

Disposable Point Of Use Diagnostic Lab Cards

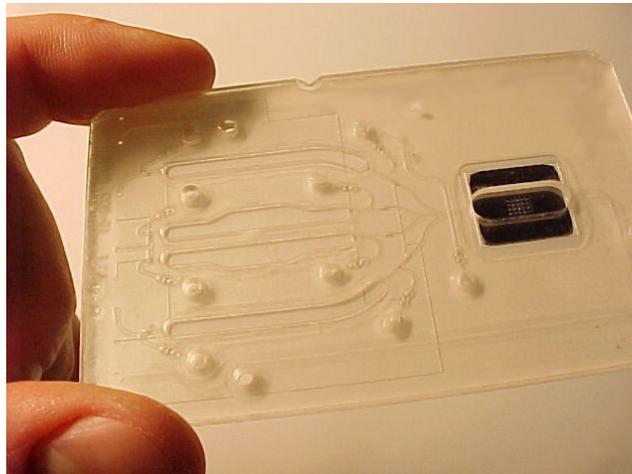


Micro Nano Breakthrough
2004

Fred Battrell, Ph.D.

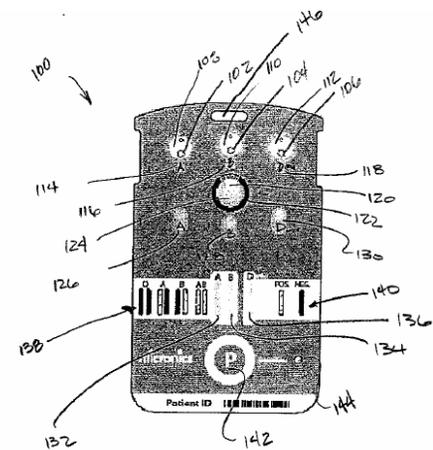
Welcome to Micronics

- The leading provider of **custom lab card development services** on behalf of:
 - diagnostics, pharmaceutical & biotech companies
 - reagents & instrument companies
 - national laboratories & military initiatives



Micronics' Enabling IP

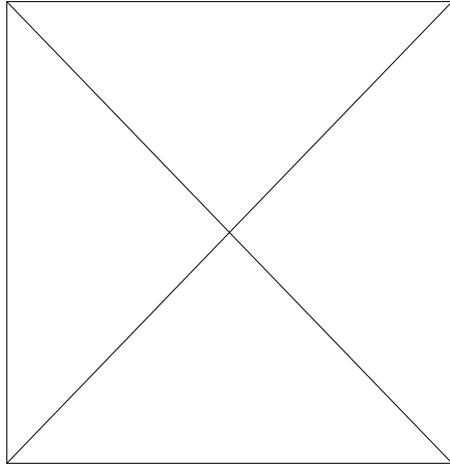
- Includes surface modifications, prototyping, production
- Patent estate applied to custom lab card development
- 41 US patents issued/allowed in areas of
 - Laminar flow diffusion-based separation & detection
 - Microcytometry
 - Microfluidics – on/off card pumps, valves, heating & cooling
- >40 US/international patent applications pending
- Fundamental and blocking
- Exclusive worldwide rights, all fields



Micronics' Microfluidics...

- Ability to move fluids in laminar – non turbulent – flow
- Takes advantage of predictable properties of liquids
- Allows mixing, separation, measurement of relative & absolute concentrations
- Minimizes volumes of fluids required – samples, reagents
- Results in faster assay time
- Multiplexes for processing of larger volumes
- Qualitative and quantitative

Laminar Flow Diffusion Interfaces

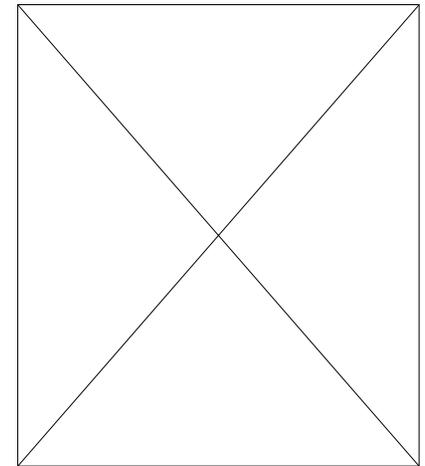


Micronics' **H-Filter®** platform

- separation
- mixing

Micronics' **T-Sensor®** platform

- embedded controls
- detection



- **microFlow™ System**
 - Ultra-low pulse pump system
 - Precise fluid control
 - Picoliter volumes
 - Sold worldwide to researchers in private and public sector
 - Serves as instrument base for many active card designs
- **Access™ and Active™ Cards**
 - Laminar flow diffusion lab cards
 - Active (instrument driven) and passive (manually activated) disposable lab cards
 - Enables reformatted assay development



Micronics' POC Microcytometer™

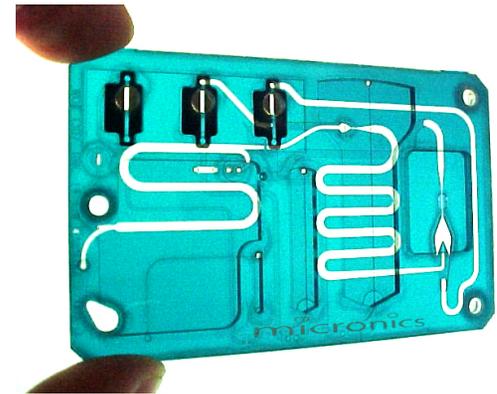
(Active Lab Card)

Miniaturizing flow cytometry

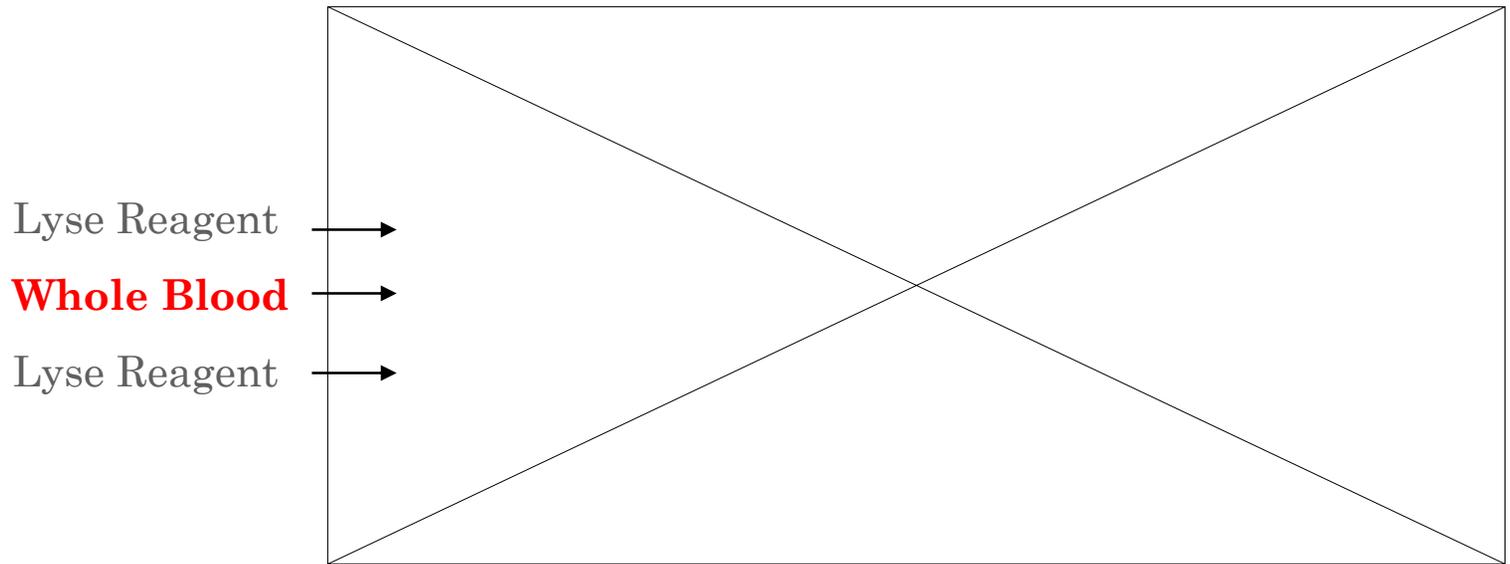
- The gold standard in cell counting / analysis

- **Microcytometer lab card**

- Small, disposable plastic card
- Portable detection instrument
- Requires minimal training
- Reagents/ waste stored on card
- Eliminates time, reagents; reduces cost
- Allows direct sample injection; sample-to-result in **3 minutes**



Micronics' Red Cell Blood Lysing Structure



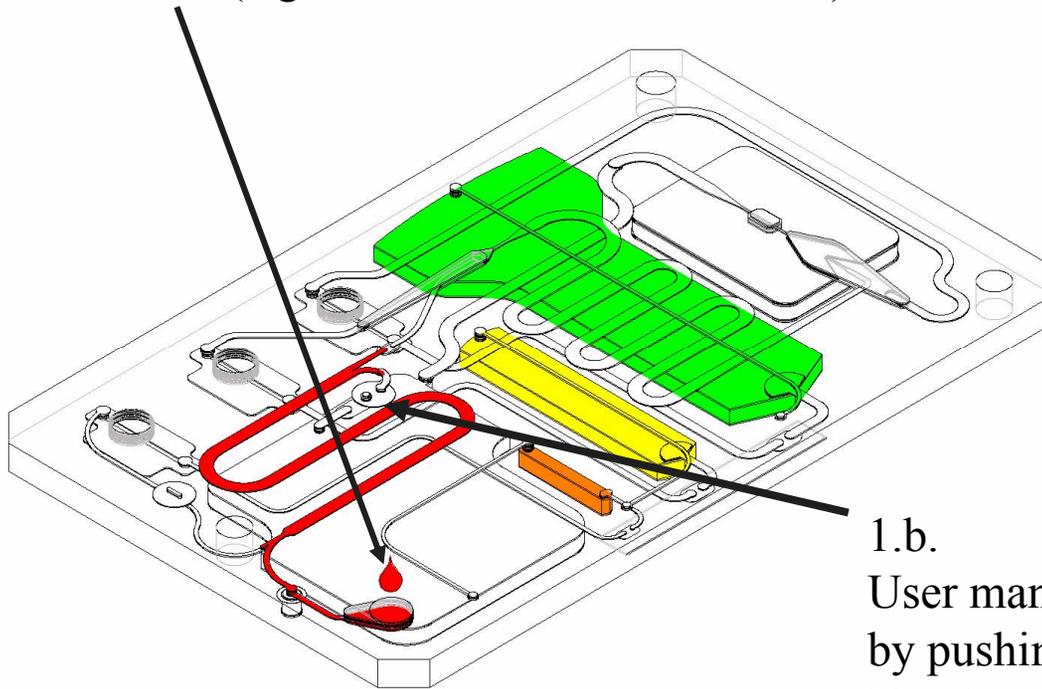
Whole blood is focused into a **Thin Ribbon** surrounded by lyse reagent.

The red blood cells rupture, leaving the white blood cells which continue onward to the cytometer focusing chamber.

#1 Sample Acquisition

Manual sample insertion and activation

1.a. Droplet of “raw” sample 30 μL placed onto card (e.g., whole blood, saliva, urine)



1.b. User manually activates “bellows” by pushing with finger to draw **blood sample** into card

2. Card Inserted into Portable Instrument

User pushes “start”

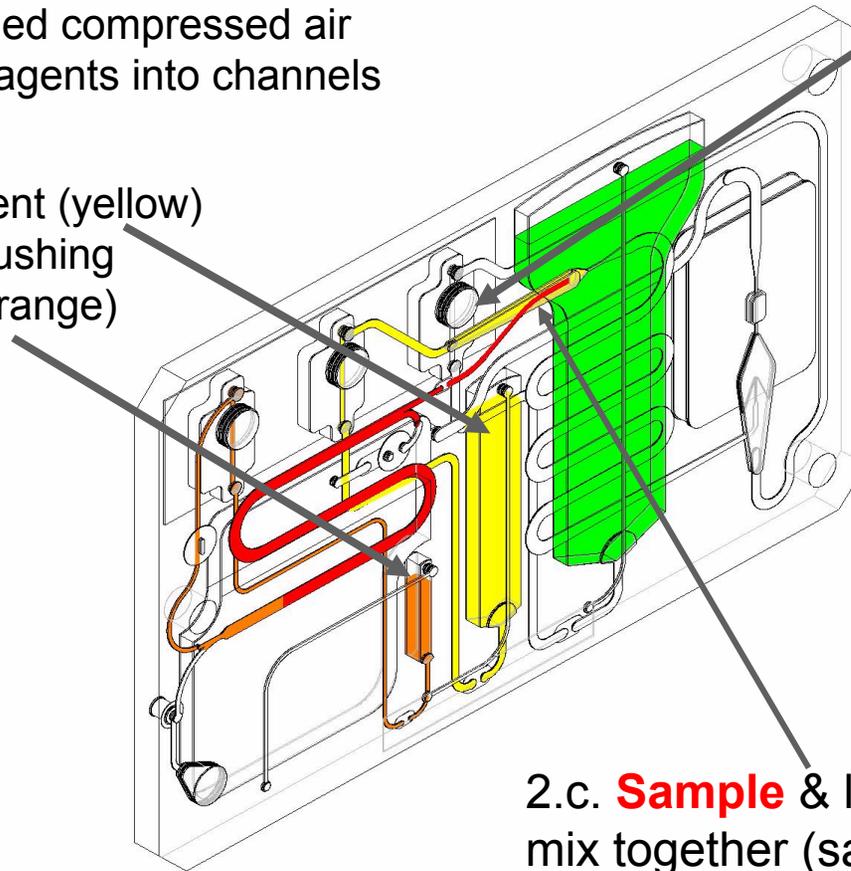
2.a.

Instrument-controlled compressed air pushes on-card reagents into channels

On-card lyse reagent (yellow)
On-card sample-pushing (saline) reagent (orange)

2.b.

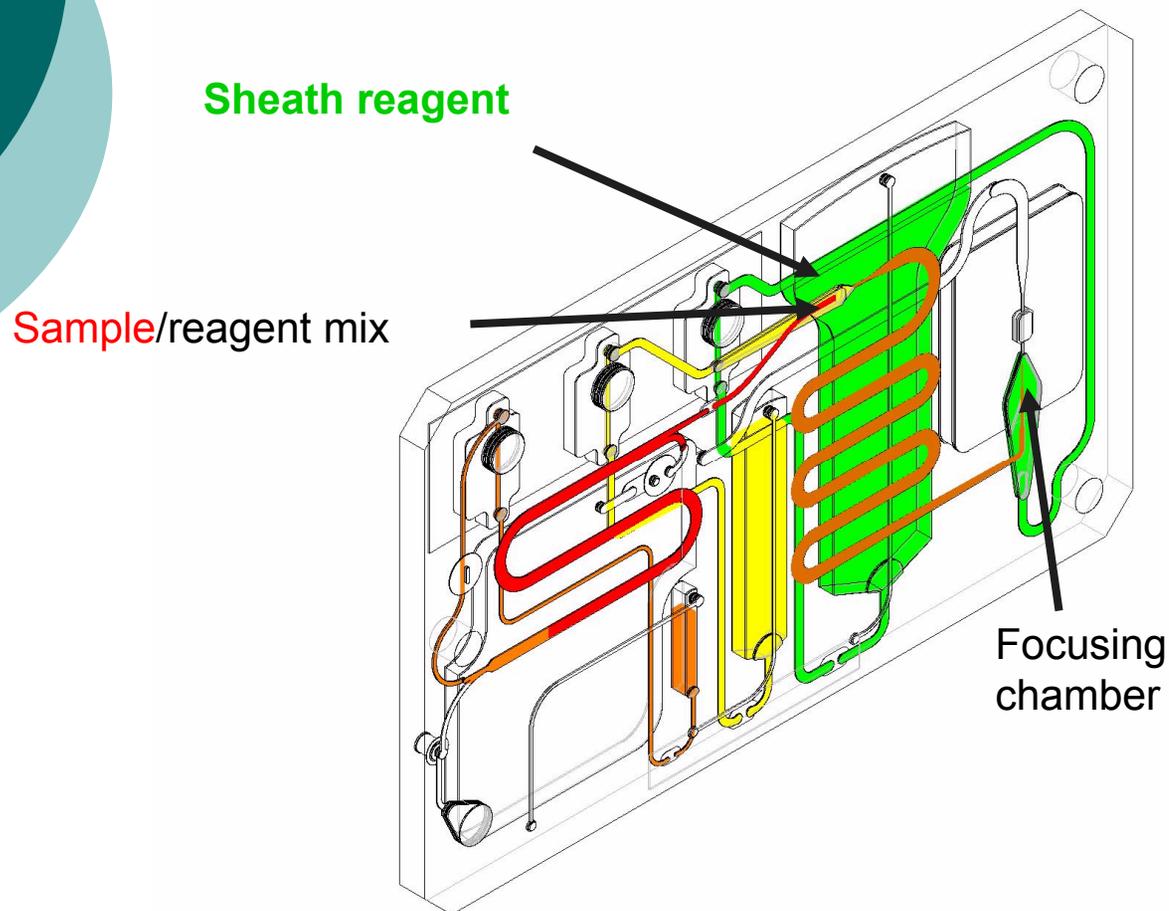
Reagents flow across embedded Flow Sensors that measure reagent volume & flow rate (instrument controls for accuracy)



2.c. **Sample** & lyse reagent mix together (saline left behind in channel)

3. Continuous lysing of blood and cell analysis

Card under instrument control

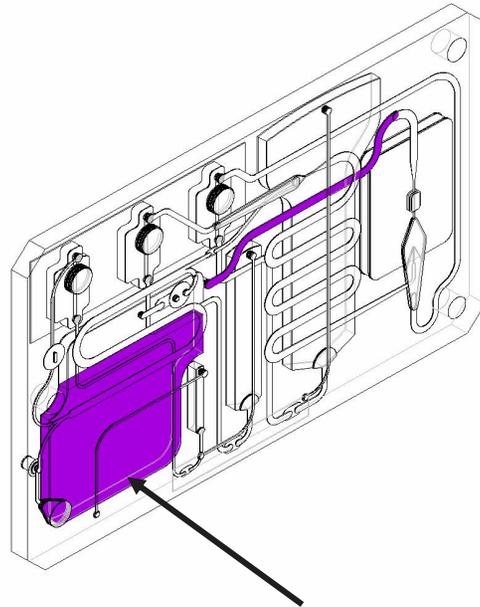


On-card **sheath**
(saline) reagent

Sample/lyse reagent
mix continuously
moved in the channel
by instrument control

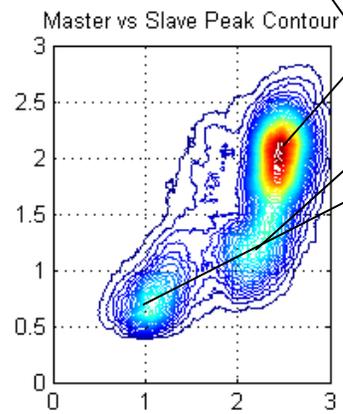
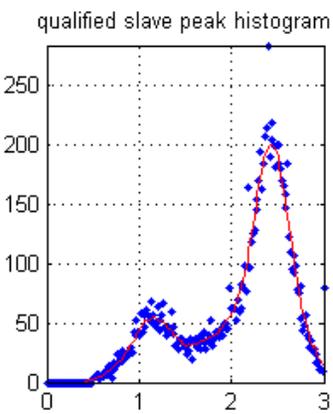
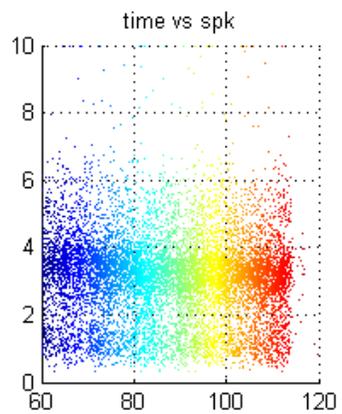
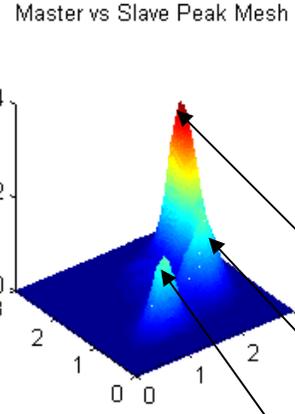
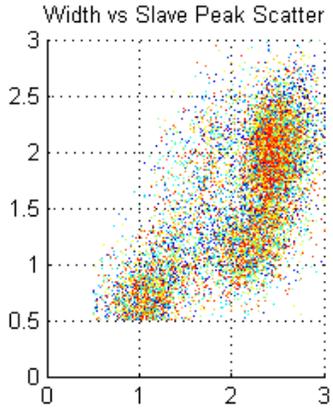
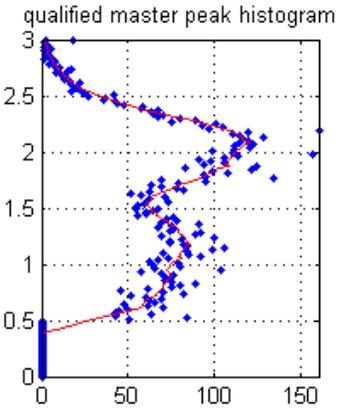
Sample/lyse reagent
mix interacts with the
sheath reagent and
continues to flow into
the focusing chamber
for laser interrogation

4. Post-analysis, waste storage on card



Following cell count analysis in the Focusing Chamber, all mixture of sample and reagents are diverted into **Waste Chamber** for on-card storage to ensure safe handling and disposal

Microcytometer Card Results – WBC Assay Using Microfluidic Lysing Technique

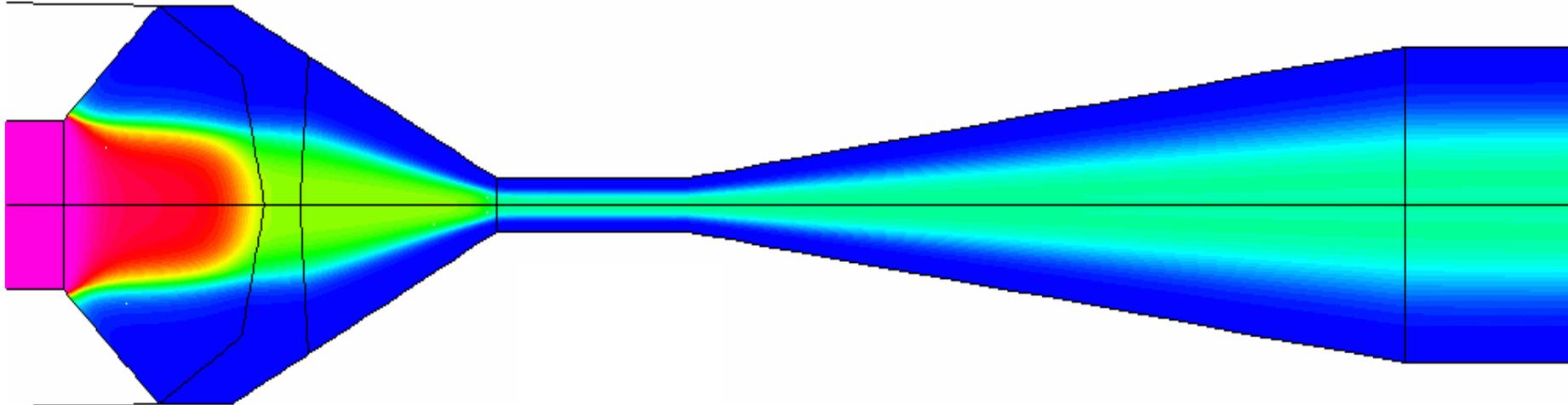
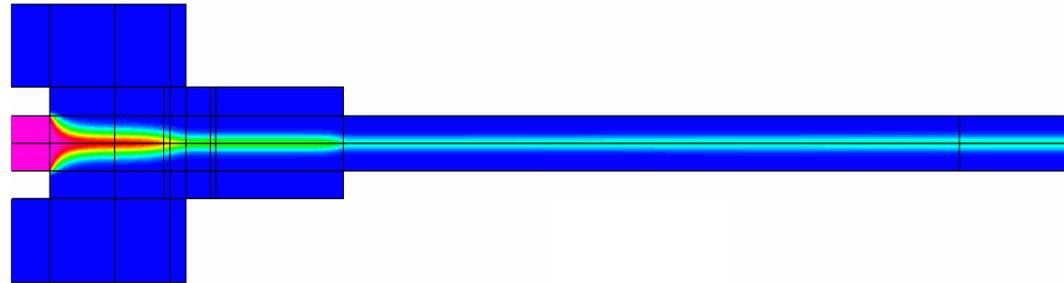


Neutrophils

Monocytes

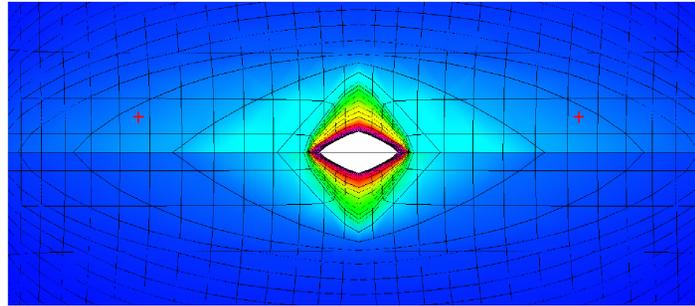
Lymphocytes

Thin Ribbon Core, CFD Model

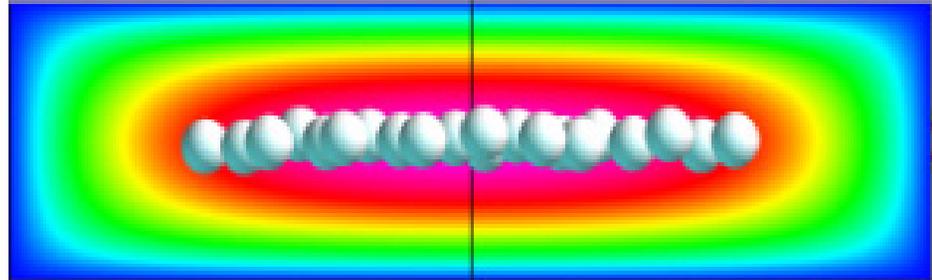


Fluid Core Comparison

Cytometer core
Single cell

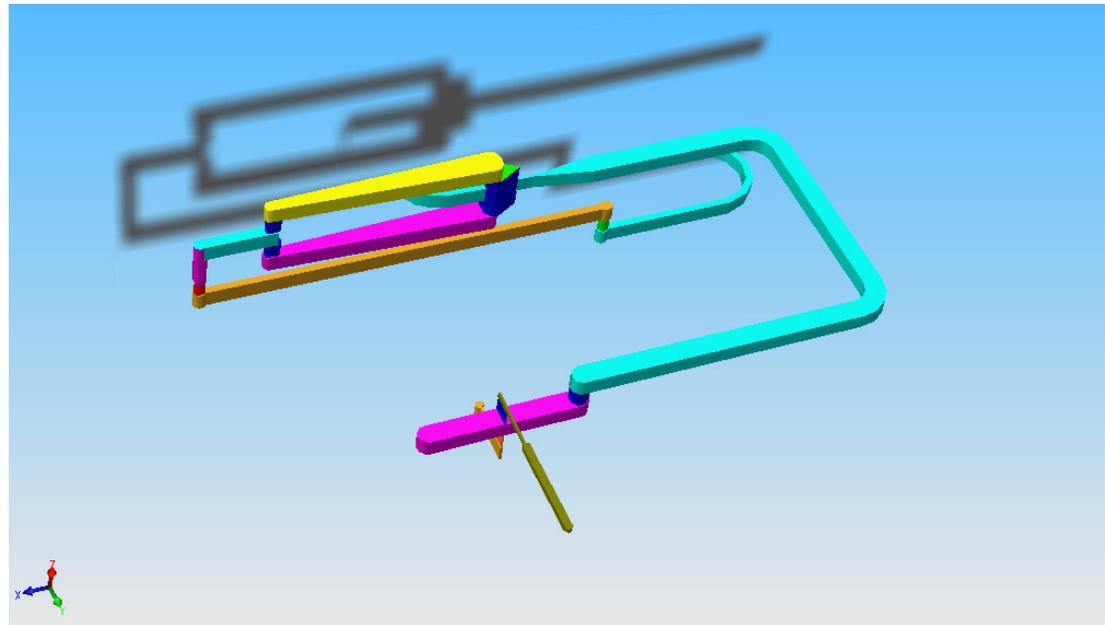


Ribbon Thin Core

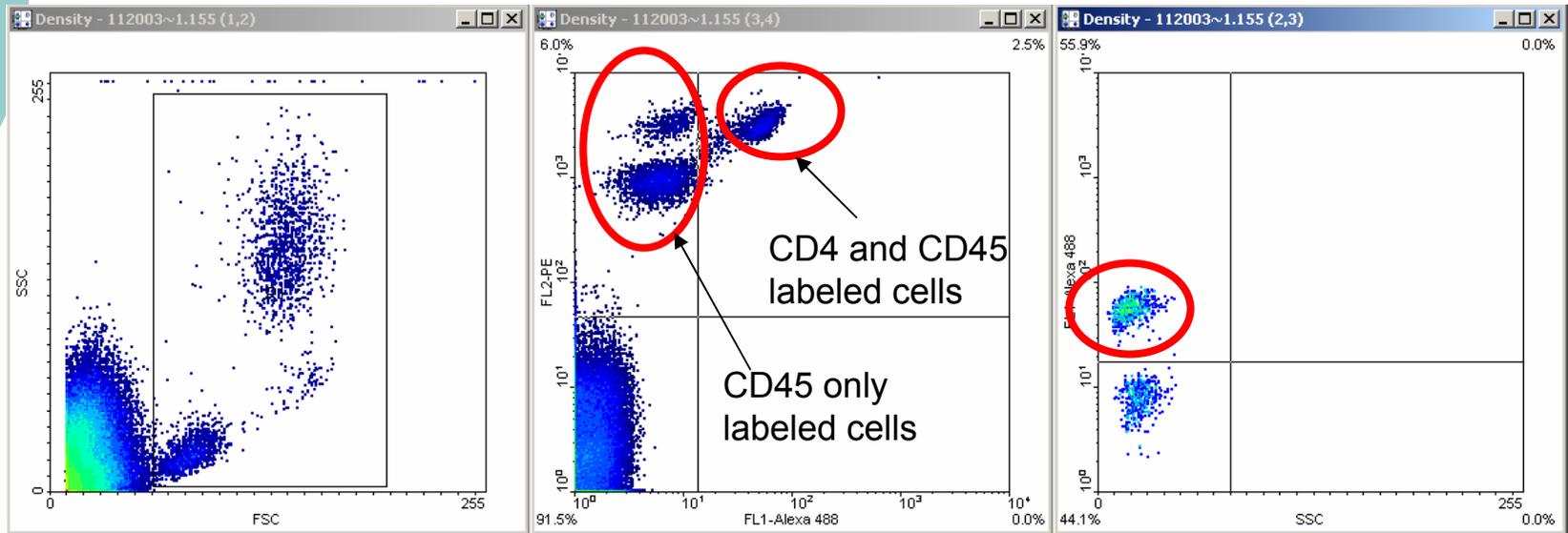


Microfluidic Extruded Circuit for Cell Labeling & Sorting

- Show 3D Model



Lab Card/Assay Confirmation (CD4)



Scatter, with Gate
Drawn around White
Blood Cells

Fluorescence (CD4 is
X-axis, CD45 is Y-axis)

CD4 labeled
Lymphocytes

Macro vs. Microfluidic

Immunofluorescence Staining of Blood Cells

Assay Step	Standard Bench Scale Assay Conditions	Micronics Lab Card Formatted Assay
Whole Blood Sample	100 μ l	12 μ l
Dilution w/ PBS	400 μ l	
Mabs (labeled antibody)	5 μ l	0.6 μ l non-optimized
Dilution w/PBS		258 μ l
Incubate	20-30 minutes @ 4°C	20 seconds
Centrifuge	5 minutes	
Remove supernatant by aspiration	30 seconds	
Lysing solution	1.4 mL	500 μ l non-optimized
Incubate at room temperature	3-5 minutes	20 seconds
Centrifuge	5 minutes	
Remove supernatant by aspiration	30 seconds	
Dilution w/ PBS & vortex	600 μ l	
Centrifuge	5 minutes	
Remove supernatant by aspiration	30 seconds	
Dilution w/ PBS & vortex	400 μ l	
Cytometric Measurement	2-3 minutes	2-3 minutes

Micronics' POC Blood Typing (Passive Lab Card)

The Issue

- Mis-transfusion* poses 2nd highest risk for major transfusion errors
- 30 mL of ABO-incompatible blood can be fatal

Micronics' solution - **ABO™ Card**

- 10 second test
- Bedside blood type test, pre-transfusion/transplantation
- Sample, reagent and waste stored on-card



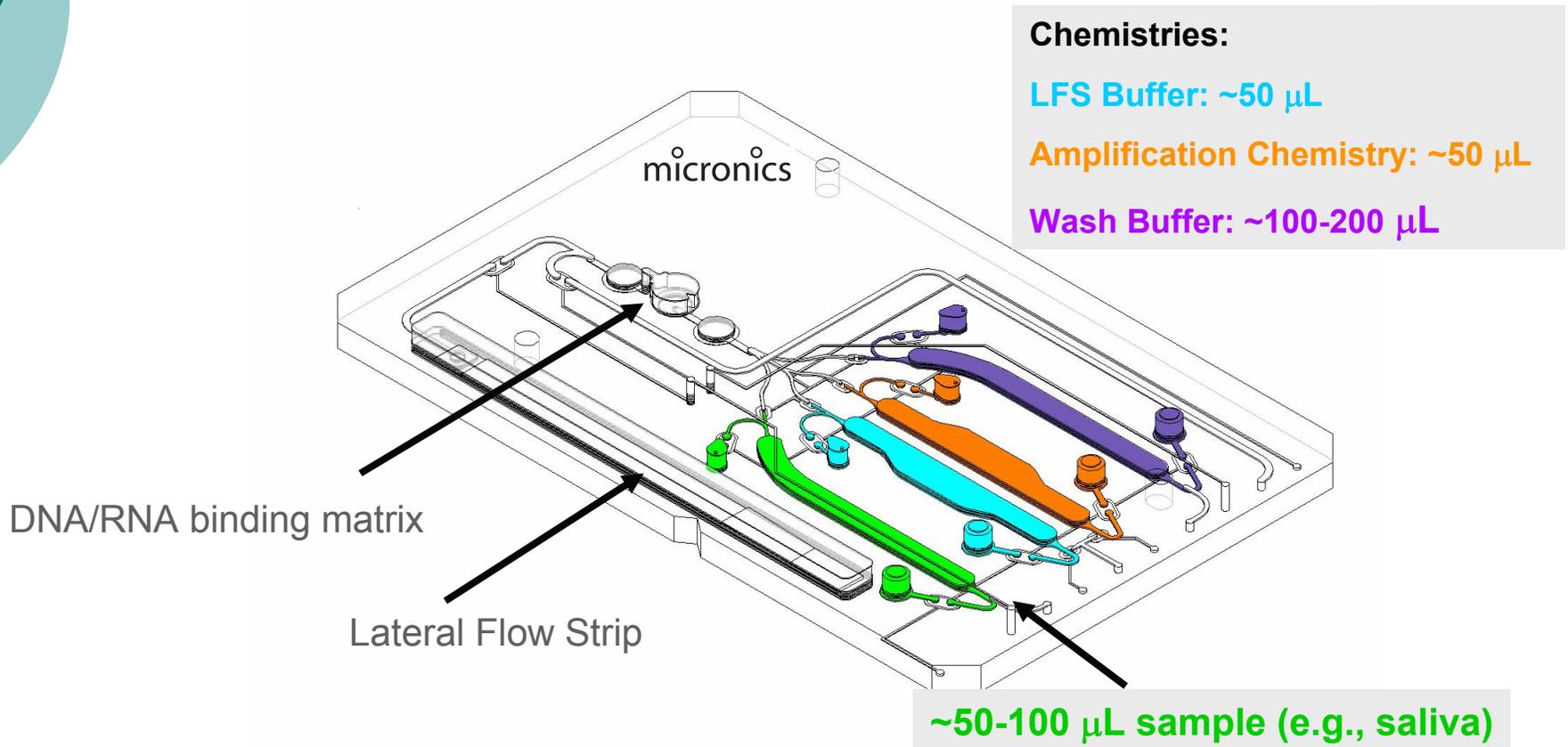
*failure to give right blood to the right patient at the right time

Manually Activated ABO™ Card



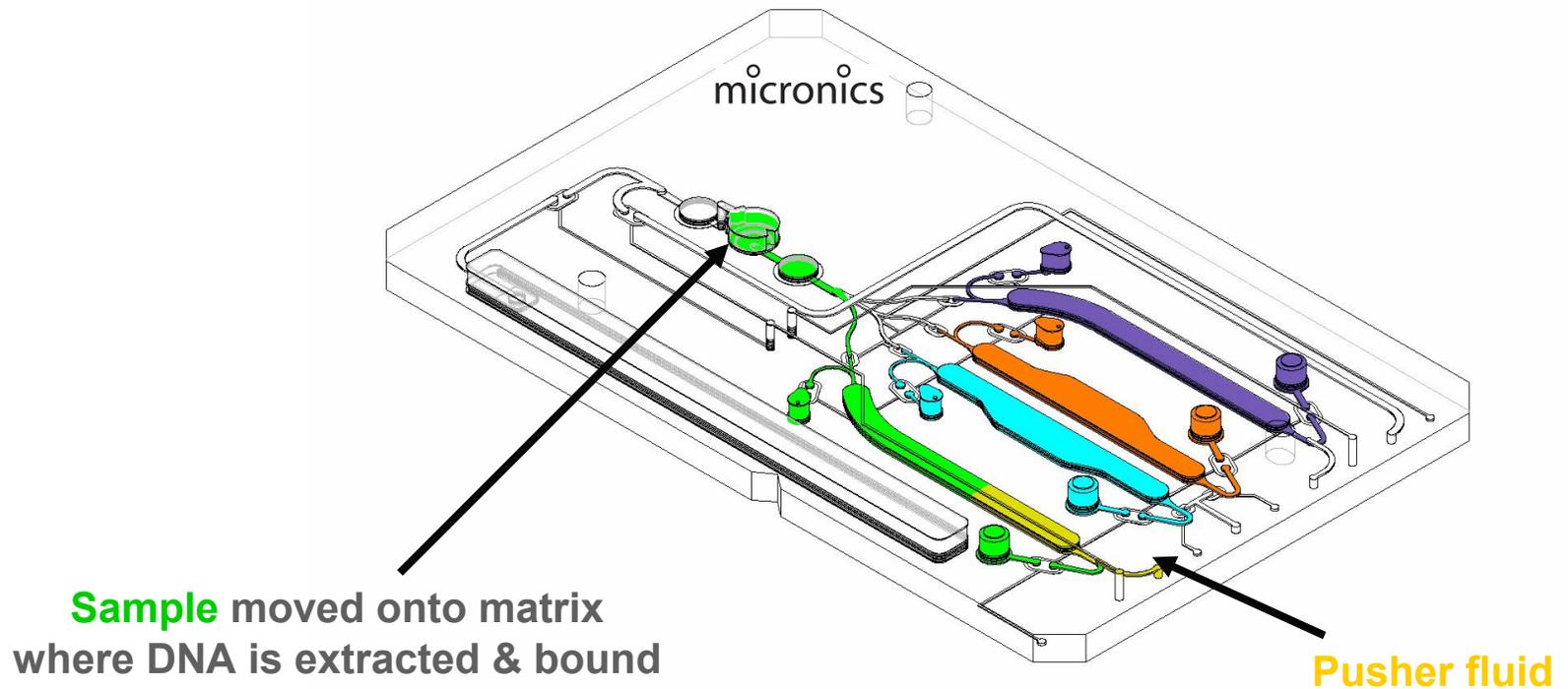
Custom Lab Card Example #1: DNA assay lab card

Step 1. **Sample** placed on card; card inserted into instrument;
chemistries activated under instrument control



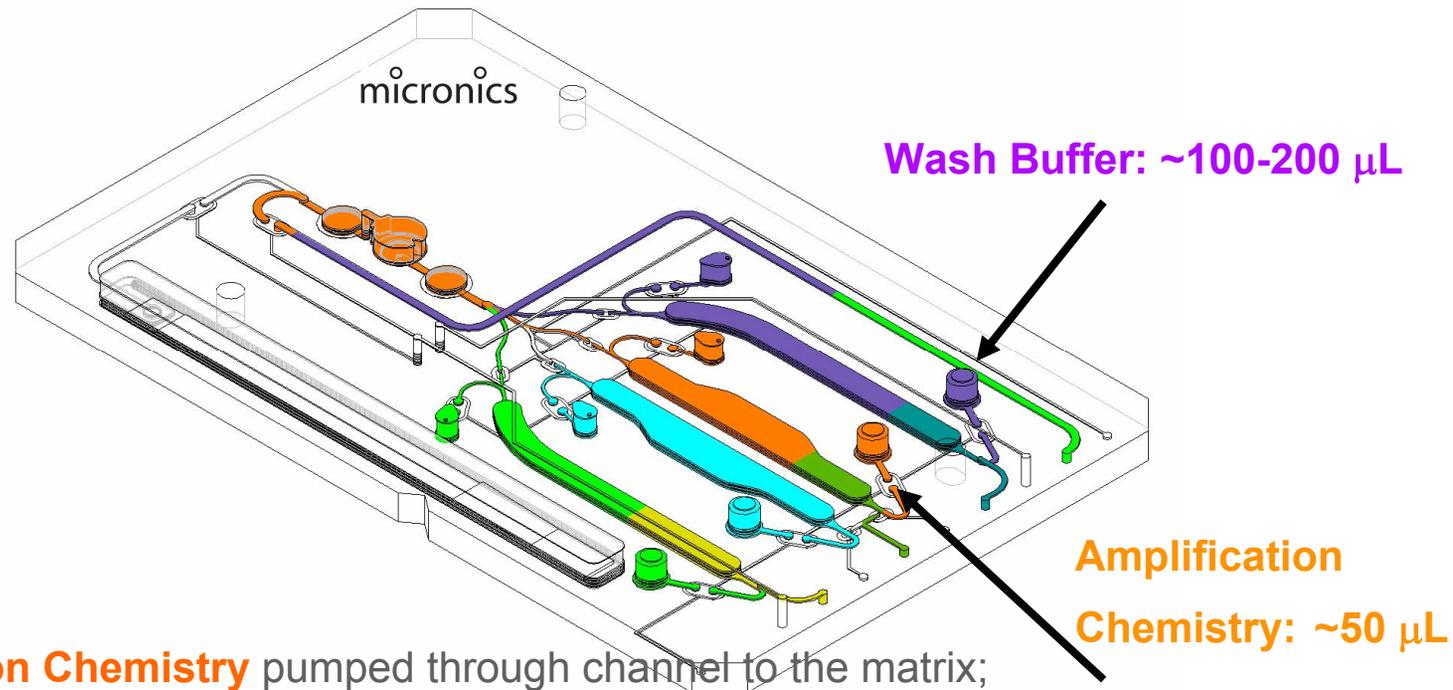
Sample Extraction

Step 2. Under instrument control, **pusher fluid** pumped onto card to drive **Sample** onto the binding matrix



Wash Buffer & DNA Amplification

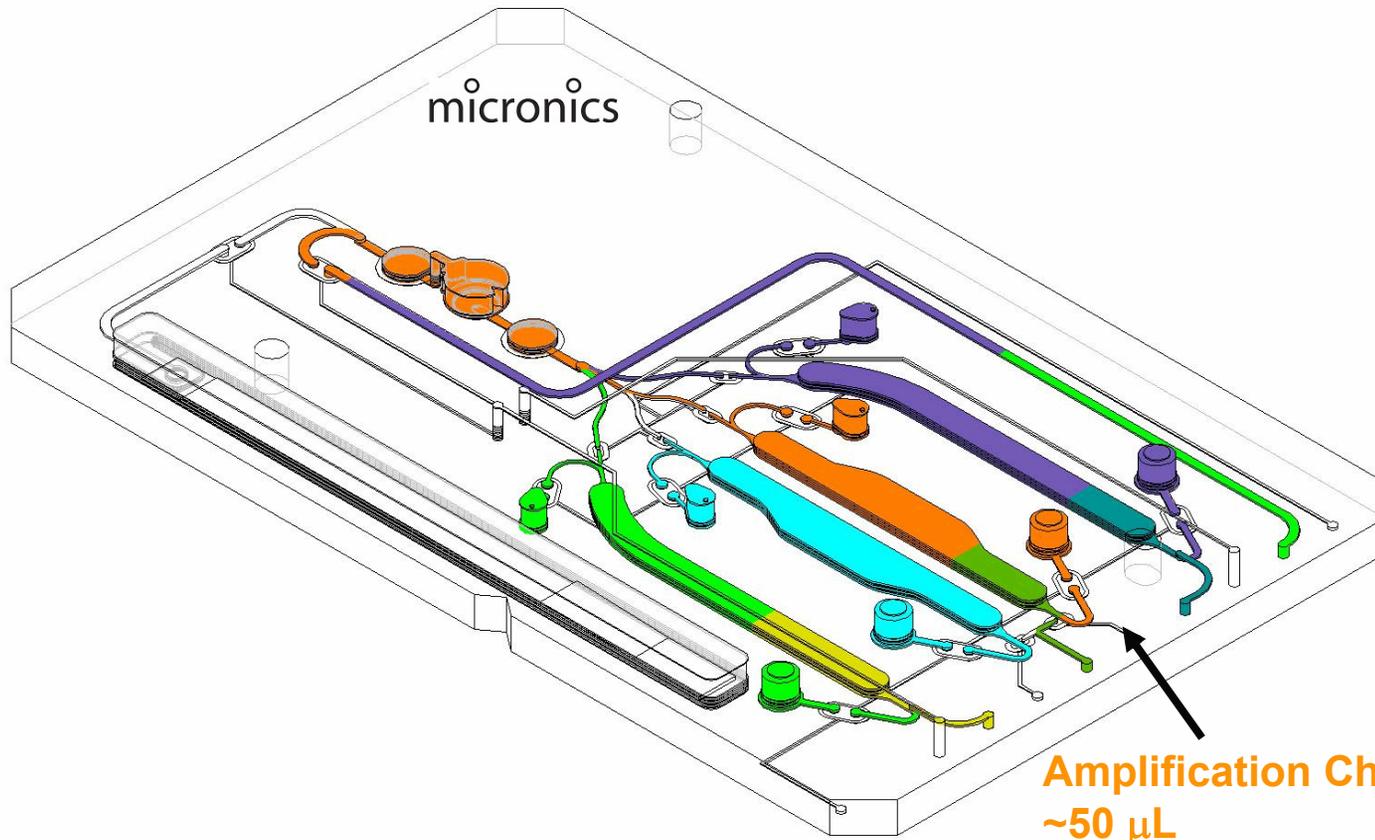
Step 3. Wash buffer activated to flow onto matrix and remove inhibitors; leaving purified DNA



Step 4. Amplification Chemistry pumped through channel to the matrix; Heater unit (not shown) brought up to the matrix component of the card for DNA amplification

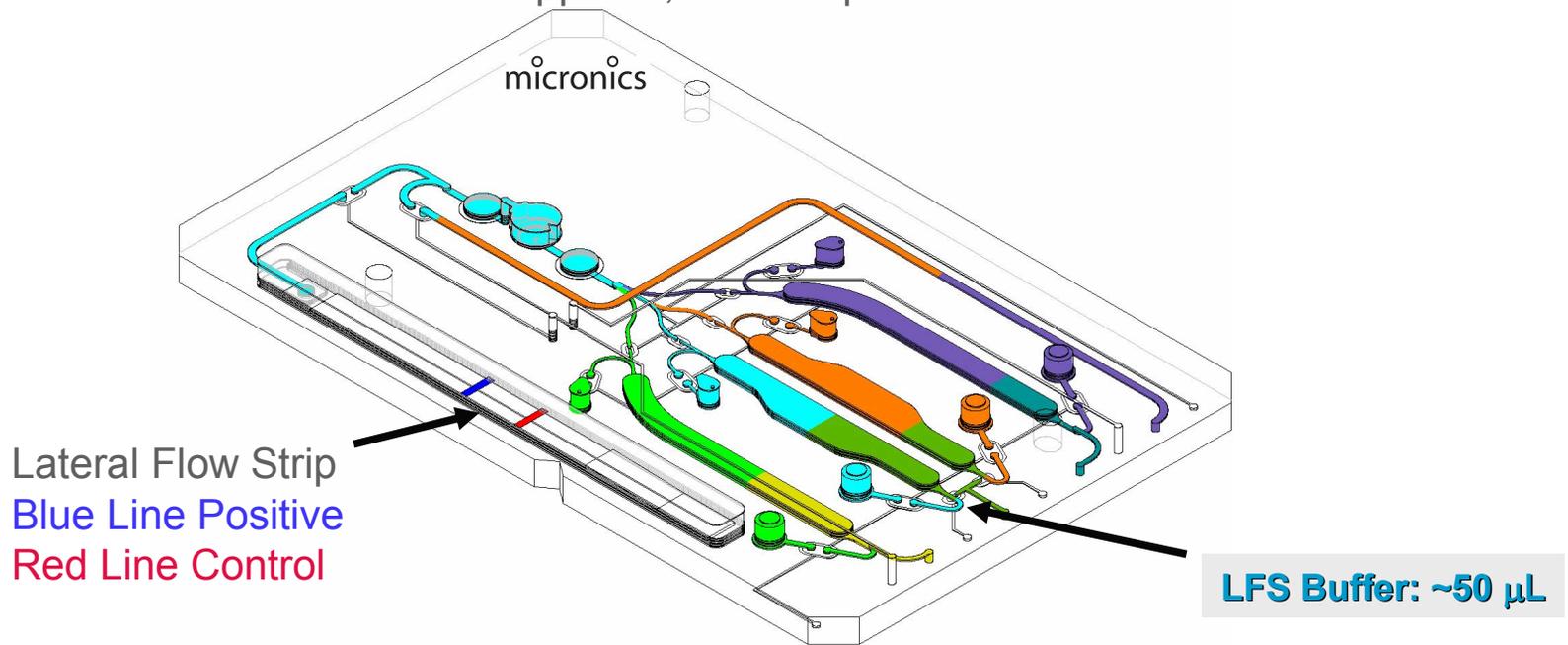
DNA Amplification

Step 4. **Amplification Chemistry** pumped through channel to the matrix; Heater unit (not shown) brought up to the matrix component of the card for DNA amplification



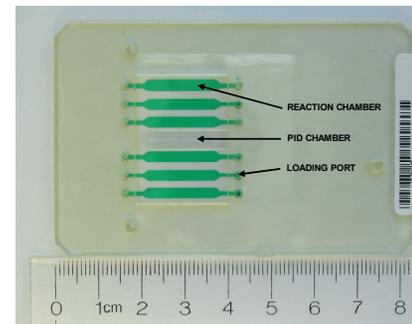
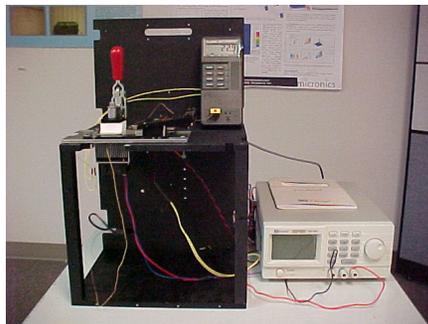
Result Read-Out

Step 5. **LFS buffer** transmits amplified DNA onto Lateral Flow Strip;
RED line serves as control that chemistry is captured;
When **BLUE** line appears, result is positive



Rapid PCR Thermalcycling

8 Minutes - 35 Cycles



Rapid Thermalcycling Micro vs. Macro

Step	Temperature	Micronics Time (Micro)	Cycles	Time (Macro)
cDNA	60°C	30 sec	1	120 sec
Activation	60°C	45 sec	1	300 sec
PCR	60 – 73 – 94.5 °C	402.5 sec (11.5 sec)	35	2,100 sec (60 sec)
Total Time		477.5 sec ~8 min		2,520 sec ~42 min

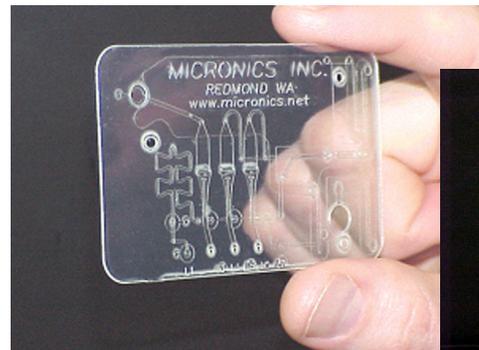


Micronics' Custom Services

- World's leading custom lab card developer
- Fluidic modeling expertise
- Sophisticated CAD design /ORACLE based tracking system
- Surface chemistries and material science library
- Laminar flow diffusion platform tools
- Micropump and valve expertise
- Macro-micro interface design
- Rapid prototyping

Examples of Custom Lab Cards

- Sample preparation
- Antigen/antibody detection
- DNA/RNA purif/amplif. – e.g., PCR, NASBA, LAMP, SMD
- Amplification-based detection of *E.coli*
- Amplification-based panel detection of sexually transmittable diseases
- Amplification-based detection of respiratory disease panel
- Fluorescent & bead-based assays for chem/bio detection
- Integrated assays – molecular/immunoassay



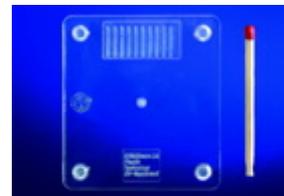
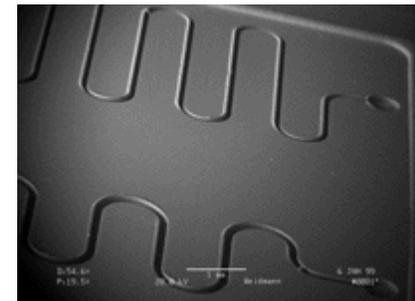
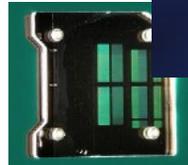
Rapid Prototyping

- Premier thin film prototyping facility
 - Capacity >100,000 cards per year today
 - Class 10000
 - Operates under design control guidelines
 - Oracle backbone
- Commercial ISO and cGMP production of lab cards
- Flexible and proven lab cards design and development
- Ability to integrate lateral flow strips, membranes, MEMs, glass, silicon, etc.
- Integrated card/instrument system development



Commercial Production

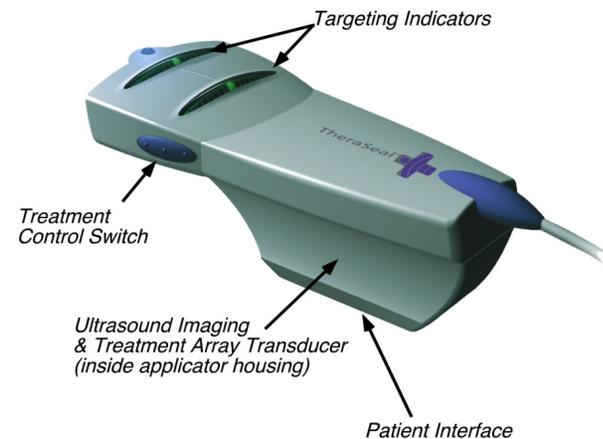
- Weidmann: High quality Injection Molding
 - Sub-micron feature size
 - Injection molding/ laminate hybrids
 - Commercial scale
 - ISO certified



WEIDMAN
PLASTICS TECHNOLOGY

Instrumentation Expertise

- Partnered with Stratos Product Development (Seattle)
- Complete engineering/design support as needed
- Handheld instrumentation experience
- Software expertise
- www.stratos.com





micronics

Micronics, Inc.
8463 154th Ave N.E.
Redmond, WA 98052
www.micronics.net
PH: (425)895-9197, x 126