Microfluidics *and Engineering a New Doctor-Patient Interface*

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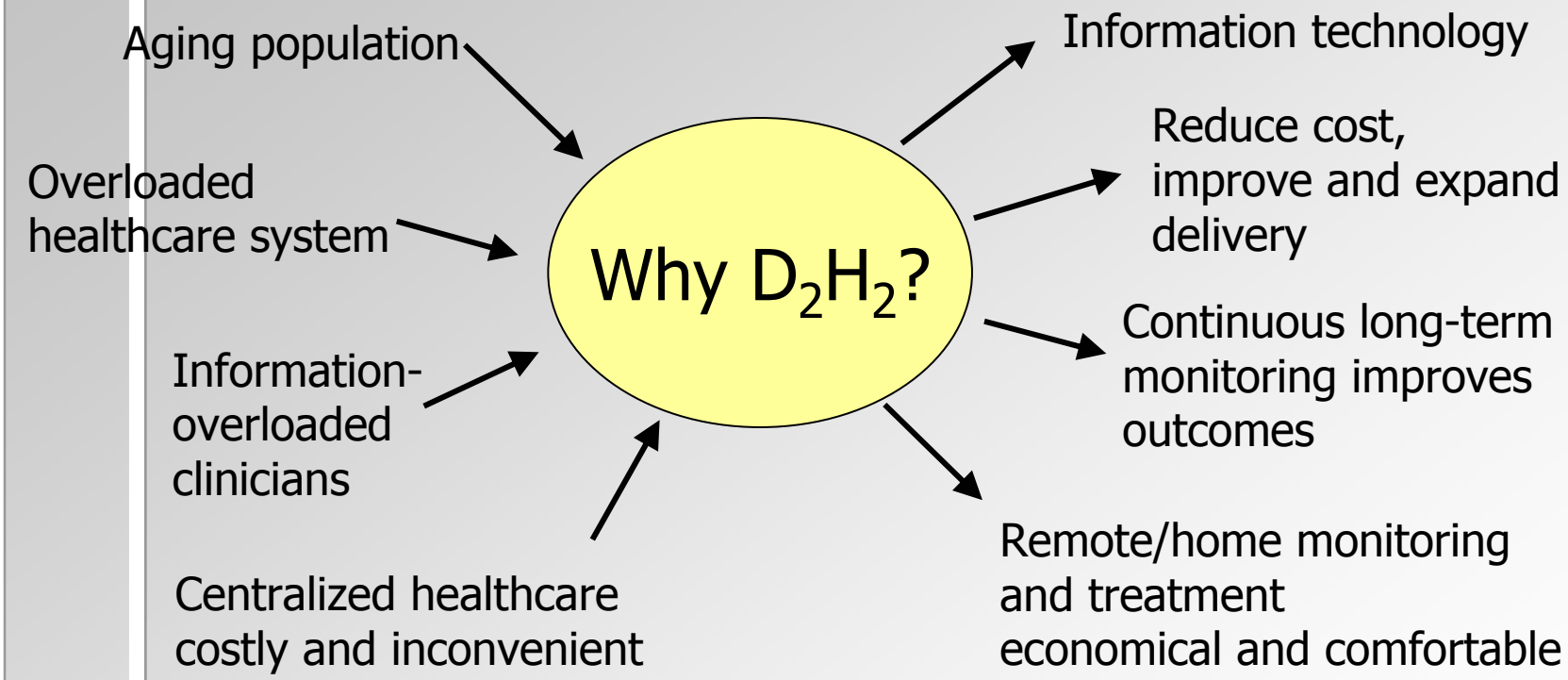
A view from the bridge

- Bioengineering is the discipline that will be able to exploit the discoveries of the life sciences and create the technology needed to apply them to the practice of medicine.

Bioengineering in transition

- 20th century bioengineers famous for developing excellent medical technology
  - Centralized
  - Expensive to buy, use and maintain
- 21st century bioengineers must look first to
  - Integration into the entire healthcare system
  - Minimizing the cost of the system

Distributed Diagnosis and Home Healthcare (D_2H_2)

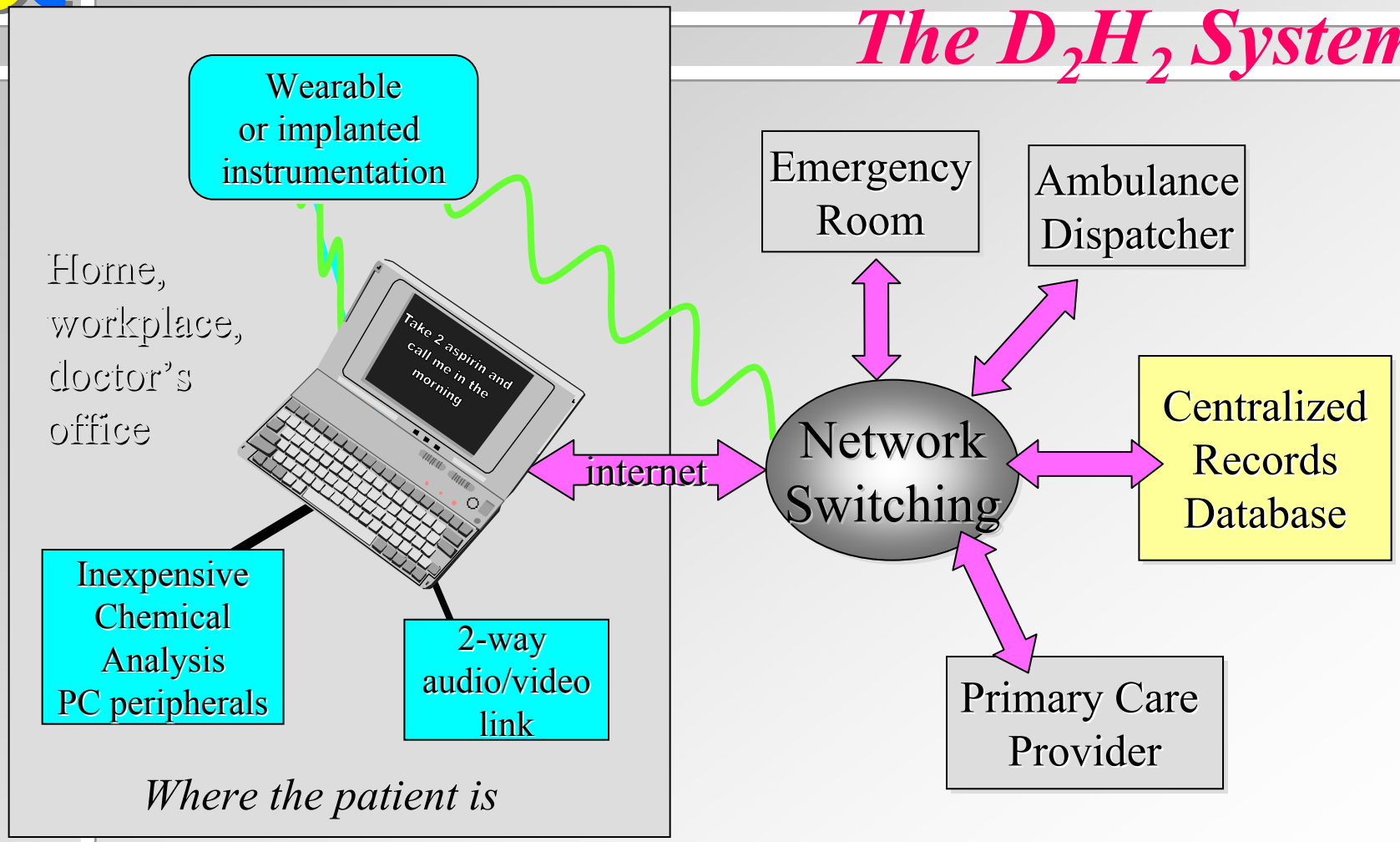


pushing

pulling

A New Doctor-Patient Interface

The D_2H_2 System



today the hospital, tomorrow the home

The Uses of D_2H_2

- Hospital inpatients (underway)
- Clinical trials of new medications
- Hospital outpatients (cancer, post-surgery)
- Chronic conditions (cancer, arthritis, AIDS, diabetes, heart disease)
- Monitoring chronic or critical drug treatment
- Pregnant women
- Early warning for emergent health conditions (e.g. heart attack, stroke, heat stress, infection, food poisoning, etc.)

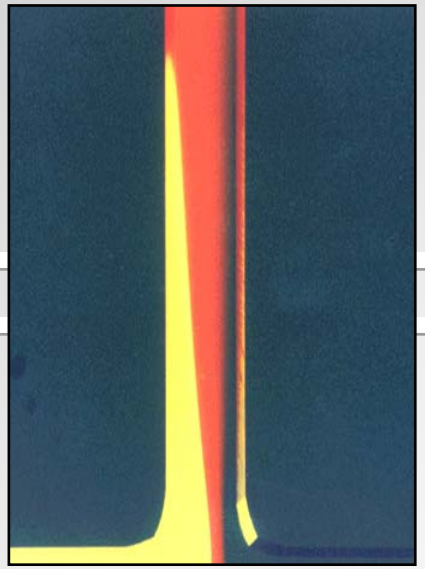
The Appeal of Microfluidics

- Potential to automate very complex procedures
- Compatibility with small sample volumes
- Potential for packing many devices in small spaces--parallel processing
- Little waste
- Possible integration with pumping, detection and processing components
- Reproducibility of function
- Potential for mass fabrication
- *Potentially* low inherent cost

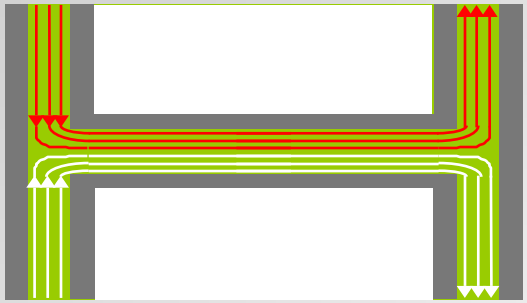
Yager Lab Medical Diagnostic Goals

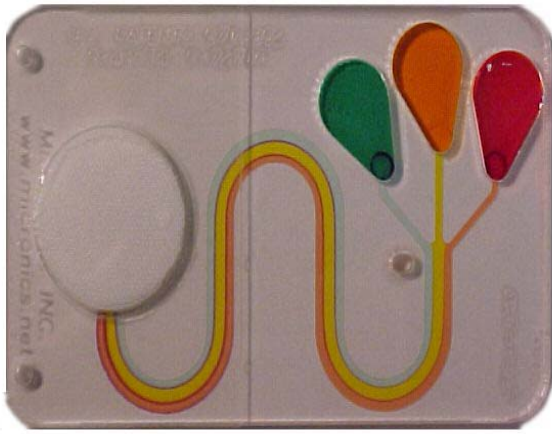
- A disposable polymeric laminate cartridge that:
  - costs less than $5
  - holds all the chemistry needed for multiple complex quantitative bioassays on a drop or two of biological fluid *(not just blood!)*
  - can be left in the glove compartment of a car all summer in Texas or winter in Alaska
  - requires only insertion in a handheld device to work
  - gives all results in under 5 minutes
  - provides laboratory-quality quantification of analytes
- A portable inexpensive measurement system that supports the use of laminate cartridges

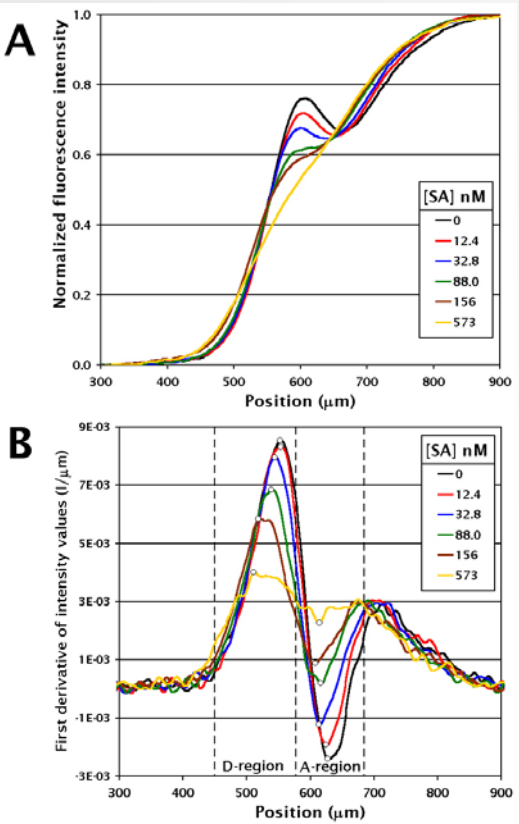
Technologies Developed Since 1994 at UW or Micronics

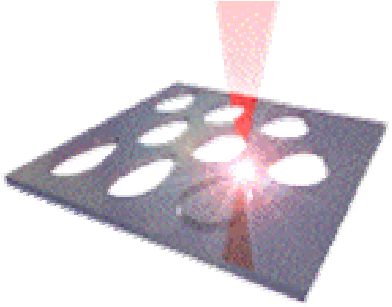


- Microfluidic methods and devices:
  - H-filter
  - T-sensor
  - Electrokinetic Fractionation
  - Electrokinetic concentration
  - Mixers
  - Valves
  - Switches
  - Flow cytometers
  - Immunoassays









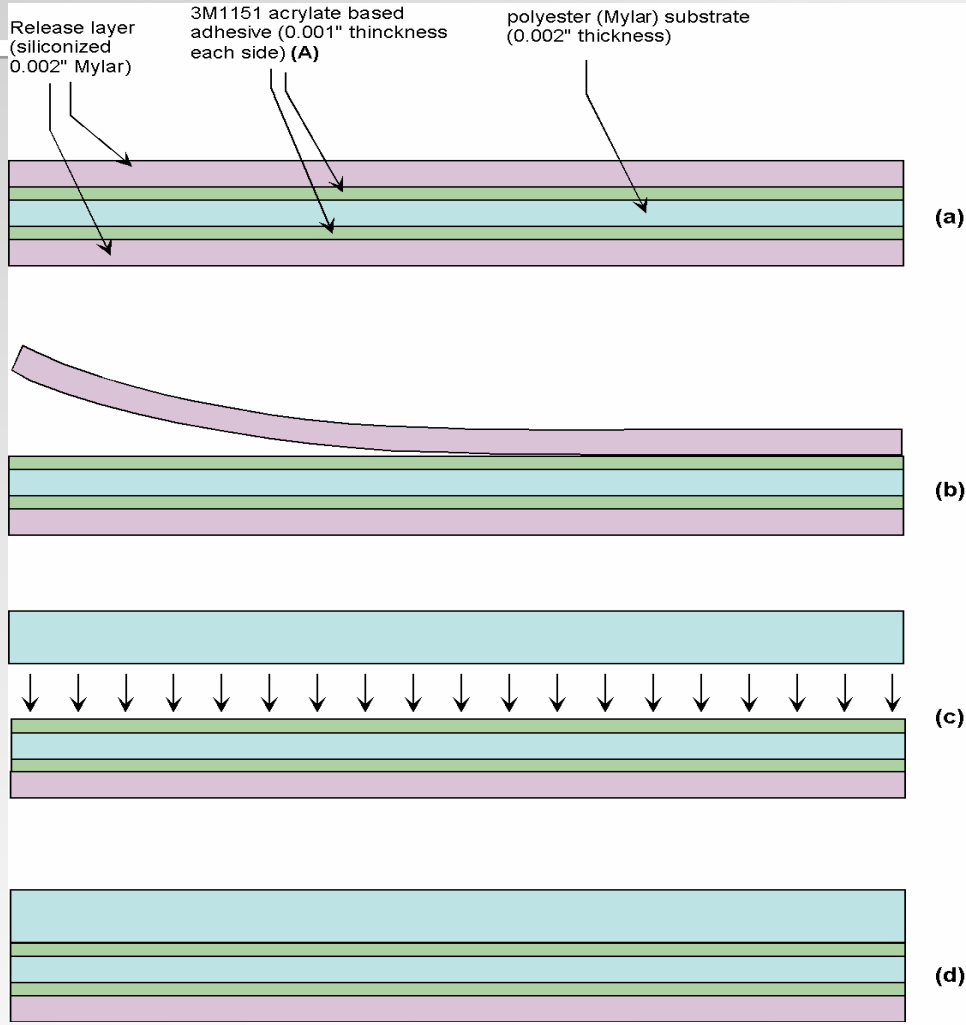
WTC/Micronics Rapid Microfluidic Prototyping

QuickTime™ and a
Animation decompressor
are needed to see this picture.

- This CO_2 laser cutting system allows conversion from CAD file to assembled multi-level polymeric laminate **in ~4 hours.**
- This is a powerful new tool for rapid prototyping.
- Assembled laminates compatible with aqueous solvents.

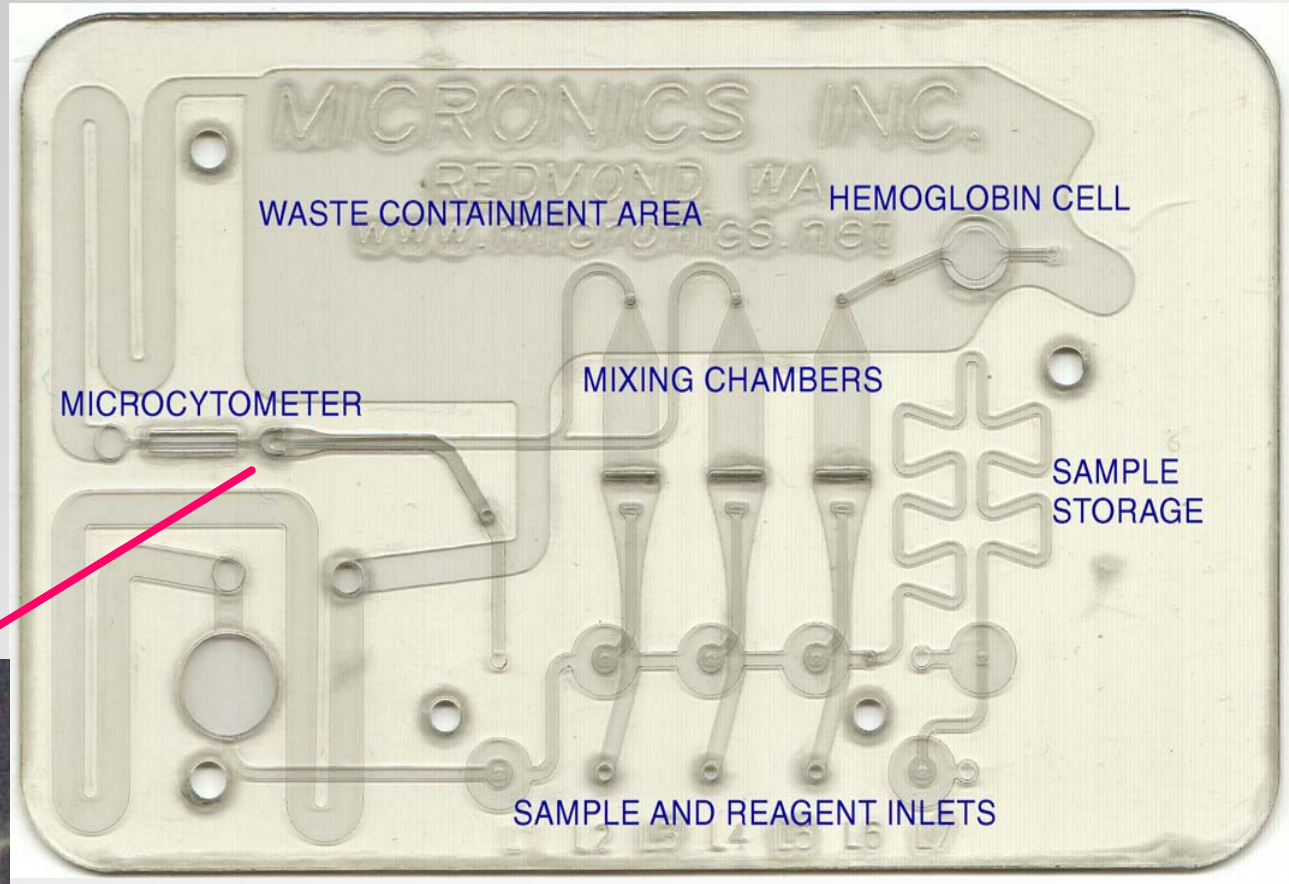
Lamination

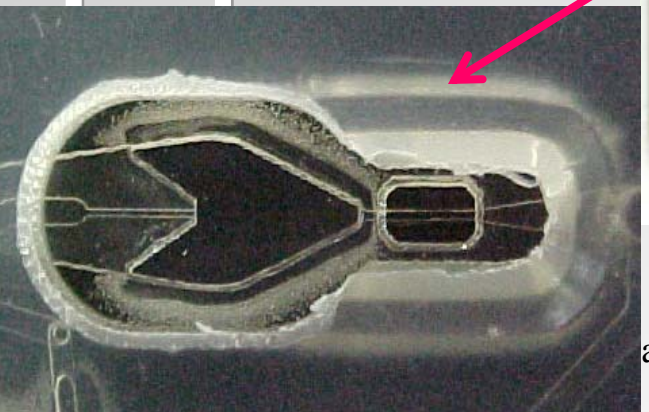
- 0.004” thick Mylar coated on one, both or neither side with 3M1151 Adhesive
- The release layers are removed and subsequent layers are stacked to form desired device geometry



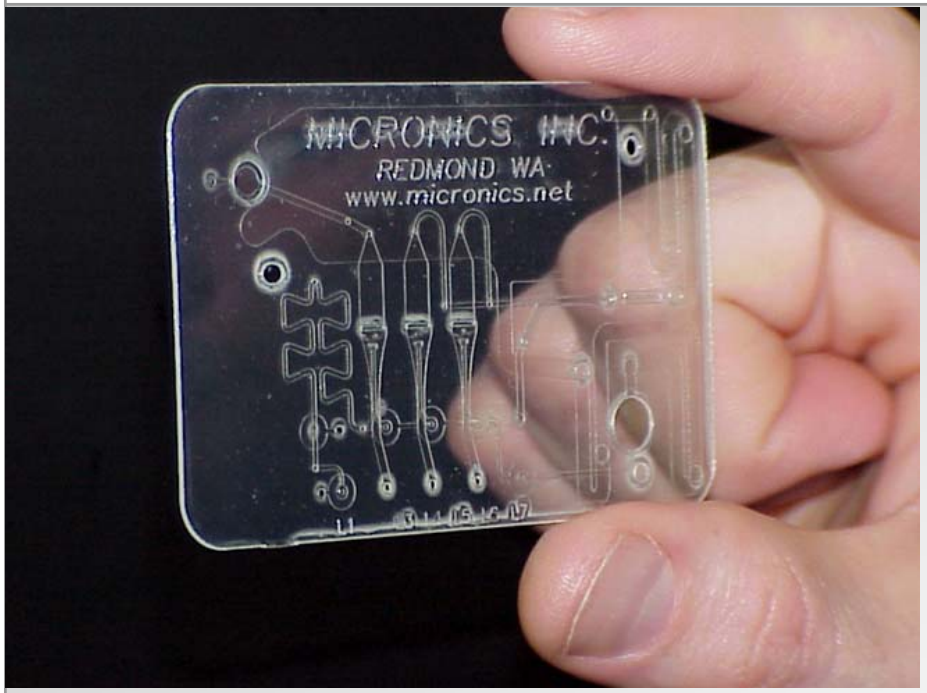
Integrated Chemical and Cellular Analysis in a Single Disposable

Micronics' 7-layer Mylar laminate hematology cartridge-- *rapid prototyping and mass fabricability*

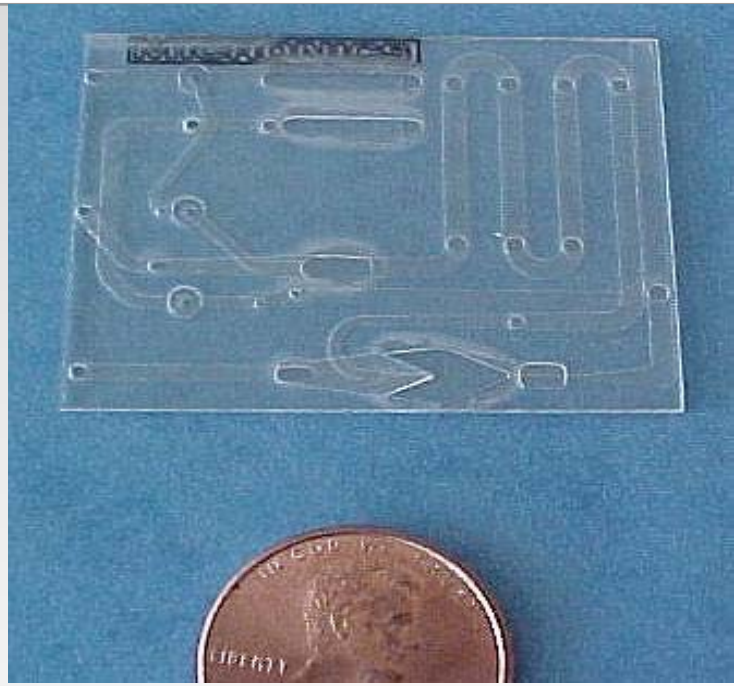




This technology is *just small enough*

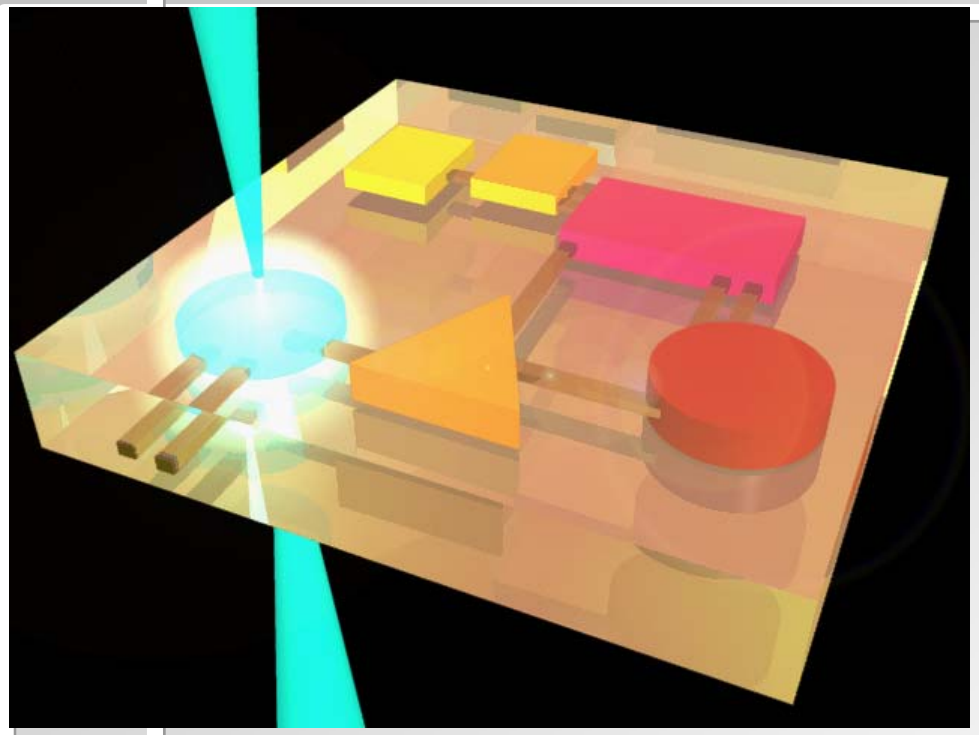


- Micronics demonstrates complete fluidics for a flow cytometry system on a 7-layer microfluidic card



- Under development with Micronics and Honeywell for DARPA is a complete cytometer to fit in a wristwatch.

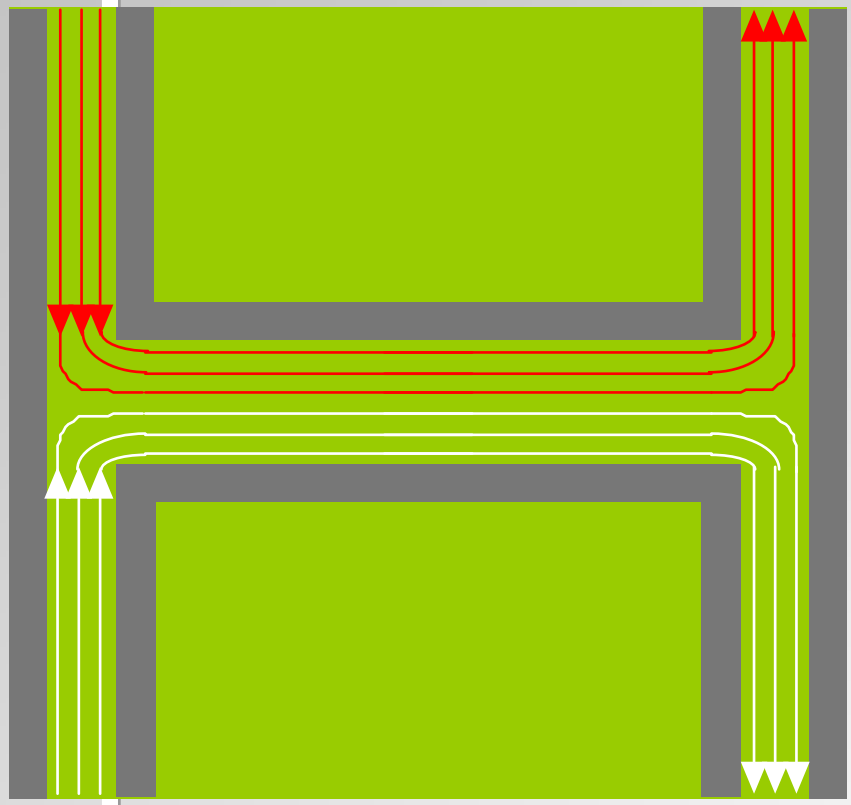
A "New" Paradigm? Laminate-Based Chemistry



The design of microfluidic systems (be they analytical or synthetic) is *completely analogous* to current design of chemical plants scaled in acres.

- Experiments are designed to be optimal for the small samples used, including multiple processing steps carried out entirely within the laminate.
- Optical and electrical access for real-time analysis by standardized off-laminate instruments are provided.
- If the experiment is <u>not</u> optimal, *redesign and retesting in one day* is straightforward.
- Because the laminates themselves cost only a few dollars, if the experiment works well, the device can be duplicated inexpensively for use at home *and shipped <u>as a file</u> for use by collaborators elsewhere the next day.*

Develop an H-filter-based microfluidic device capable of extracting a wide range of analytes *from the mucins in saliva*



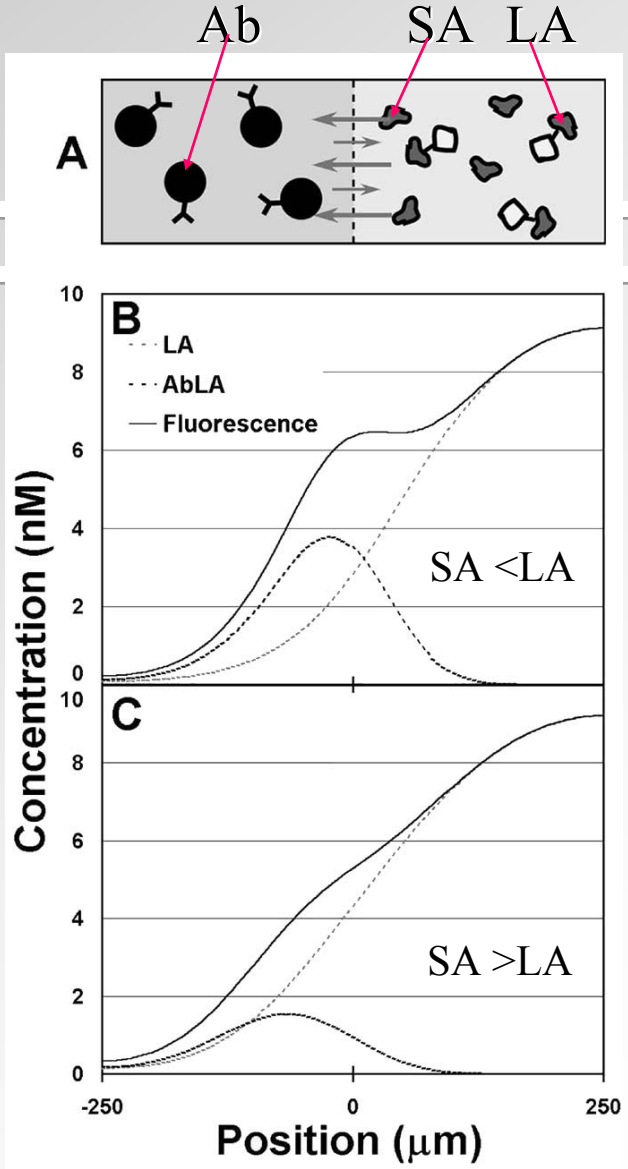
- In a thin channel at low Reynolds number, *turbulence cannot exist*.
- Flow streams can run next to each other without mixing; the reversible apposition of two fluids as at left is possible only in devices with small dimensions.
- Diffusion is the dominant transport mechanism between adjacent flow streams.
- Large particles diffuse slowly and stay on their side of the channel; small molecules rapidly diffuse across.
- ***We will extract analytes from the mucins in a flowing stream of saliva with this principle.***

The DIA:
A New Bioassay based on Microfluidics

- The T-sensor was shown early on to measure flowing analytes
- Given that immunoassays are central to diagnostics
- Attempt to see if a competition immunoassay could be implemented in a T-sensor
- The Diffusion ImmunoAssay (DIA) based on differential diffusion of small and large molecules

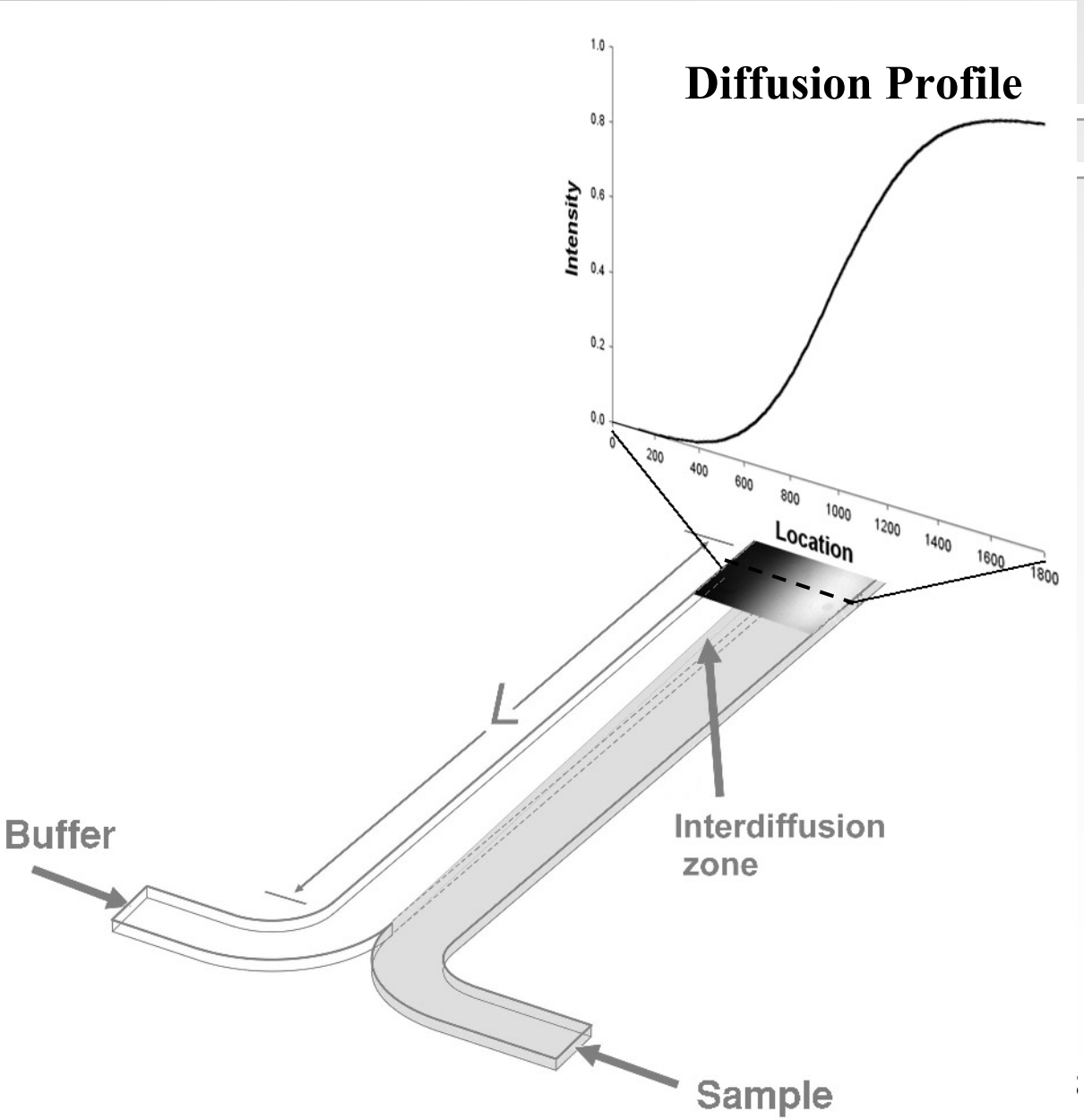
The DIA: A diffusion-based competition immunoassay

- In the assay the sample antigen (SA) is forced to compete for the antibody (Ab) binding sites with a known quantity of antigen to which a small fluorescent label has been covalently attached (LA).
- If [SA] is high enough, all of the Ab binding sites are blocked and the LA diffuses as if the Ab were not there!



Measurement with the T-Sensor

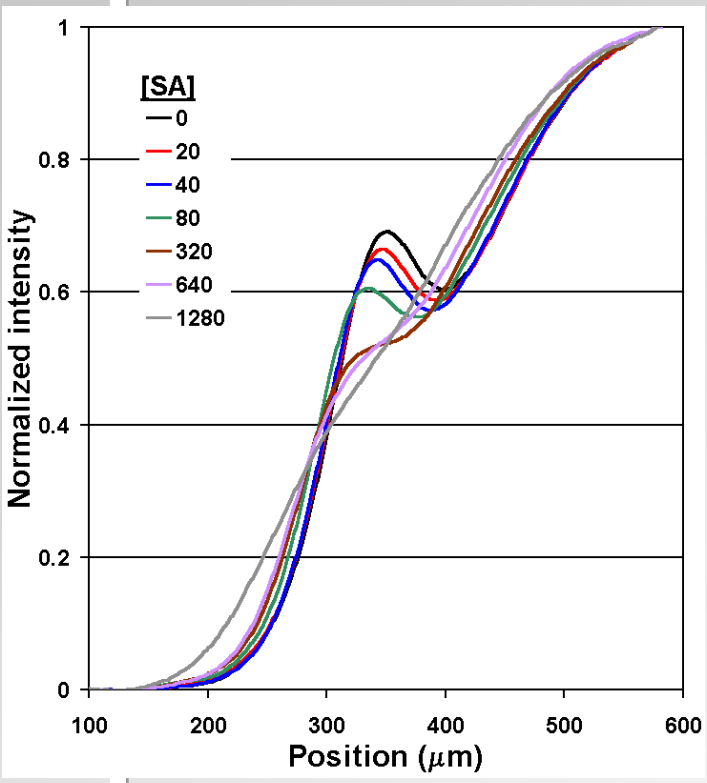
- Optical imaging along the *y* axis (transmission or reflection) allows measurement of concentrations of analytes along *d* at a known distance *L* downstream (and a known time since contact between solutions.
- Interactions between diffusing species and be measured by intensity and position.

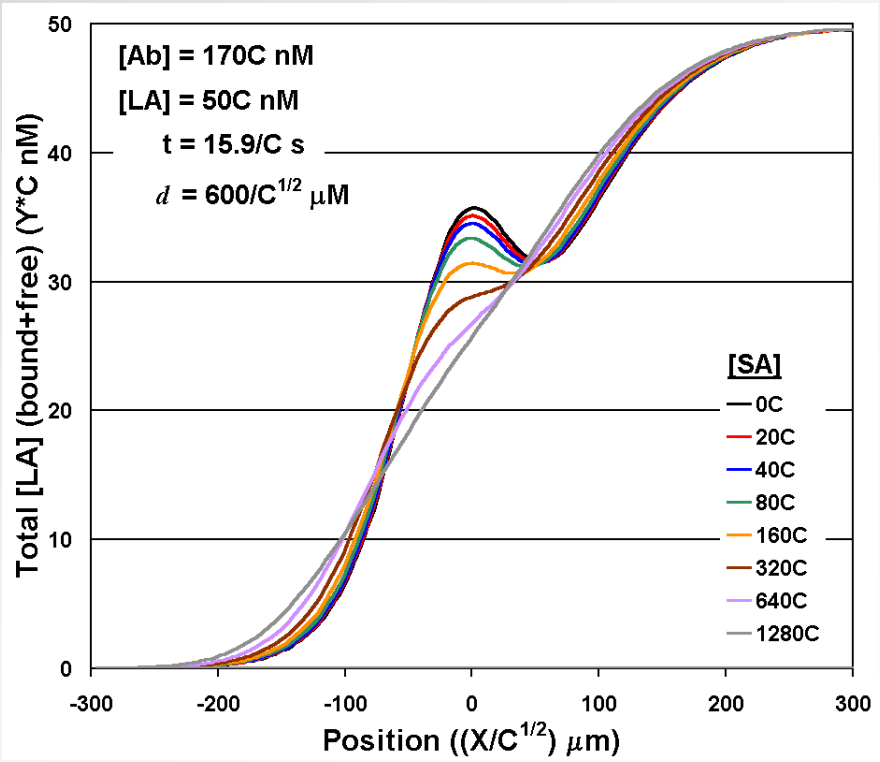


DIA for Anti-epileptic Drug Phenytoin

- **Experiment:** fluorescence intensities across the T-sensor for [SA] from 0 to 1,280 nM (1000 x lower than in blood)

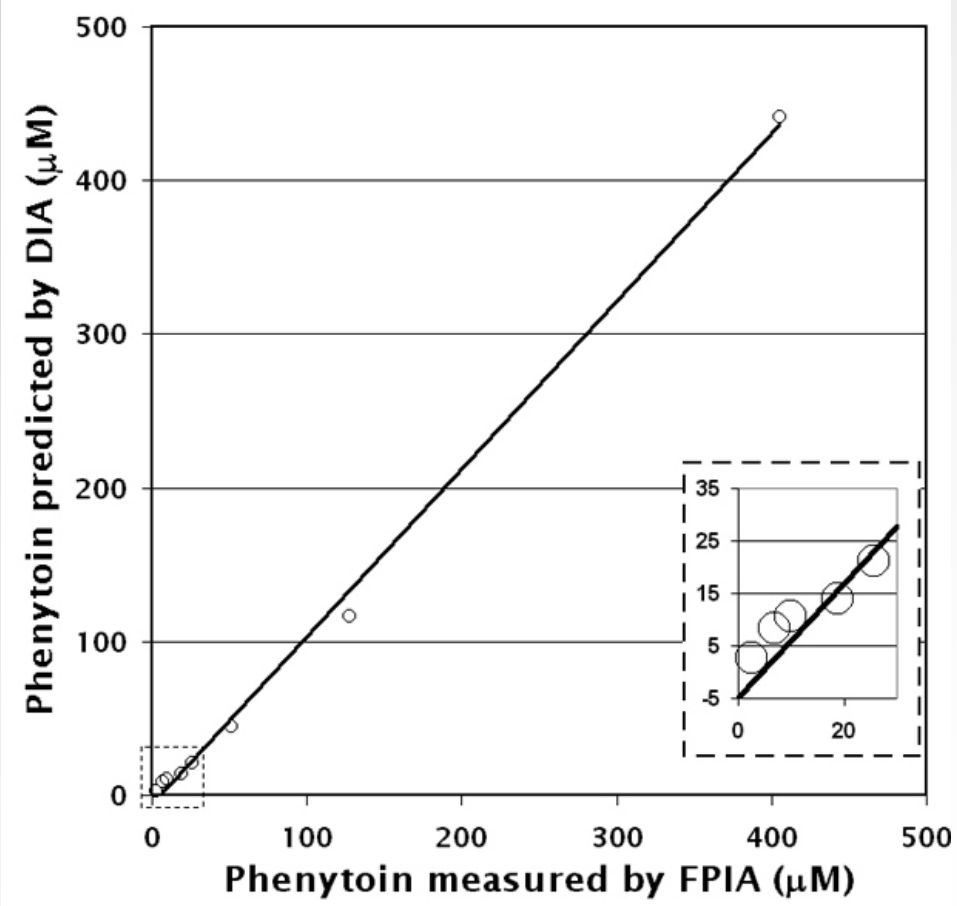
- **Modeling:** a 1D simulation of the phenytoin experiment
- Model has been used to design improved devices and optimize reagent concentrations





DIA compared to FPIA

- DIA tests of blood samples compared to FPIA measurements.
- DIA compared favorably with FPIA for wide range of sample concentrations.
- The open circles were NOT used to form the calibration line



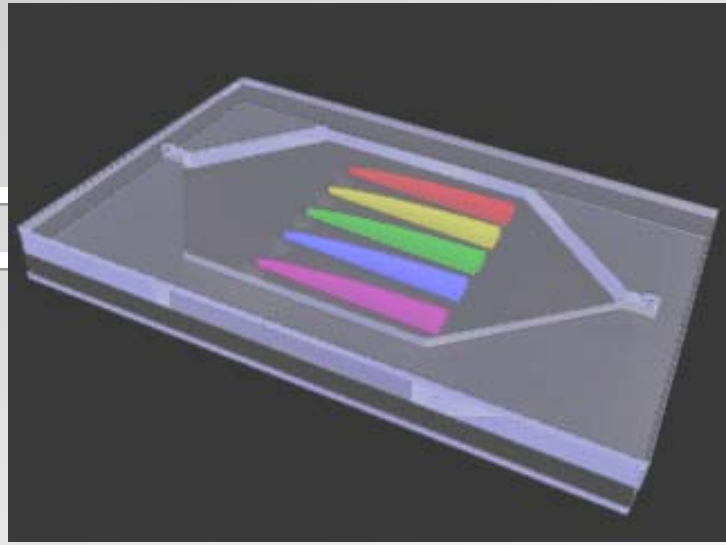
NCRR DIA Project Status

- Implemented successful immunoassay in a T-Sensor for detecting a small analytes.
- Implemented successful immunoassay in hydrogels in a 96-well format for detecting small analytes.
- Demonstrated measurement of phenytoin concentrations from 1 nM to 1 μM *in less than 30 seconds* in diluted whole blood.
- Currently demonstrating detection of larger molecules.

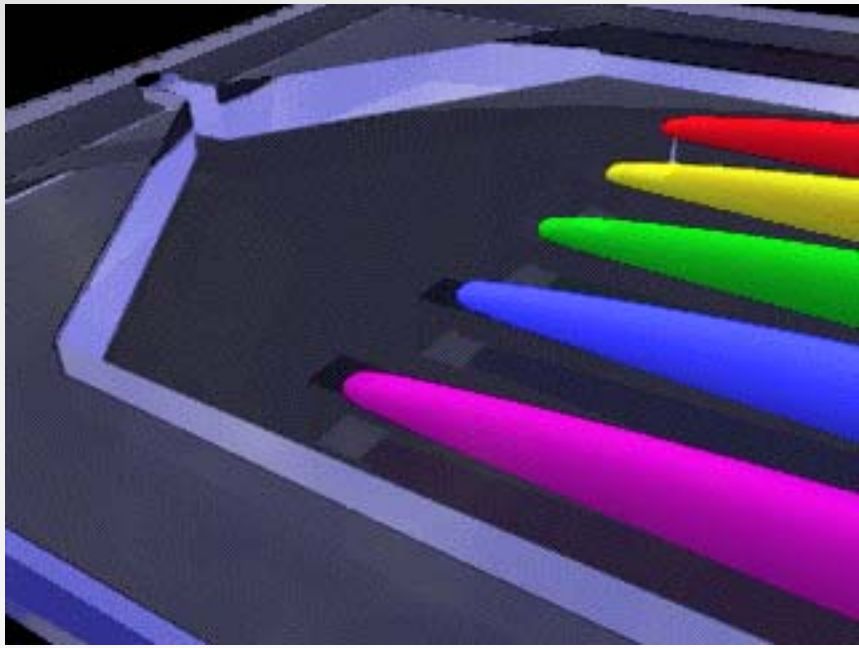
DIA Challenges

- How to multiplex the DIA and related assays without filling a room with syringe pumps?
- How to preserve reagents on the laminate until time of use?

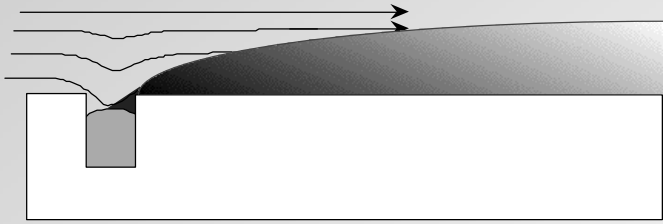
Multiplexing the T-Sensor in a Polymeric Laminate

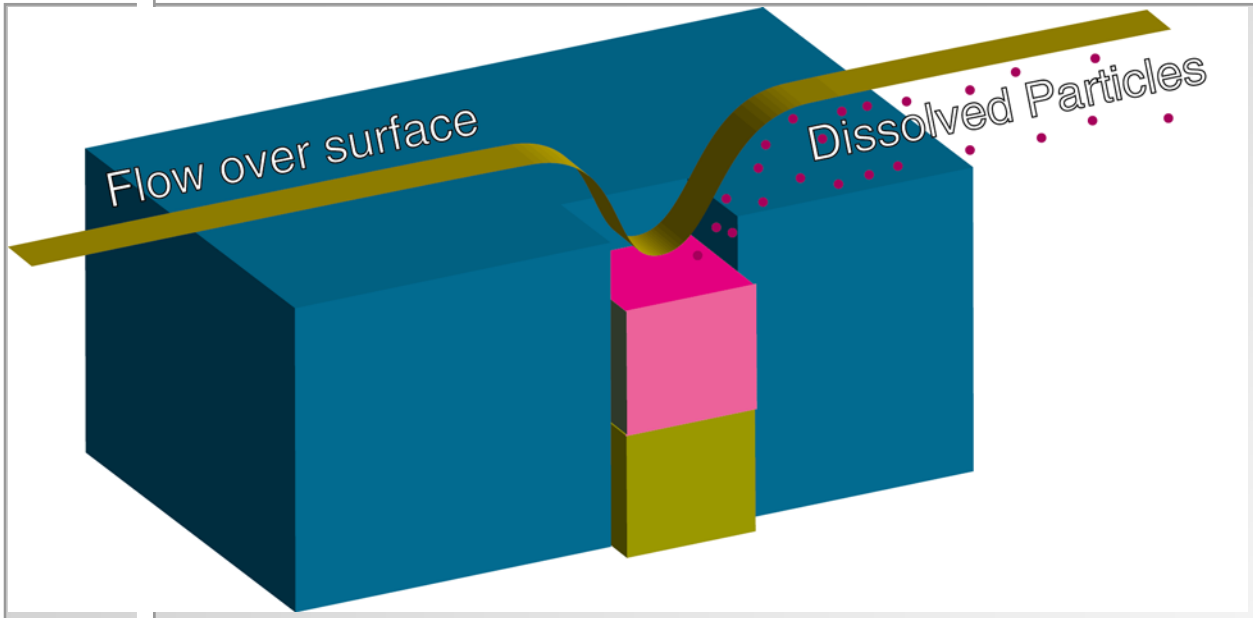


- The “Flat T-sensor” concept is the use of multiple interdiffusing streams.
- Molecular binding and other interactions can be detected at the points of overlap between the streams and the analyte.
- The device is “started” by wetting out.
- *This approach greatly simplifies the fluid controls required!*



Dry Reagent Delivery





How to create controlled streams of multiple reagents without multiple pumps? Blocks of one or more materials dissolve as the fluid flows over them.

- We have begun to explore the use of dissolution of dry plugs of material to produce interdiffusing streams.
- Flow over dry reagents will cause controlled dissolution of the reagents, creating “plumes” of reagents downstream
- These plumes can either be used for reaction in solution, or for creating patterned capture surfaces downstream

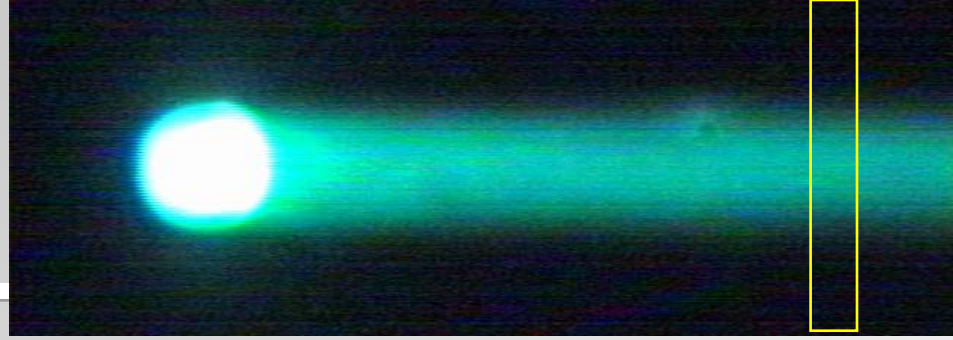
Dissolution of sample in cavity in PDMS

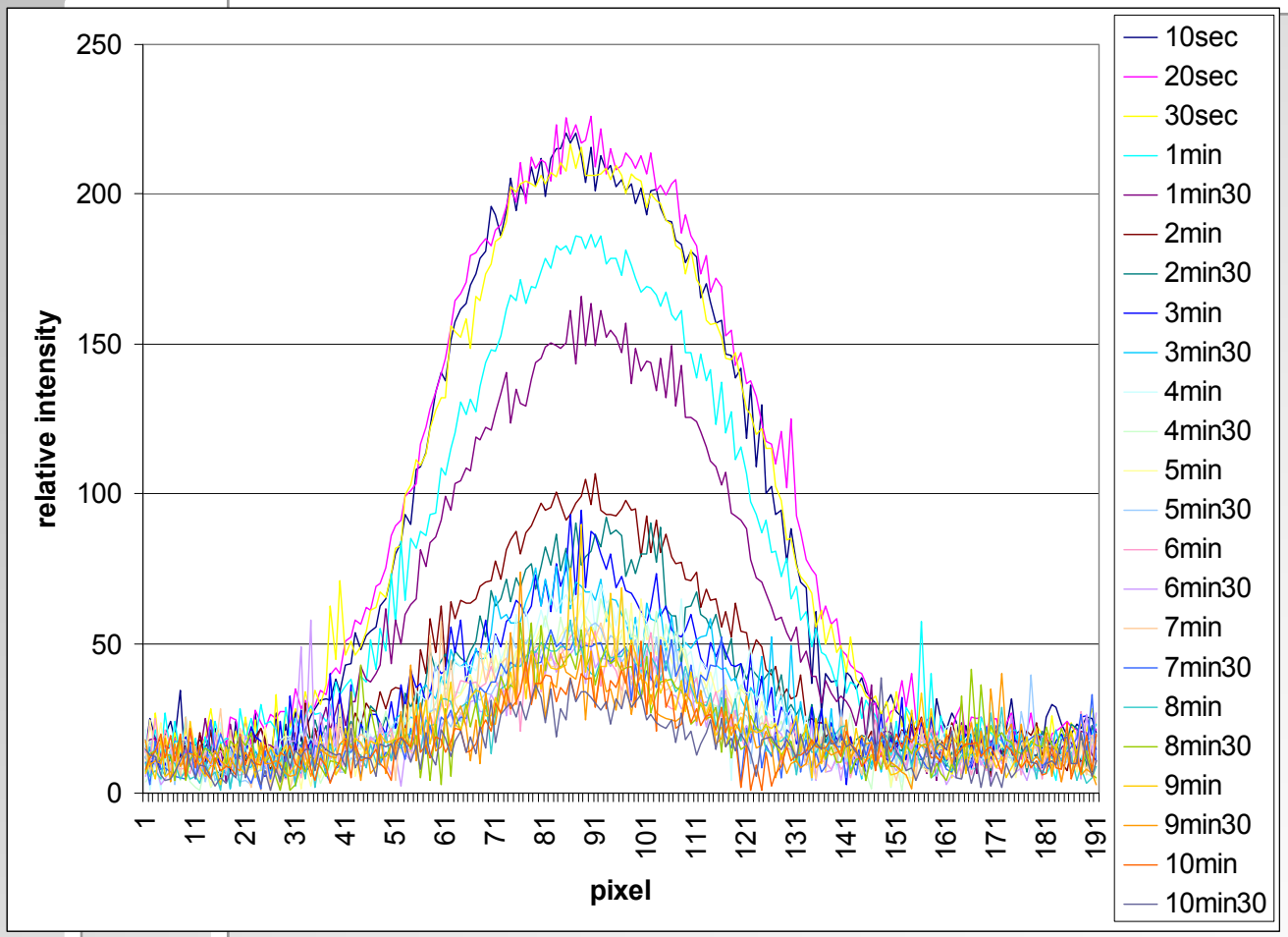
- 200 µm x 200µm cylindrical hole in PDMS
- Filled with dry trehalose-dextran matrix and fluorescein as a label
- Dissolution measurable for >10 minutes
- Dissolution controllable by fraction of dextran
- After wet-out, shape of plume appears constant even though overall intensity drops.

QuickTime™ and a DV - NTSC decompressor are needed to see this picture.

Flow from left to right. Movie begins just after arrival of the solvent front

Plume stability





- Note that plume intensity drops to 50% by ~2 min.
- Further control possible by shape and depth of hole, dextran concentration, diffusivity of solute, flow rate, etc.

Modeling Dissolution

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

- Modeling is ongoing in collaboration with Bruce Finlayson of ChemE
- Shown--a 2D CFD model (FEMlab) of dissolution of low viscosity reagent in cavity in channel.

Optimizing the Modeling

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

- The solution in the cavity is now 11 times more concentrated in solute, which increase the viscosity 11 times.
- The viscosity changes as the solute concentration drops.

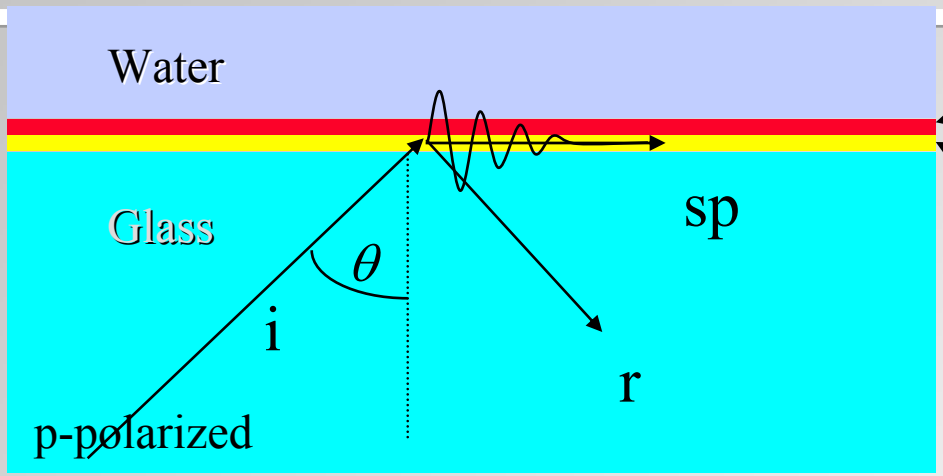
Limits to the DIA

- The DIA and all other T-sensor embodiments are "homogeneous assays" in which all the chemistry (hopefully) occurs in the solution phase.
- To date the minimum analyte concentration measured with the DIA is about 0.3 nM.
- Many analytes of interest in biological systems are present at much lower concentrations
- ***To measure these, some form of solid phase must be employed to capture the analyte to allow it to accumulate prior to measurement.***

Surface Plasmon Resonance (SPR)

- No labeling required of reagents
- Accumulation of things on walls for sensitivity
- Generic detection of anything--small molecules large molecules, beads or pathogens
- Basic principles well known
- Commercial products already available (e.g., Biacore and TI's SPREETA)
- Inherently inexpensive
- Easily coupled to microfluidics
- Amenable to multiplexing for many samples

Surface Plasmon Resonance Operating Principle (after Kretchmann)



Bioactive Layer or Hydrogel

Metal (typically gold)

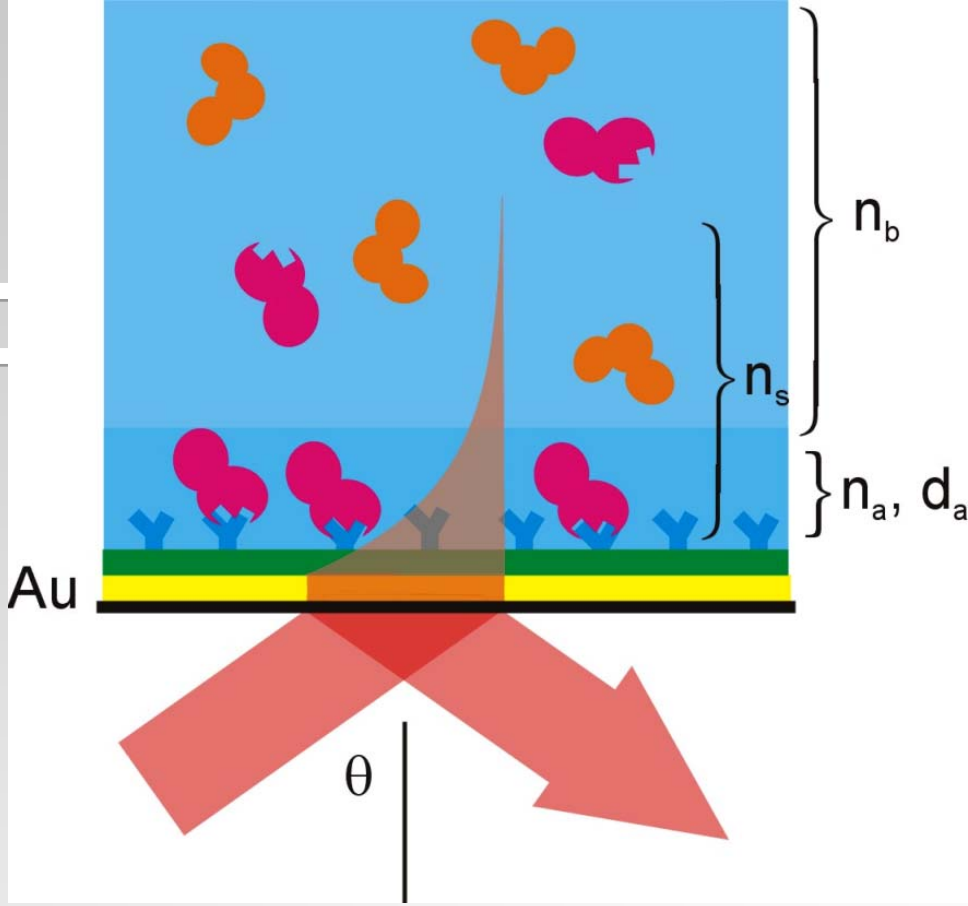
Surface plasmons are strongly damped (absorbed), so the light energy only propagates a few micrometers beyond the coupling point.

For a given metal film thickness, light at one frequency will, at a particular angle θ, resonate with plasmons in the metal film.

The resonance greatly reduces the reflectivity.

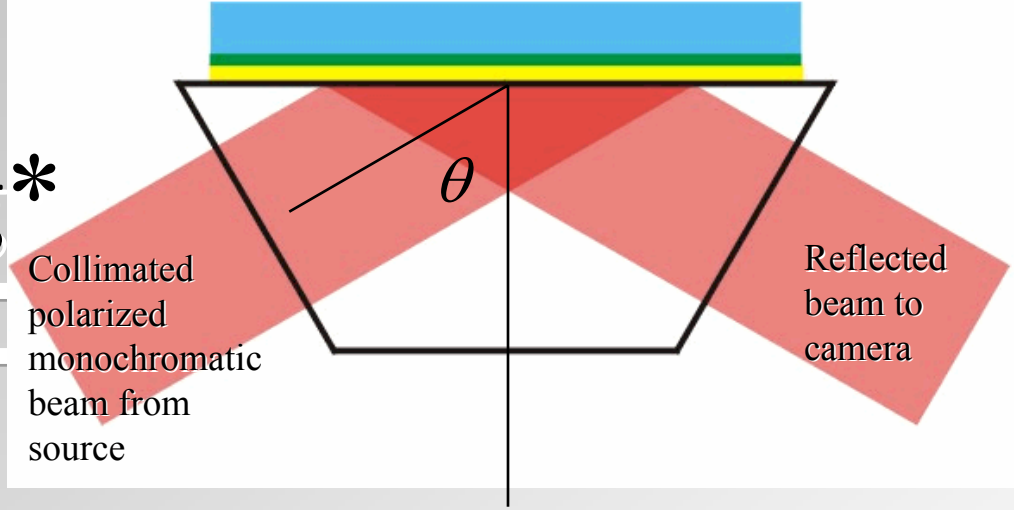
The refractive index *in the plasmon field* on the far side of the film strongly influences the conditions for resonance

Real SPR



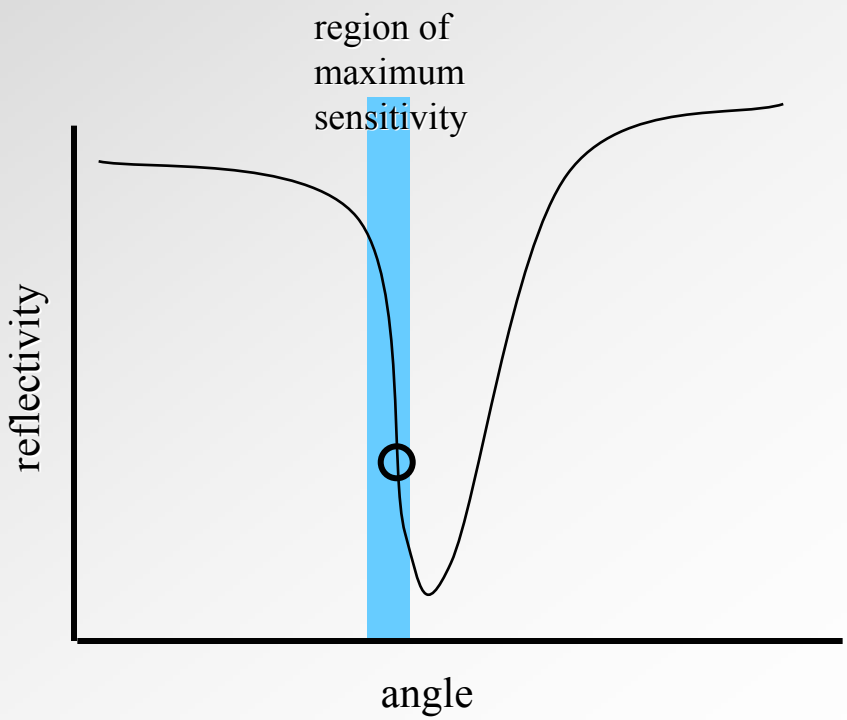
- Schematic representation (not to scale) of detection of surface binding events using SPR.
- Capture molecules are attached to the surface of the film that overlies the gold film.
- The capture layer may be a monolayer or a thick layer as in the dextran films sold by Biacore for use in their instrument.
- The field that senses the refractive index (n) penetrates a large fraction of 1 μm.
- It therefore senses both the refractive index of the surface film n_a, but *also the refractive index of the solution beyond that surface film* out to the point where the field strength drops to zero.

SPR Imaging*

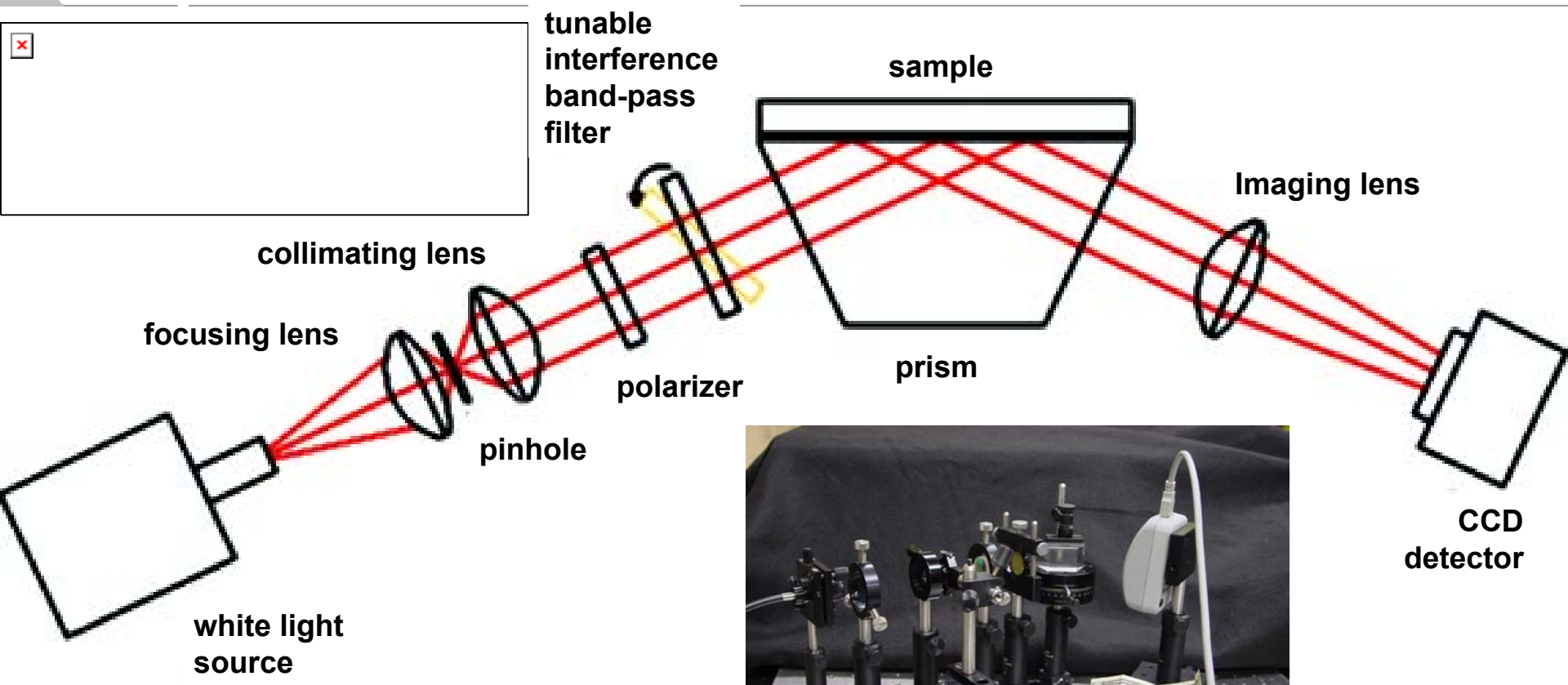


- On the assumption that analytes will increase the refractive index *n* of the sample, poise the angle θ at the point where any change in *n* produces the largest change in reflectivity
- Changes in *n* produce features whose intensity can be correlated with concentration of material at or near the surface

*the newest SPR technique

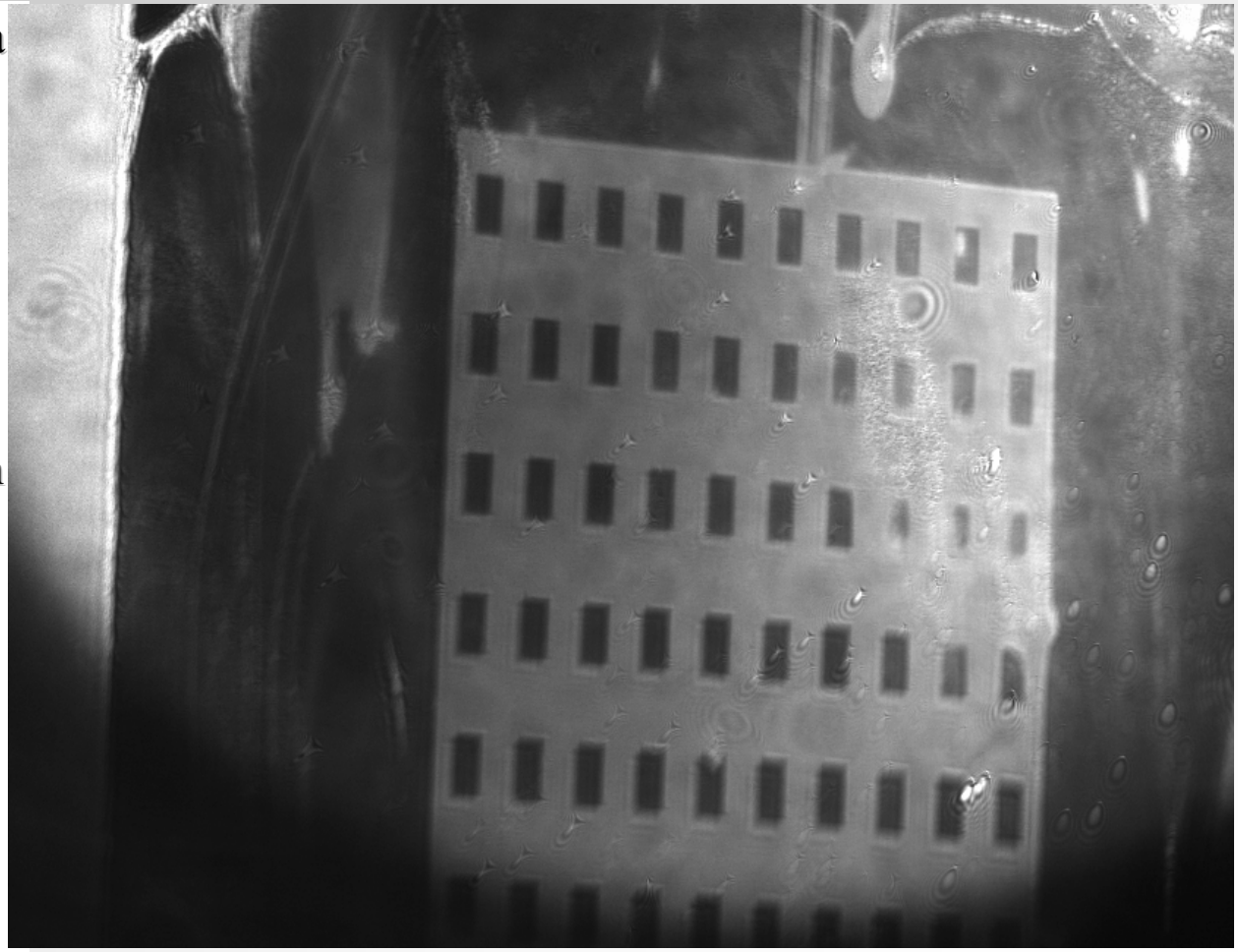


Design of Yager Lab SPR Microscope



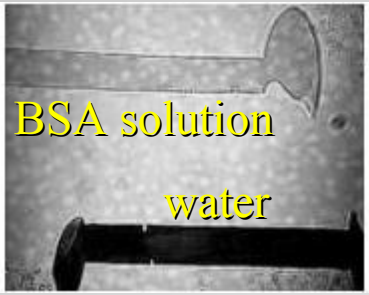
Recent SPR Microscope Images

- An SPR image taken with a B&W CCD camera.
- The pattern was made by stamping C_{16} alkane thiols onto the gold surface to produce one monolayer.
- The gold surface was then covered by a laminate-based fluid chamber, which was filled with buffer before imaging
- The stamp surface contained 500µm x 500µm pits that produced the dark rectangles in the image. The rectangular shape is due the foreshortening of the image.

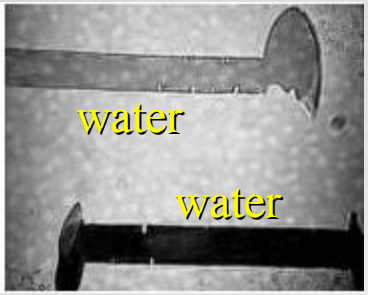


Protein Film Detection by SPR Microscopy

A



B



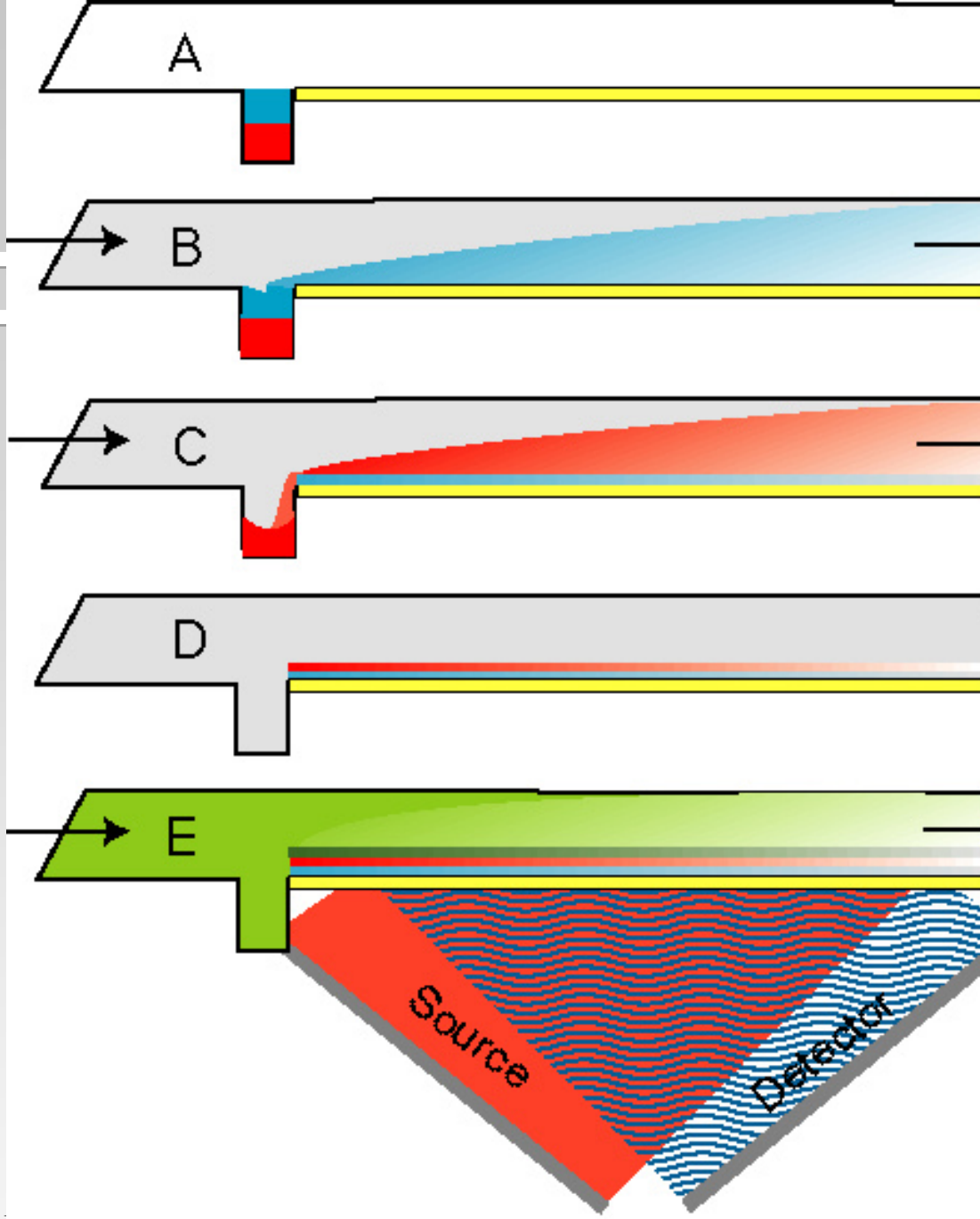
C

C-A

- Detection of adsorption of a monolayer of the protein bovine serum album (BSA) onto the Au surface
- In B a ~2 mg/ml solution of BSA in PBS flows through
- In C is the channel after extensive rinsing with water
- C-A shows the presence of the film in the upper channel, and no change (except the formation of a few bubbles) in the lower channel

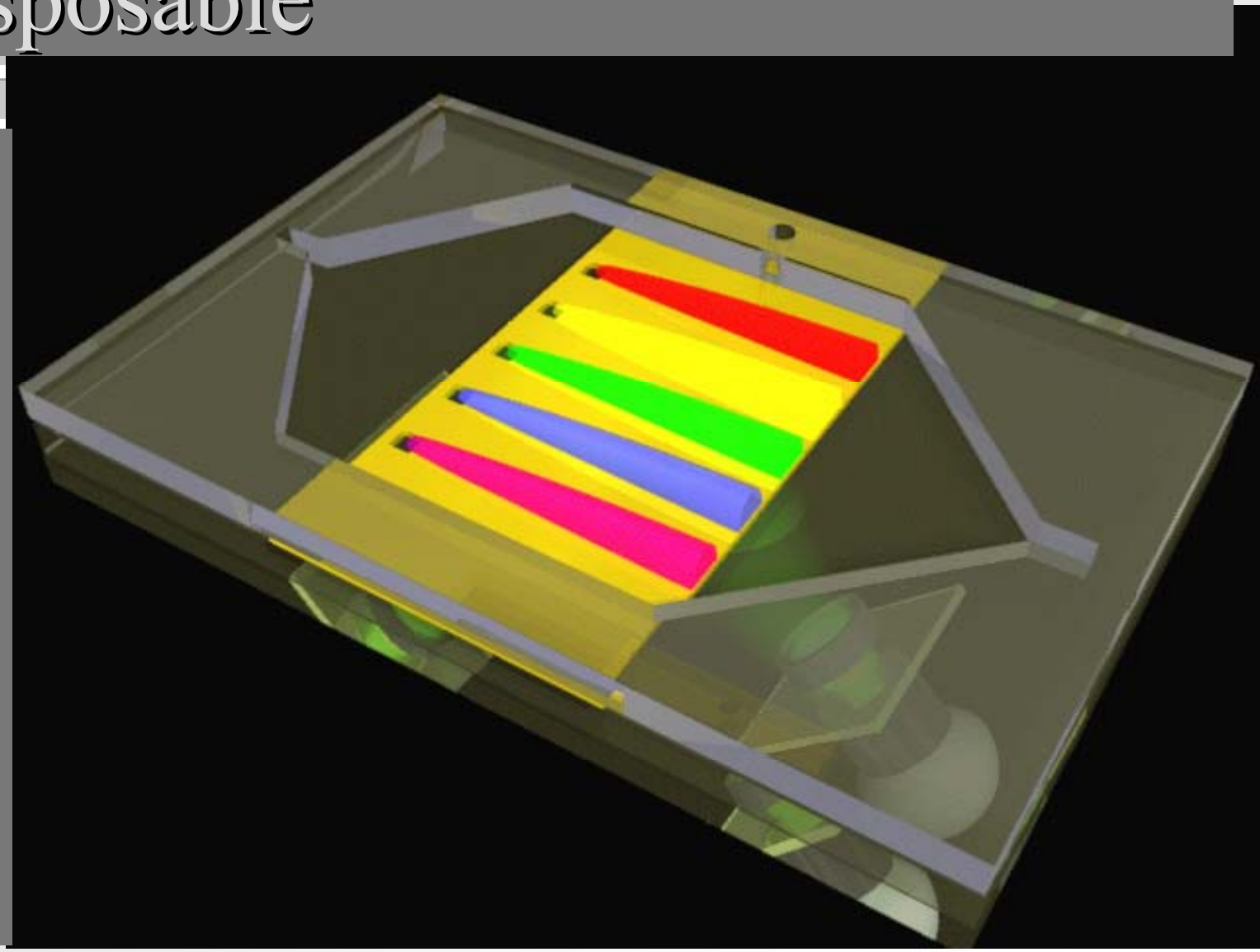
SPR Sensor Concept

- A) Antibodies (blue) are stored dry and stable in a well in the wall of a microchannel in a single-use device.
- B) When the device is wetted-out antibodies dissolve, flow downstream, diffuse to and coat the gold surface,
- C) The antibody coating varies in density depending on position,
- D) Analyte-laden sample flows over the now-activated gold surface.
- E) SPR optical probing of the position-dependent refractive index of the coated surface is made from below with "permanent" inexpensive optical components.



An inexpensive SPR-based disposable

- Reagents would be stored dry and rehydrated during wet-out
- Placing 20 channels in a 1 cm square is feasible.
- The gold surface on the laminate would be accessible from below for SPR and from above for fluorescence imaging



Yager Lab Ongoing Projects

- Development of diffusion immunoassay (DIA) to detect clinically-relevant analytes in whole blood
- Creation of systems for multiple parallel point-of-care immunoassays in an inexpensive disposable
- Surface plasmon resonance microscopy for POC diagnostics
- Controlled mixing of solutions that combine to form aggregates
- Rapid extraction of DNA from whole bacteria
- Development of systems for analysis of saliva

Acknowledgements

- Many collaborators, post-docs and both graduate and undergraduate students (see www site)
- The Washington Technology Center (1993-present)
- DARPA Defense Sciences Office (1994-1996)
- Senmed Medical Ventures / Micronics, Inc. (1994-present)*
- DARPA MEMS/MicroFlumes Program (1997-2000)
- MesoSystems, Inc. (2000-present)
- NIH NCRR (2001-present)
- Amgen Corporation (2002-present)
- NIH NIDCR (2002-present)
- Singapore's A*STAR (2002-present)
- * Yager has a financial interest in Micronics

DARPA

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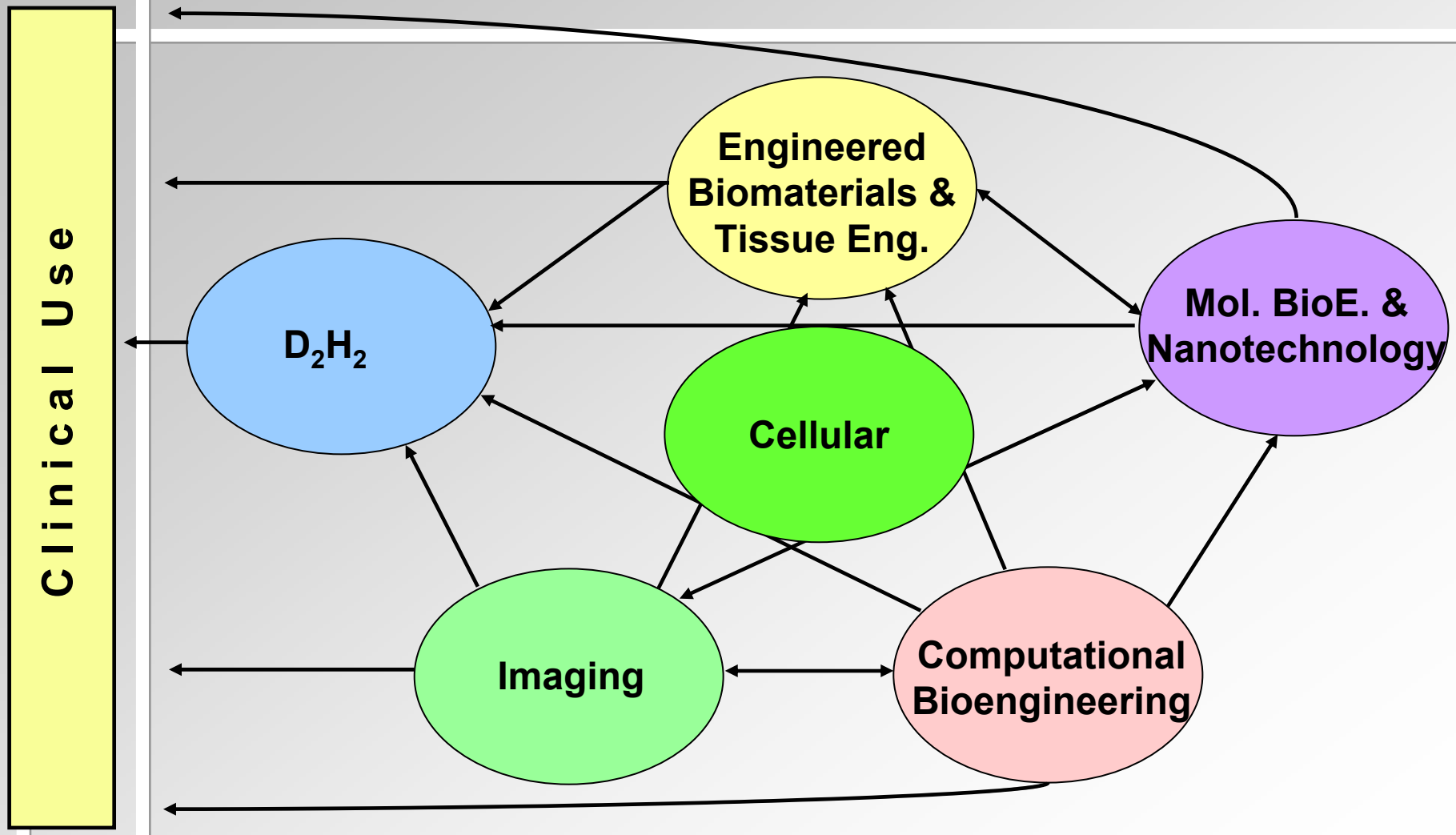
Fin

UW BioE Research Thrust Areas



Accounting for Molecular Interactions with Surfaces in Flow