Microfluidic Devices that Capture Bacteria for Growth and Kill Analysis

Michael J. Lochhead, PhD.
Accelr8 Technology Corporation
Denver, CO

AVS Symposium
Biomaterial Interfaces
Microbe-Surface Interactions
November 14, 2006

Microbe-Surface Interactions in a Clinical Diagnostics Context: Rapid Bacterial Detection and Antibiotic Susceptibility Testing

Hospital-acquired pneumonia

- ***ICU's #1 infectious killer***
- ***>300,000 cases/yr – US only***
- ***~40,000 to 90,000 avoidable deaths***

Analysis of Infectious Bacteria is still Culture-Based Days to Result

- CULTURE = same basic strategy as in the 1870s
  - 1970s: mechanization, still using cultures
- Typically ***24 to 72 hours*** to result

Physician starts empiric therapy long before lab results available

- **Incorrect initial therapy yields poor patient outcomes**
- **Contributes to emerging antibiotic resistant organisms**

Instrument Development Strategy

Combine *traditional microbiology techniques* in a novel, automated device format

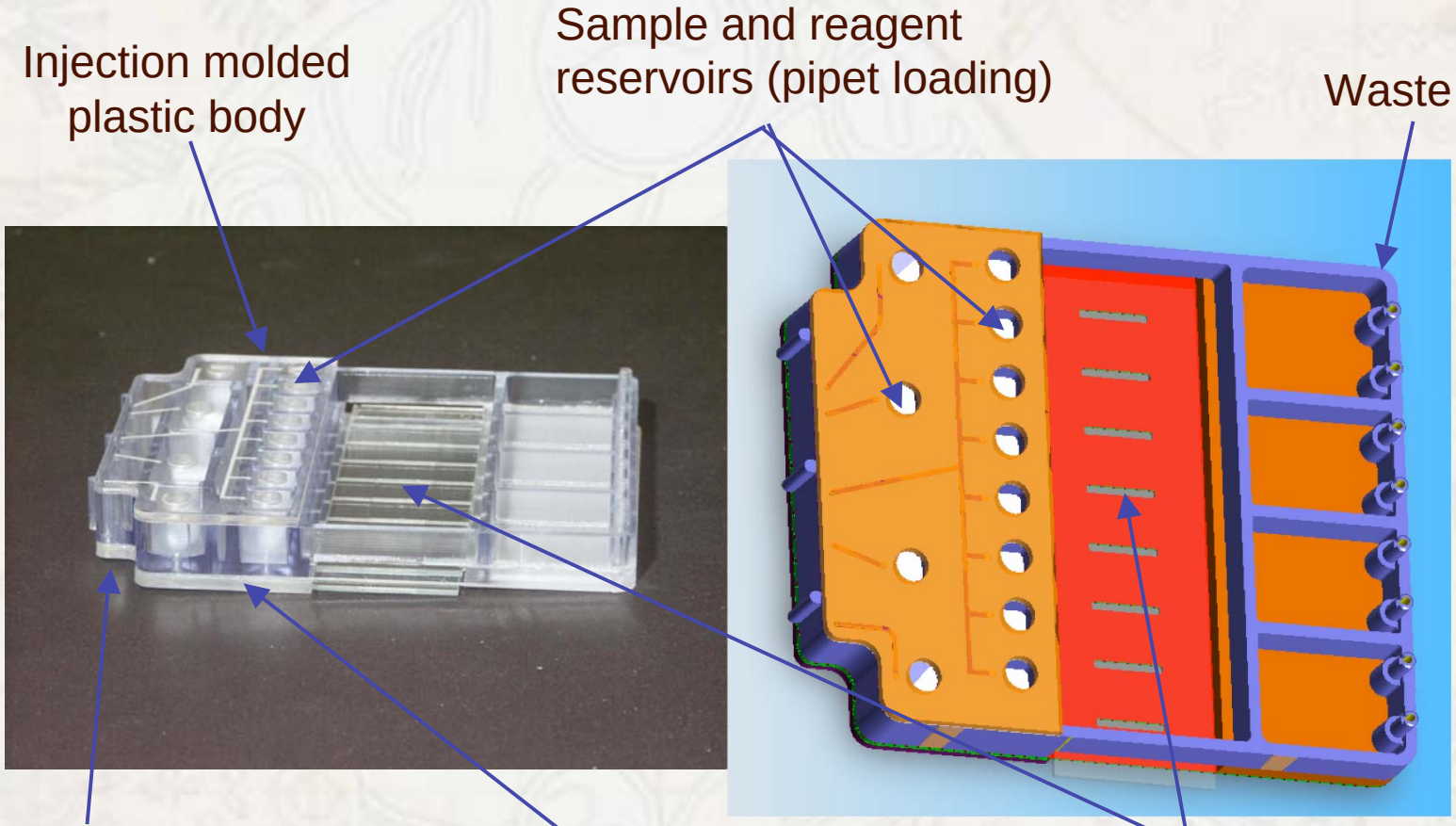
Microscopy-based analysis

- *Label-free (darkfield) microscopy*
- *Fluorescence microscopy*
- *Automated image analysis algorithms and database management*

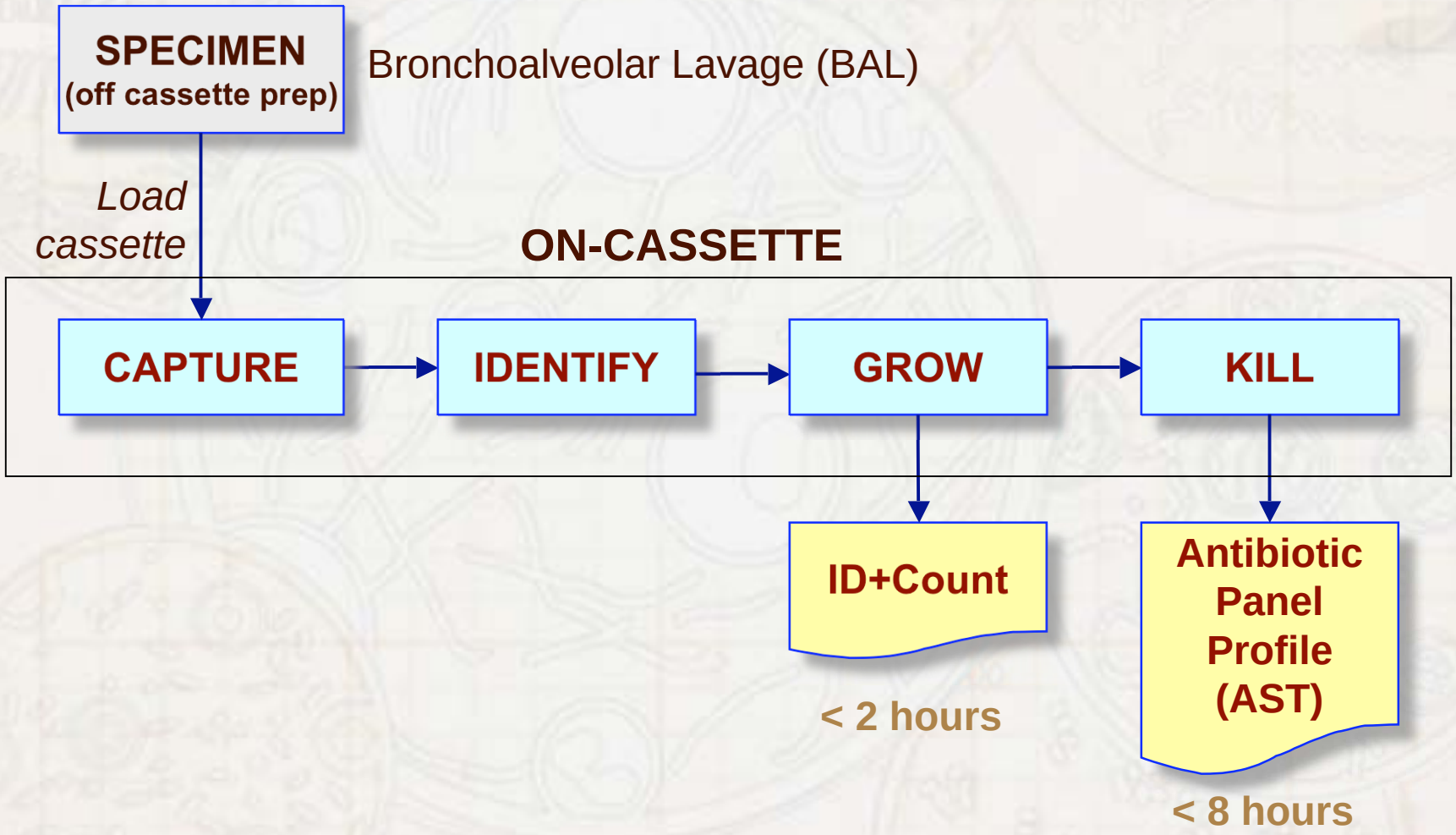
Disposable fluidic cassette

Single-Use Fluidic Cassettes

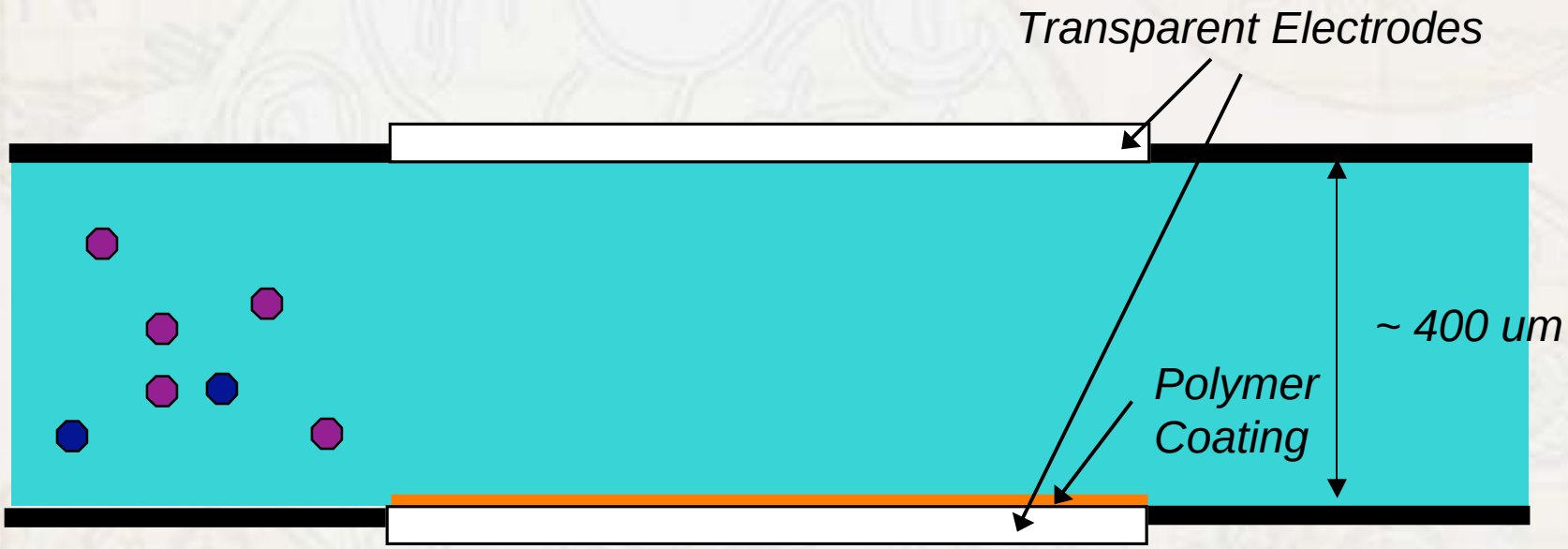
Multiple technologies satisfy different functions



5-Step Assay


SPECIMEN
(off cassette prep)
Bronchoalveolar Lavage (BAL)
Load cassette
ON-CASSETTE
CAPTURE
IDENTIFY
GROW
KILL
ID+Count
< 2 hours
Antibiotic Panel Profile (AST)
< 8 hours

Assay Details:
Sample Chamber Schematic

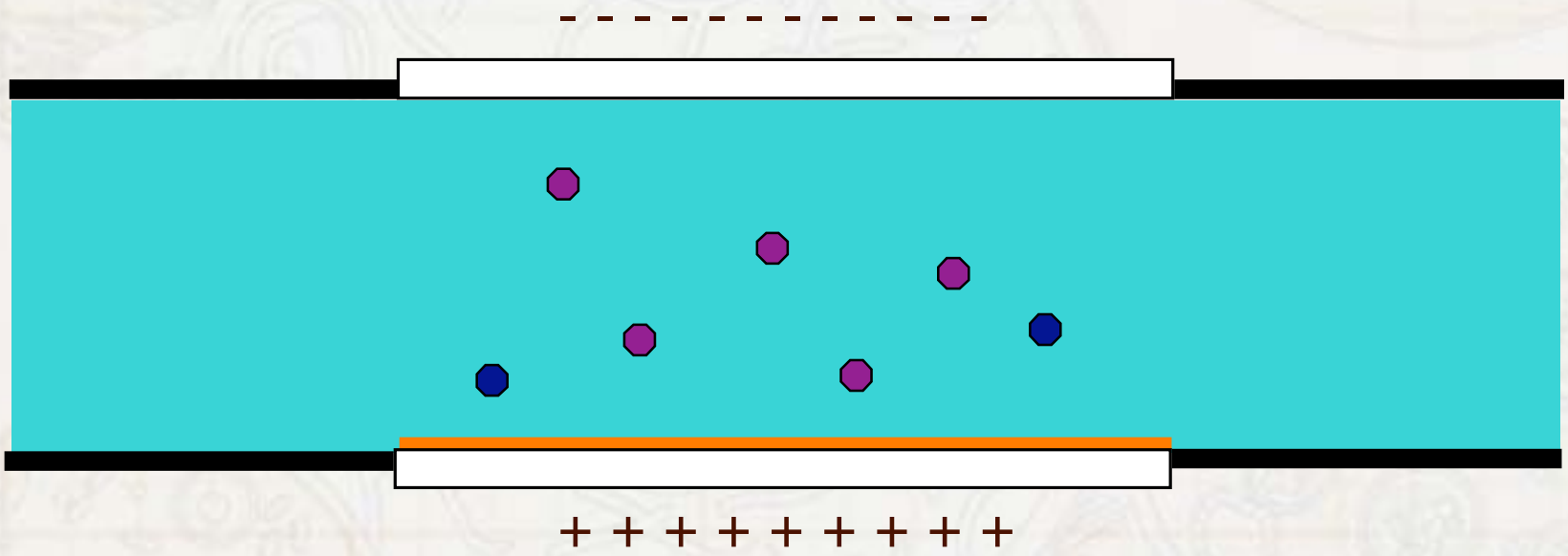


Bacterial Sample
- Off-line prep
- Suspended in proprietary buffer

Hydrodynamic (pressure-driven) flow through flow channels

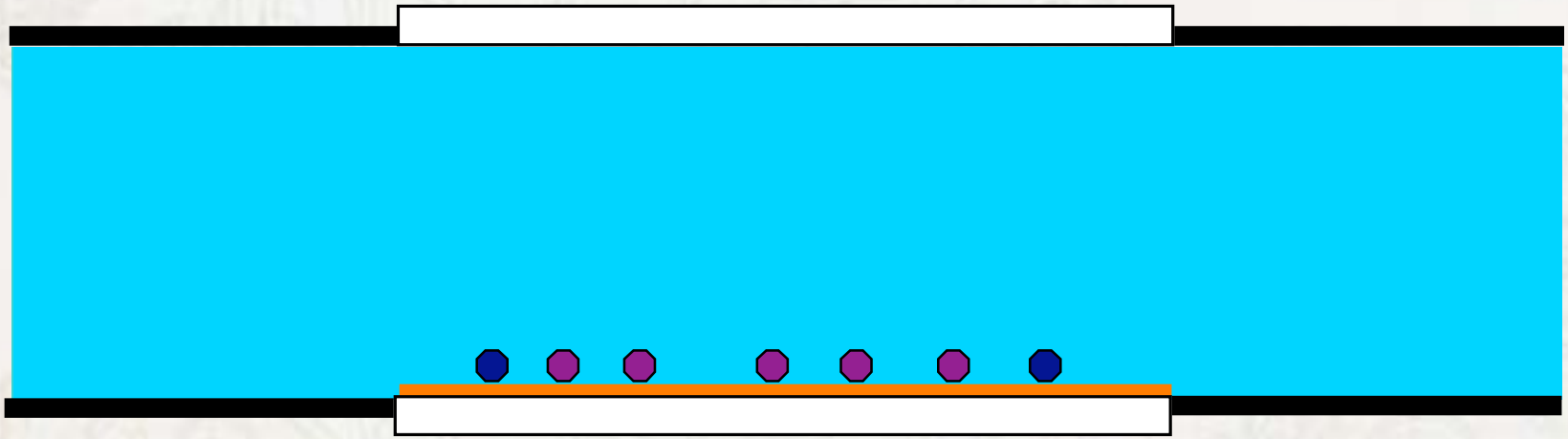
Assay Details:
Electrokinetic Concentration

Apply Low Voltage (< 2V)
Redox reactions create E field



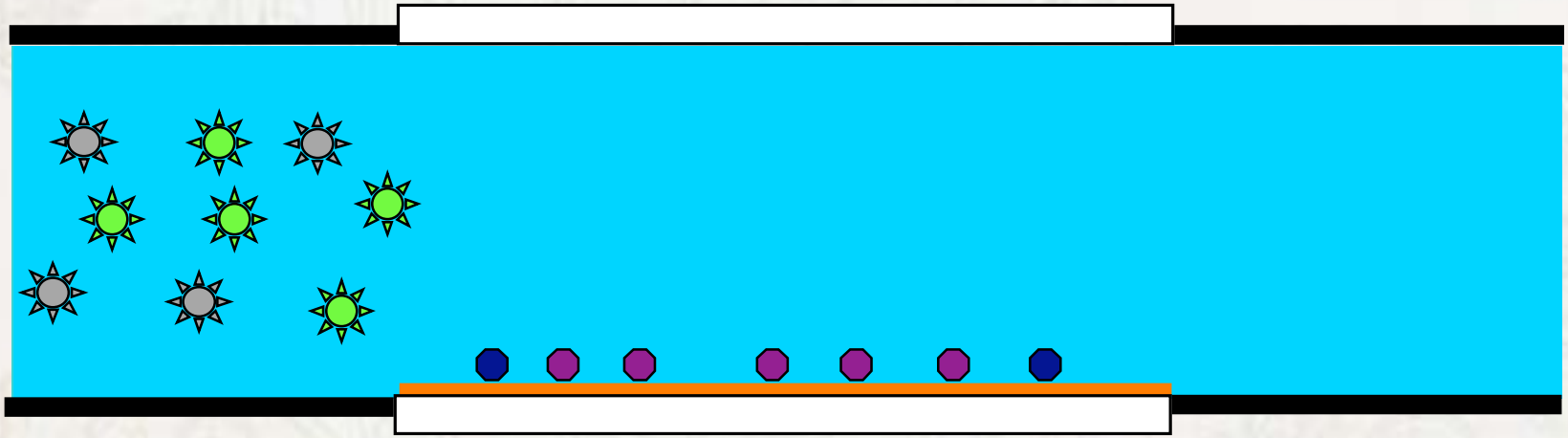
- **Electrokinetic concentration to capture surface**
- **Polymer coating facilitates adhesion**

Assay Details:
Bacterial Count and Identification



Real-time image acquisition throughout process

Assay Details:
Bacterial Count and Identification

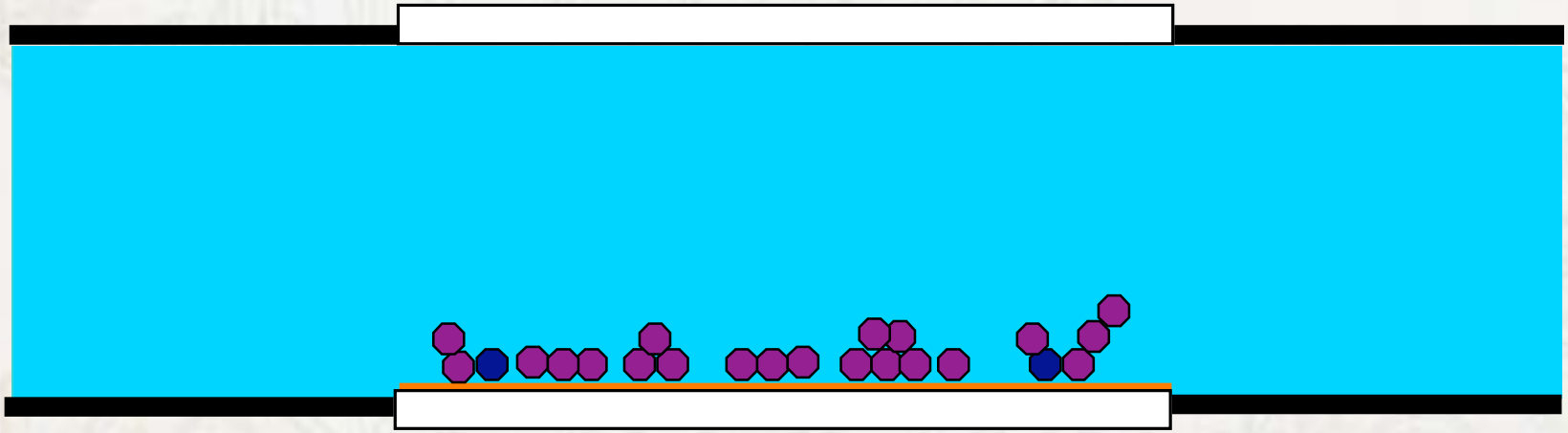


Fluorescent Reagent Cocktail

- Antibodies
- Gram stain
- Mortal stain

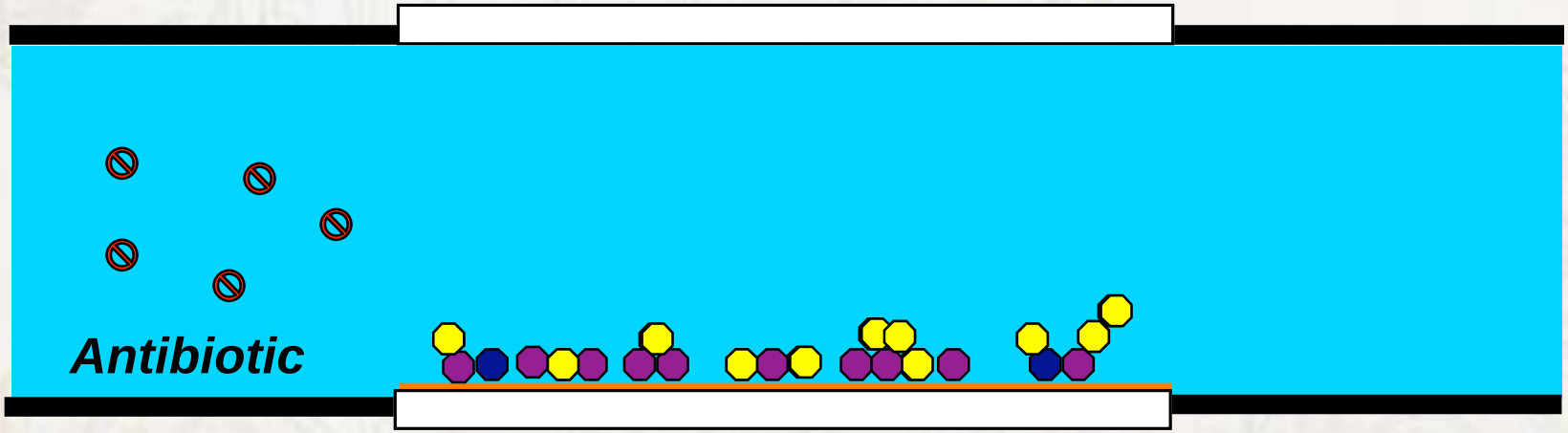
On-board incubation and programmed washes

Assay Details:
Bacterial Growth



- **Monitor growth of *individual* clones**
- **Generate quantitative growth curves**

Assay Details:
Antibiotic Susceptibility Testing



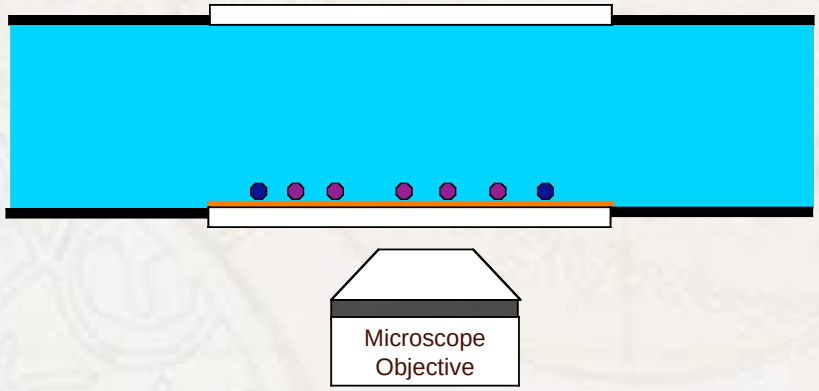
- Flow in antibiotic
- Monitor drug efficacy
  - *Change in growth kinetics*
  - *Mortal stain signal*

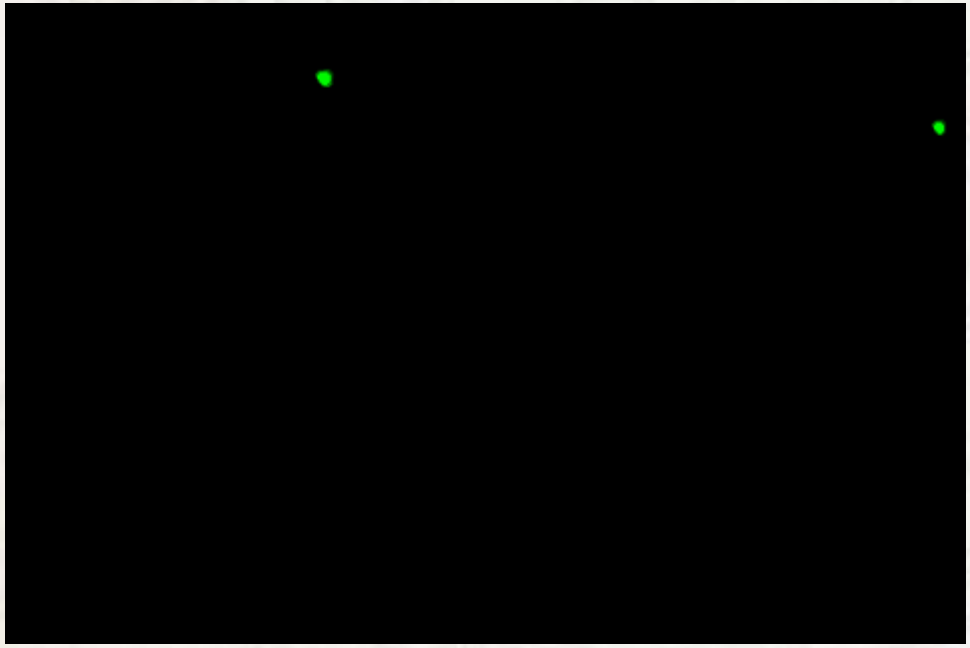
Electrokinetic Capture

CAPTURE

Elapsed Time

< 5 minutes

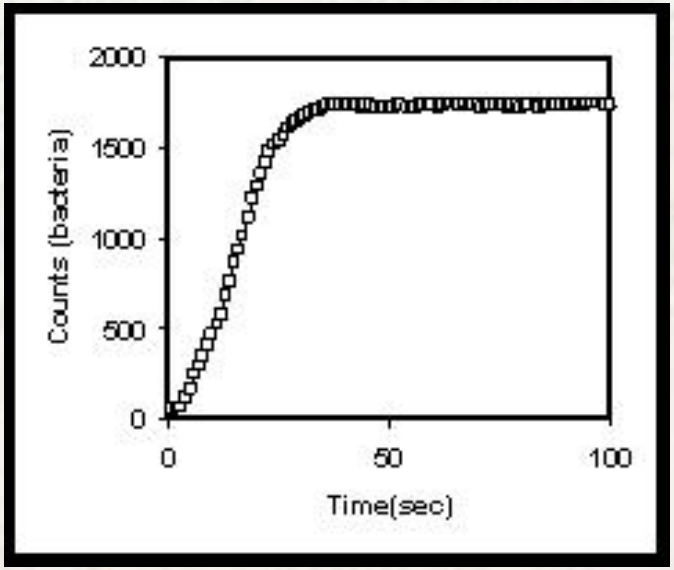




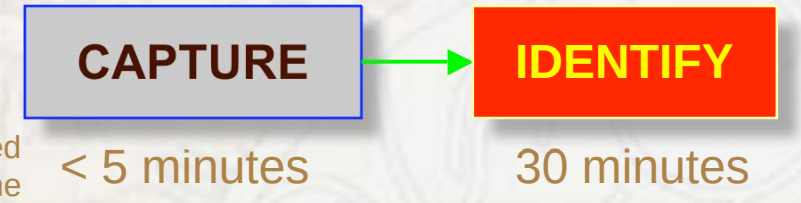
Bug size ~ 1 – 5 microns; 20X objective

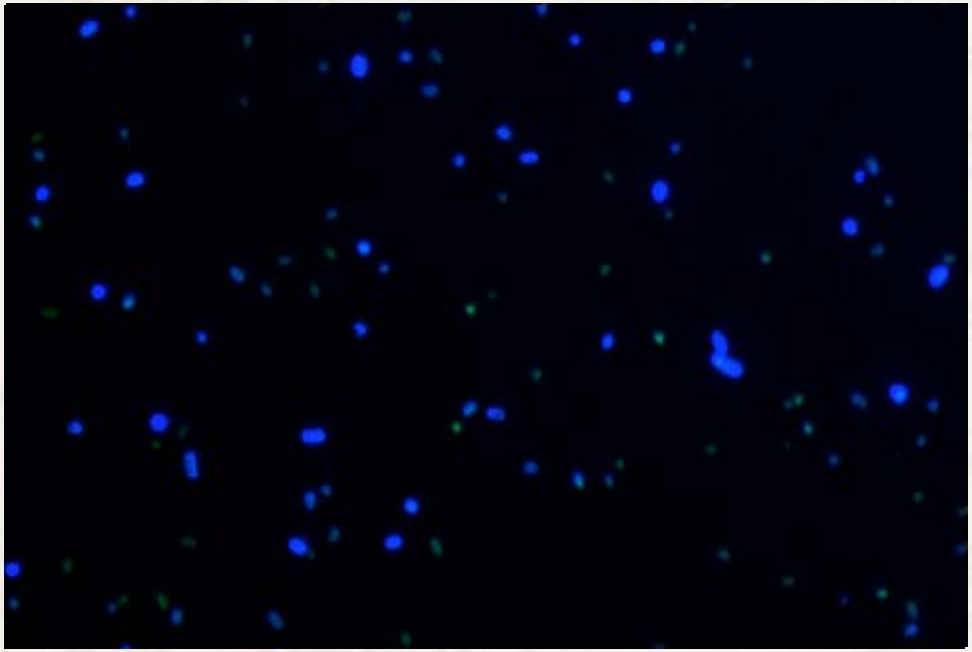
TIME-LAPSE PHOTOS

- **Darkfield Microscopy (false color images)**
- **Elapsed time < 2 minutes**



Species Identification





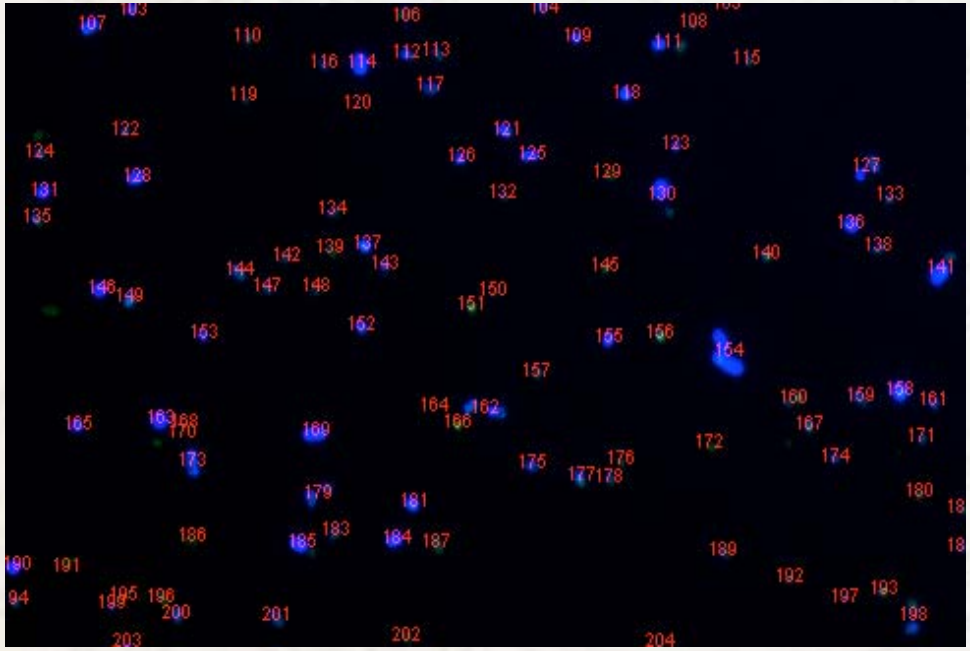
Label with antibodies against each species.

Antibodies have fluorescent "tags" of different colors.

Map and Count

CAPTURE → **IDENTIFY**

Elapsed Time: < 5 minutes | 30 minutes



Assign a physical address to each individual bacterium.

Enables analysis of the same individuals over time.

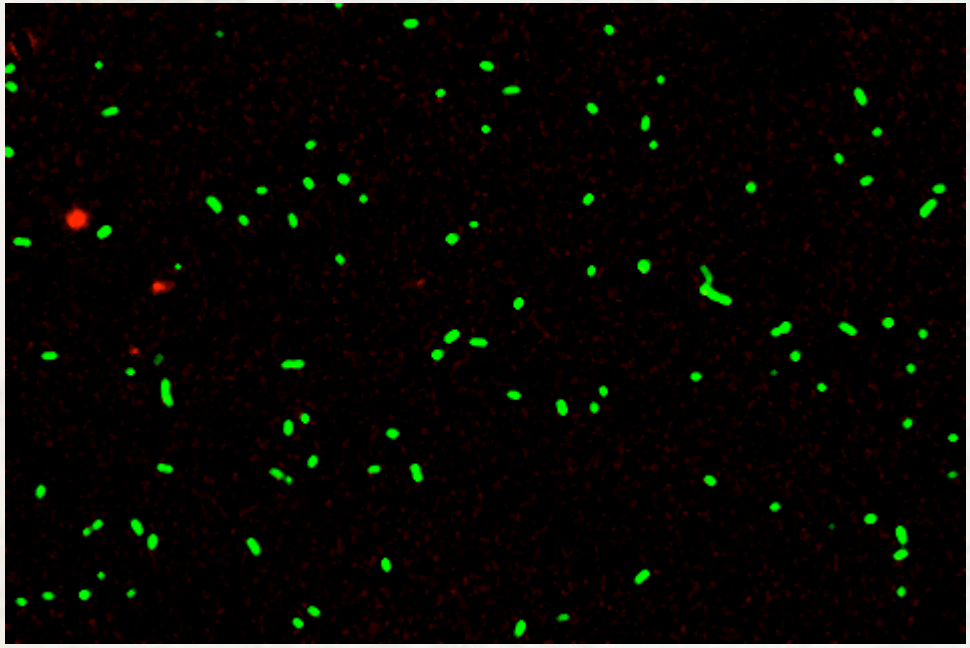
Quantum Microbiology™

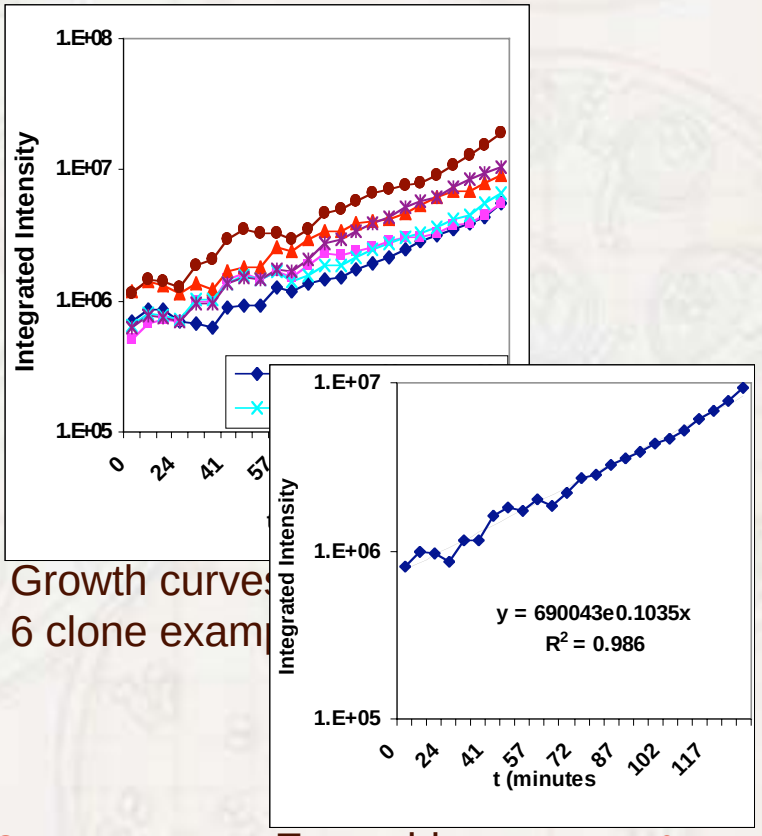
Growth Measurement

CAPTURE → IDENTIFY → GROW

Elapsed Time

< 5 minutes | 30 minutes | 60-90 minutes



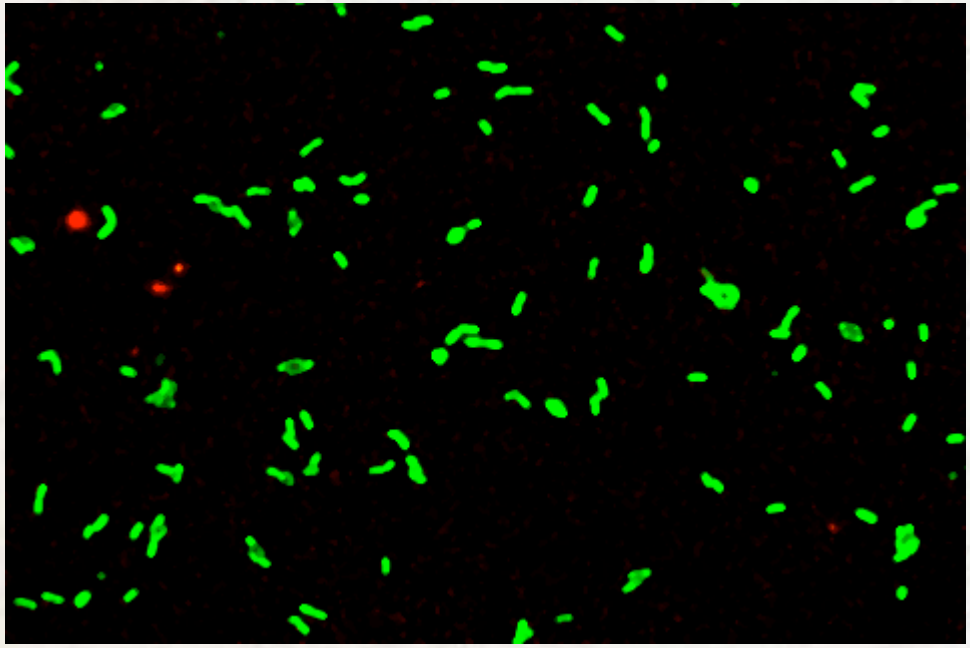


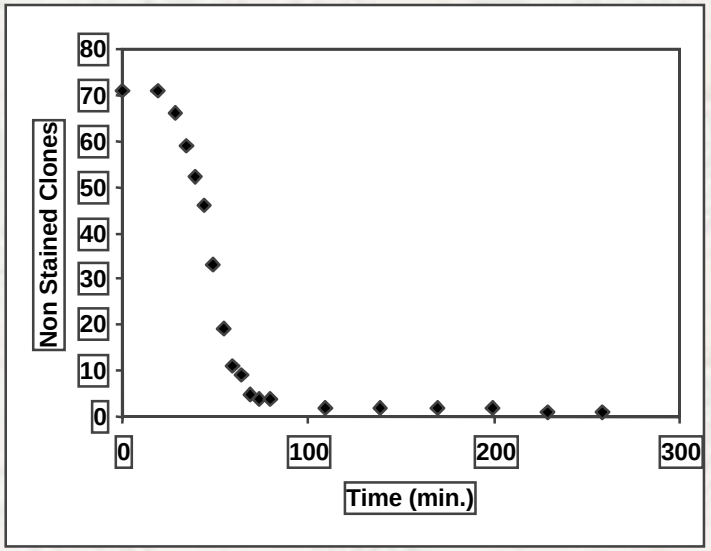
Growth curves
6 clone exam

Ensemble average

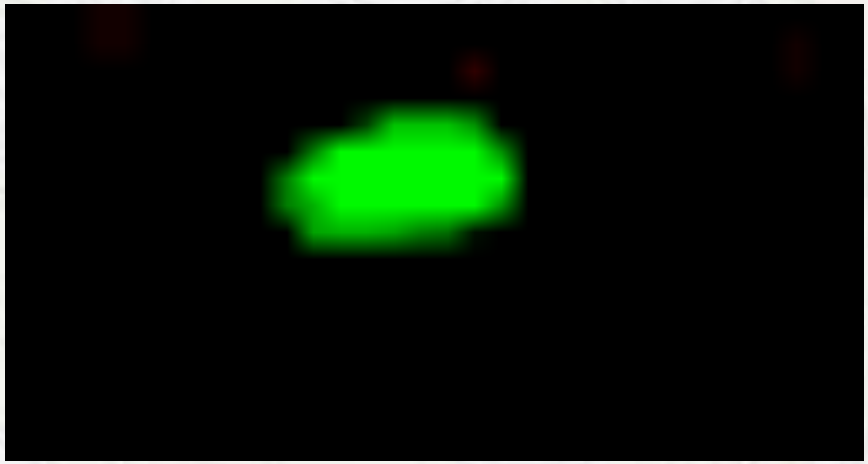
Antibiotic Kill Kinetics

	CAPTURE	IDENTIFY	GROW	KILL
Elapsed Time	< 5 minutes	30 minutes	60-90 minutes	2-5 hours





Single Clone: Growth and Kill



Growth rate	***k_0 = 2.6/hr***
Cell count	***N = 2***
Time of cell kill	***N_1 = 27-37 min.***
	N_2 = 37-45 min.

Accelr8 Rapid Bacterial Detection System Summary

- ✓ Whole organism identification and antibiotic susceptibility testing
- ✓ Bench-top, laboratory instrument
- ✓ Traditional microbiology techniques
- ✓ Disposable fluidic cassette

- ✓ Advanced surface chemistry

Microbe-Surface Interactions: Diagnostic Device Context

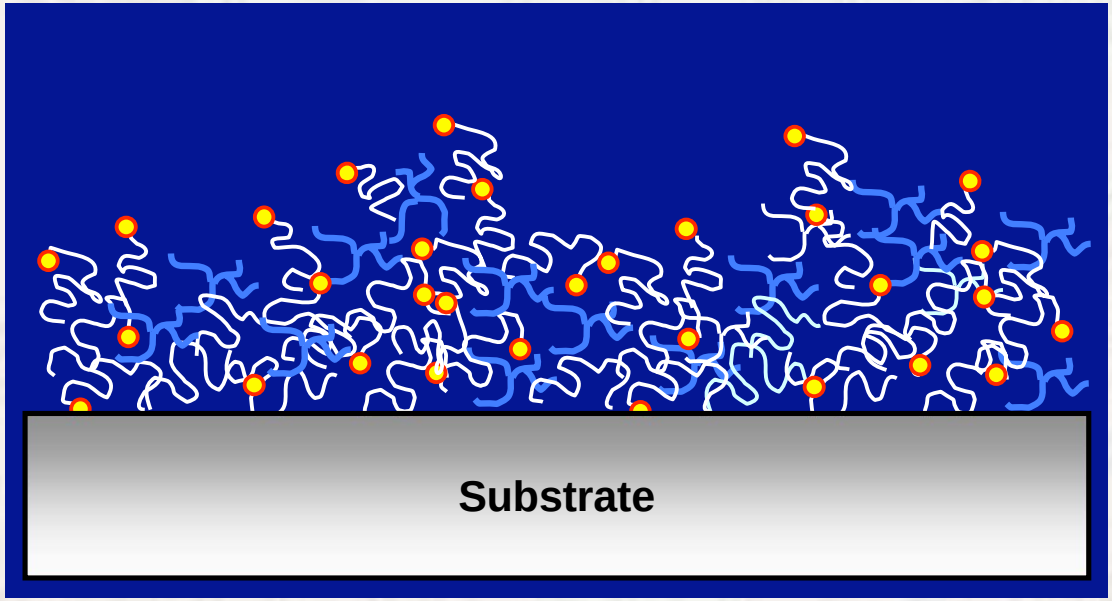
- ✓ ***Prevent*** **adhesion to assay surfaces in some places**
- ✓ ***Promote*** **adhesion to assay surfaces in others**
- ✓ **Maintain viability for AST applications**

- ✓ **Relatively short exposure times (hours)**

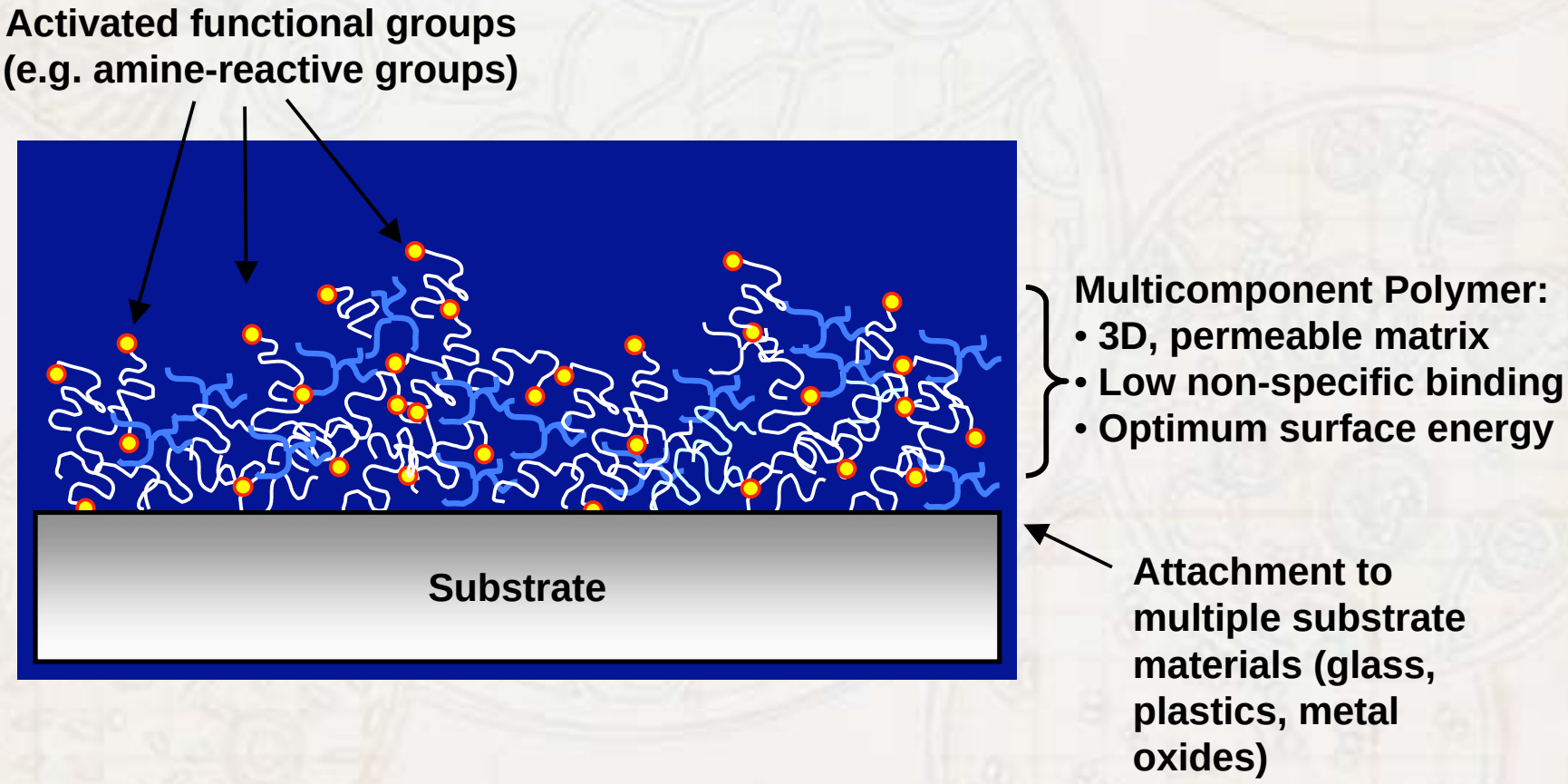
OptiChem® Surface Chemistry

U.S. Patents 6,844,028 and 7,067,194

- Poly (ethylene glycol) PEG-based technology
- Multicomponent, cross-linked formulation
- Thin hydrogel architecture
- Readily functionalized



OptiChem® Surface Chemistry
Engineered for low non-specific binding

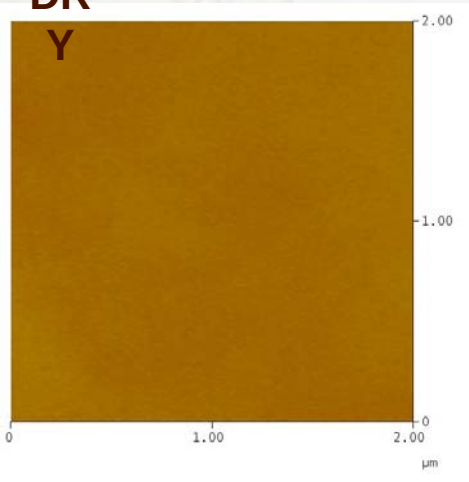


OptiChem® Coating Architecture:

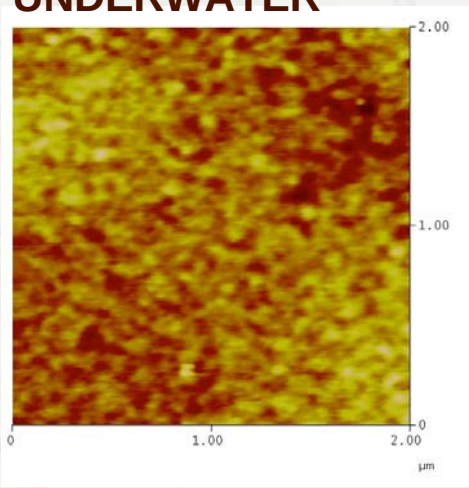
AFM surface topography (under water, tapping mode)

Dry Thickness:
~ 10 nm
(ellipsometry)

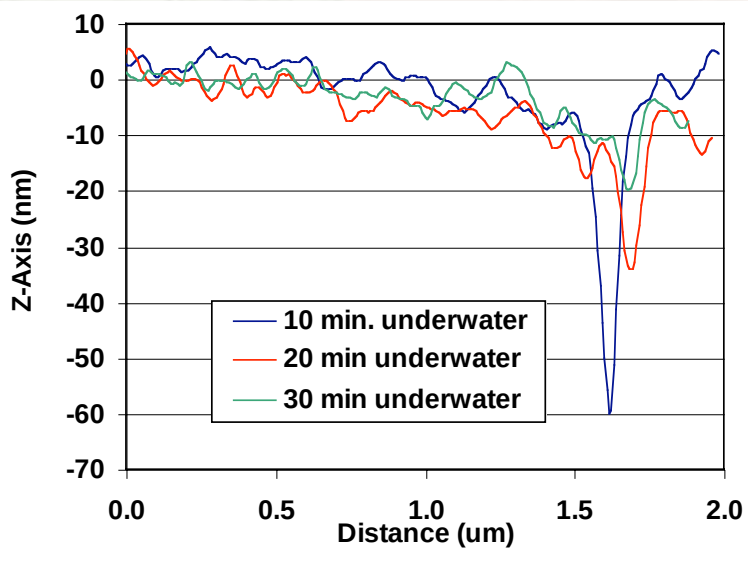
DRY



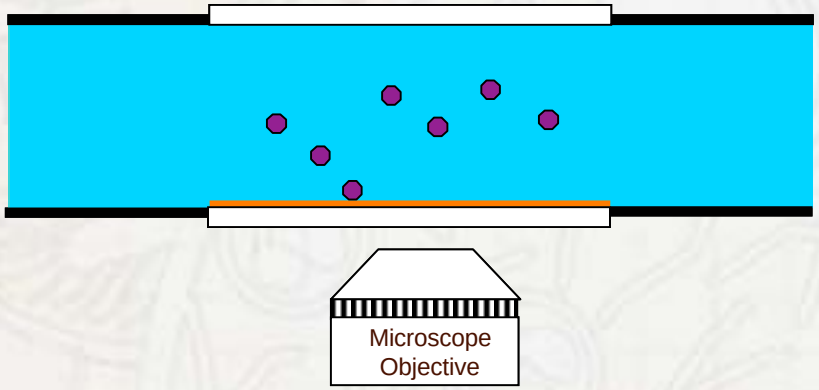
UNDERWATER



Hydrated Thickness:
50 to 100 nm

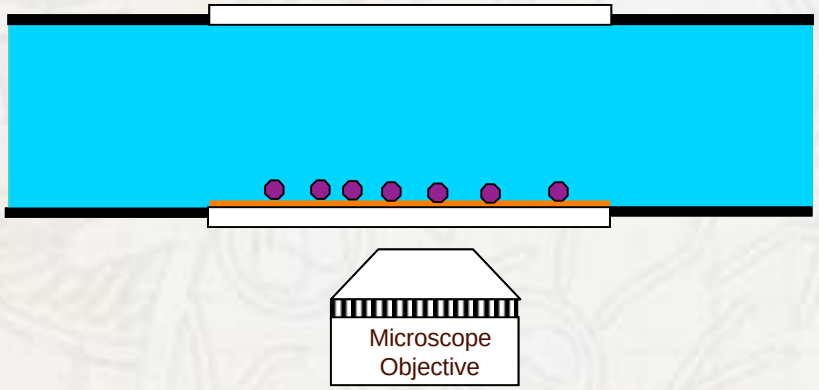


Bacterial Retention Assay



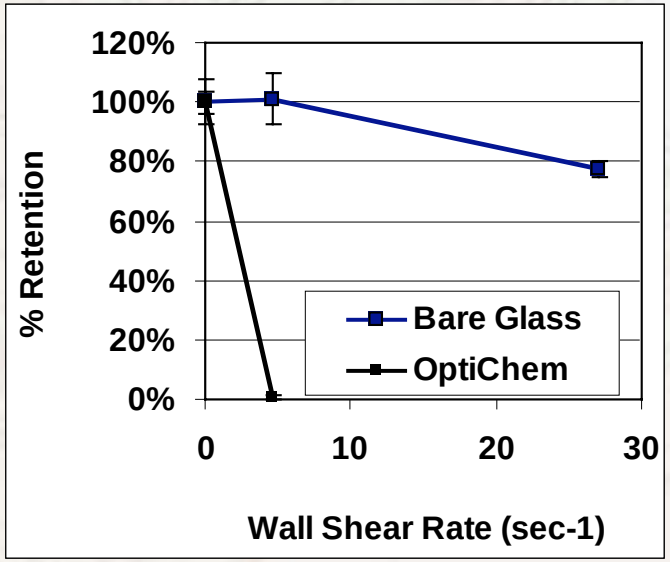
- ✓ **Gravity settle for one hour to polymer surface**

Bacterial Retention

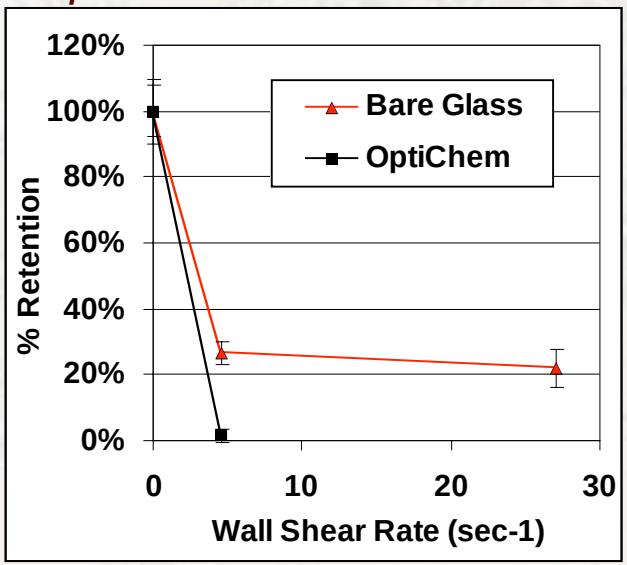


- ✓ **Controlled shear-rate washes**

S. aureus



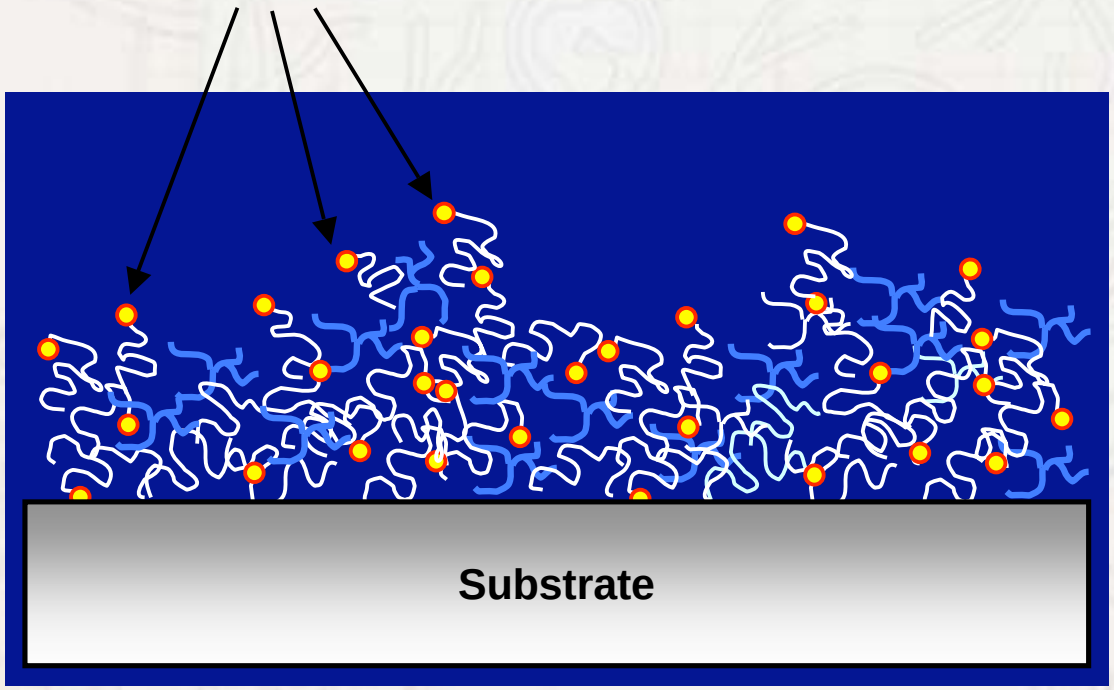
K. pneumoniae



OptiChem® Functionalization

Commercialized for Microarray Apps:

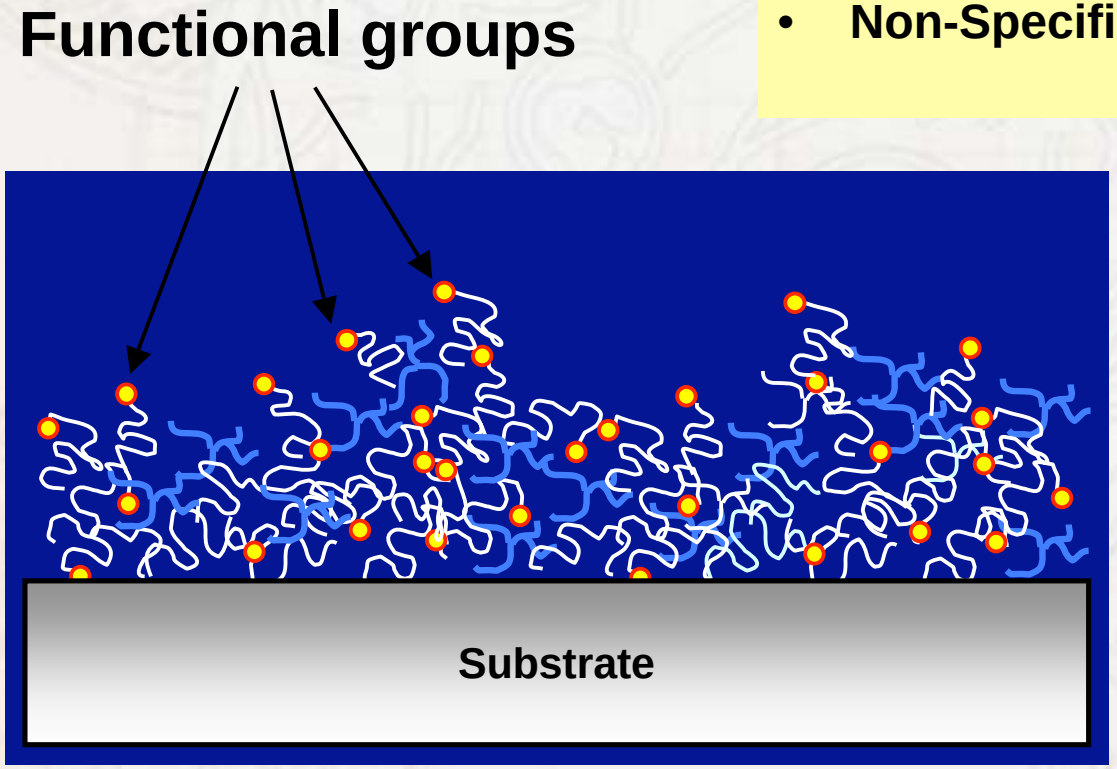
- **N-hydroxy succinimide (NHS)**
- **Streptavidin**



OptiChem® Functionalization

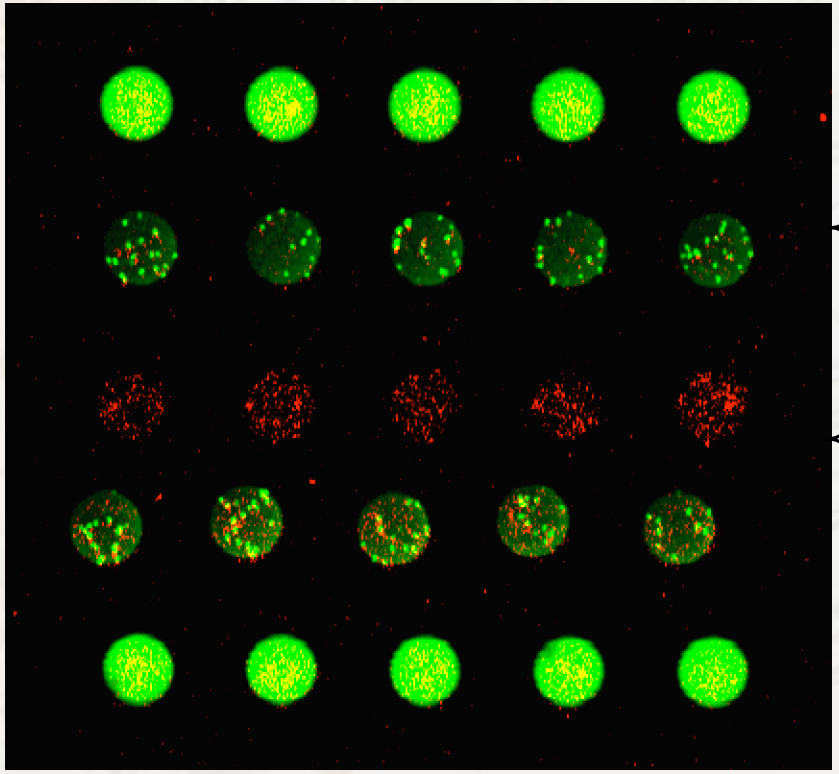
Functionalize for Bacterial Capture:

- **Specific: antibodies, lectins, etc.**
- **Non-Specific: polycations**



OptiChem Functionalization
Microarray, Whole Organism, Sandwich Immunoassay

1. Print array of specific monoclonal antibodies
2. Sample: Mixed *E. coli* + *S. aureus*
3. Mixed primary antibodies
4. Mixed detection molecules



Location control row (Rabbit IgG)

Capture: G anti *E. coli* O157:H7
Primary: R anti *E. coli*
Detection: G anti Rabbit A555

Capture: M anti *S. aureus* (mAb)
Primary: M anti *S. aureus*, biotin
Detection: Streptavidin-A647

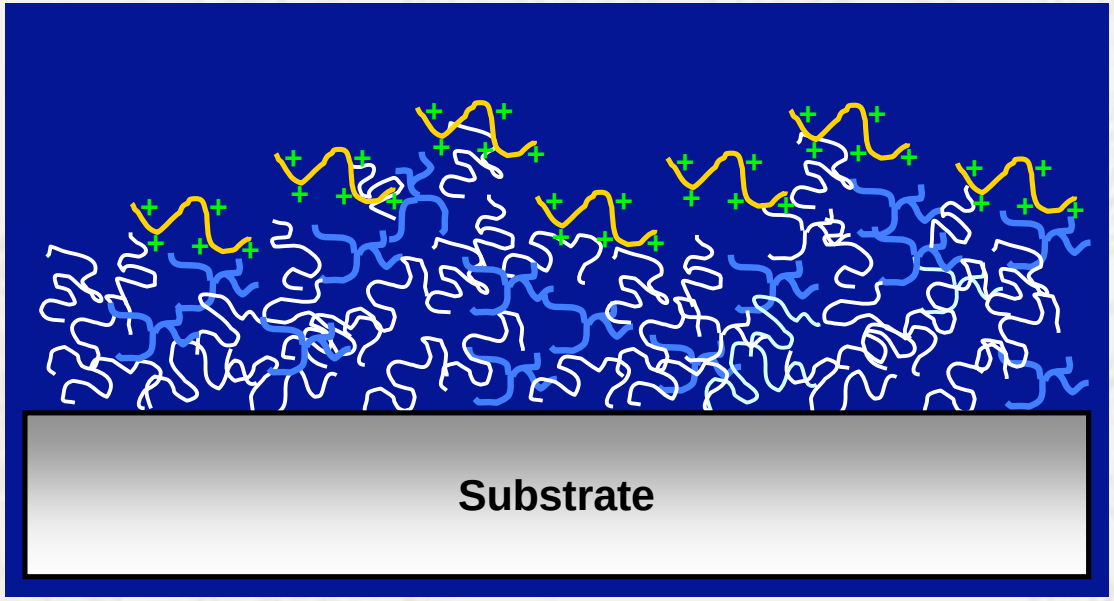
Mixed capture

Location control row (Rabbit IgG)

OptiChem® Functionalization
Non-Specific Capture of Whole Organisms

Cationic OptiChem Concepts:

- **Polypeptides (e.g., poly-L-lysine)**
- **Amine functionality**
- **Basic (cationic) proteins**



OptiChem® Surface Chemistry

- ✓ **Multicomponent, cross-linked, PEG-based films**
- ✓ **Outstanding inhibition of protein and bacterial cell adhesion**
- ✓ **Readily functionalized for specific attachment**
- ✓ **Scalable coating technology**

Acknowledgements:

- **Chad Greef**
- **Steve Metzger**
- **Dave Howson**
- **Ken Emoto**
- **Jim Mascali**
- **Misty Lindsey**
- **Nisha Kulpathipranja**

Accelr8 Technology Corporation

Mike Lochhead

mlochhead@accelr8.com

www.accelr8.com