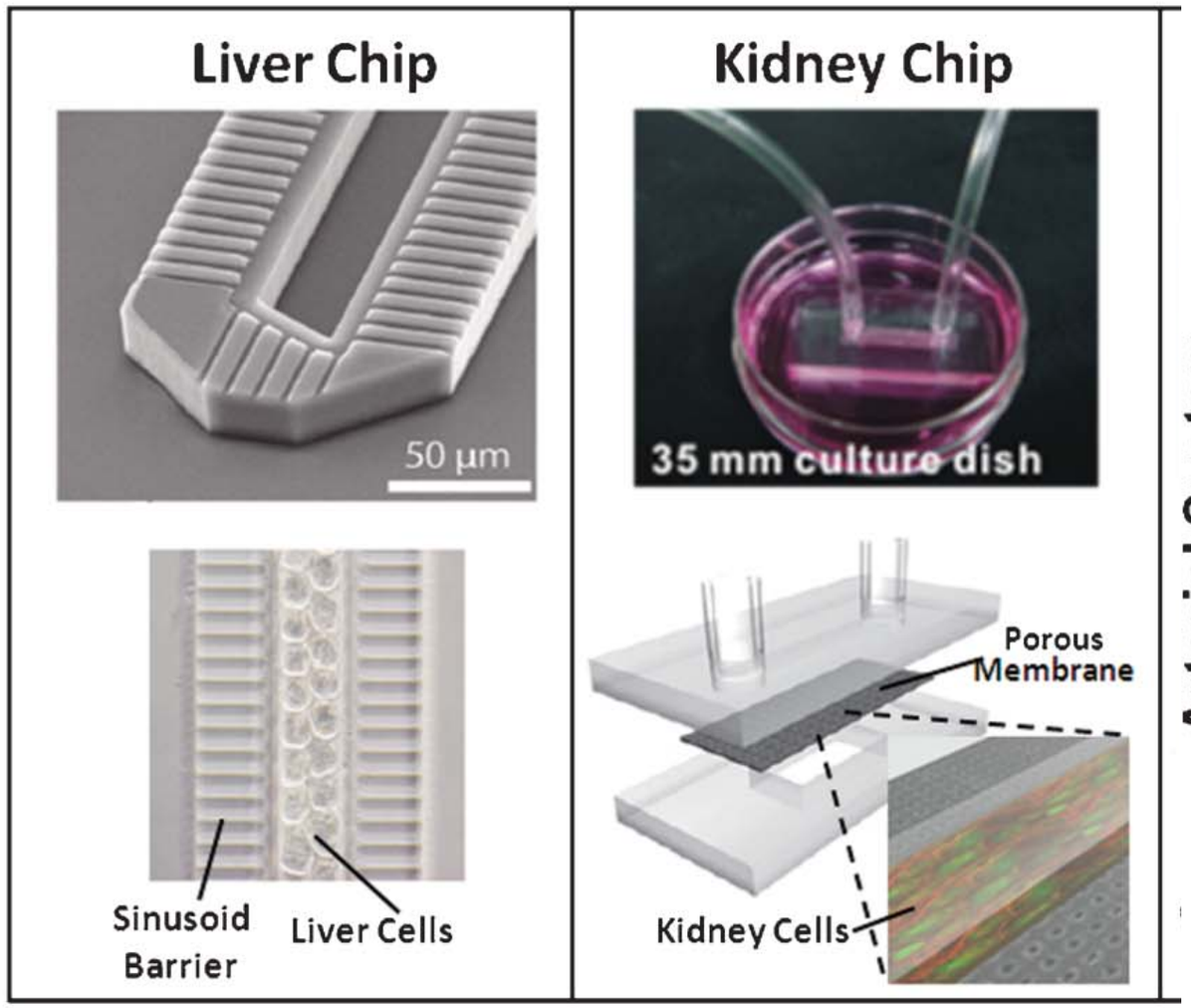
Heyries, K. A. et al. Megapixel digital PCR. Nat Meth 8, 649–651 (2011).

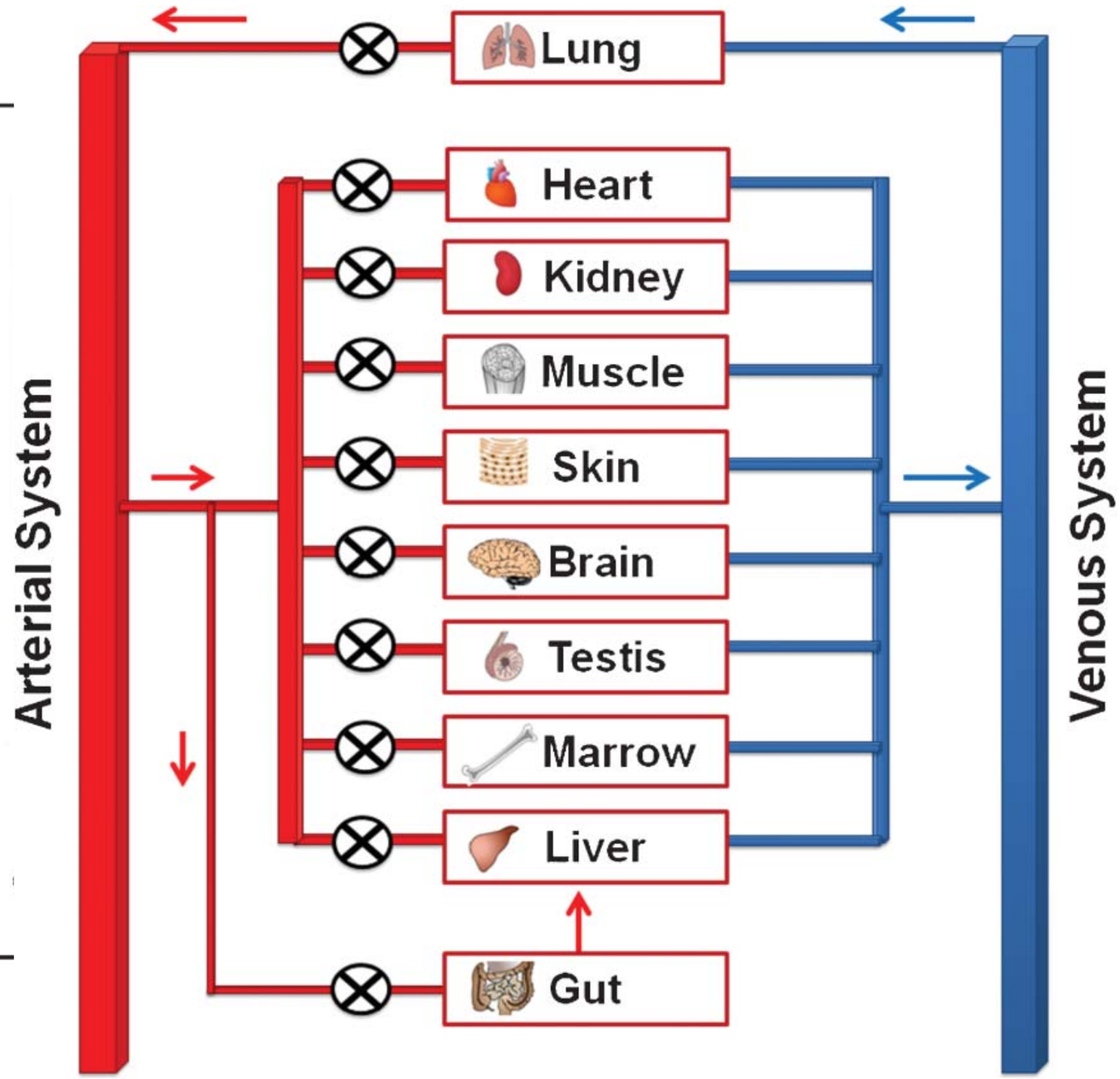
Artificial Organs (Organs-on-Chip)



Huh et al. *LOC* (2012).

In vitro Emulation of Organs (Organs-onChip)





Huh et al. *LOC* (2012).