PURDUE Discovery Park — Bindley Bioscience Center, Birck Nanotechnology Center

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# Peptide Array Biosensor for High Throughput and Multiplexed Detection of Food-borne Pathogens

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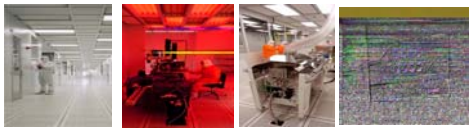
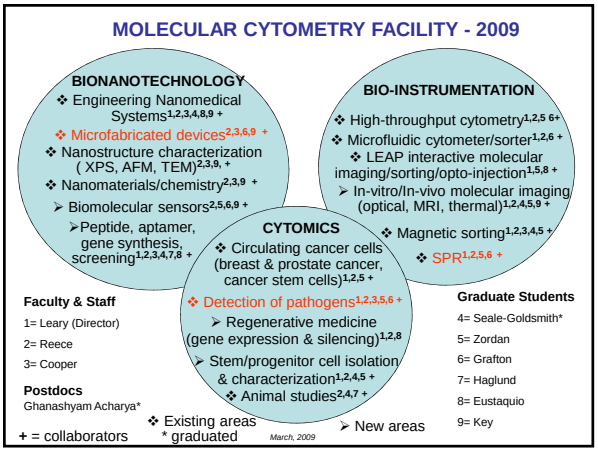


## Motivation

To develop a peptide array-based SPR imaging biosensor for the rapid and high-throughput detection of food-borne pathogens

## Ideal Biosensor

- High sensitivity without labeling and amplification
- Broad applicability to different types of analytes
- Compact, rapid, inexpensive, and easy to use




## Some Current Methods for Biomolecular Detection

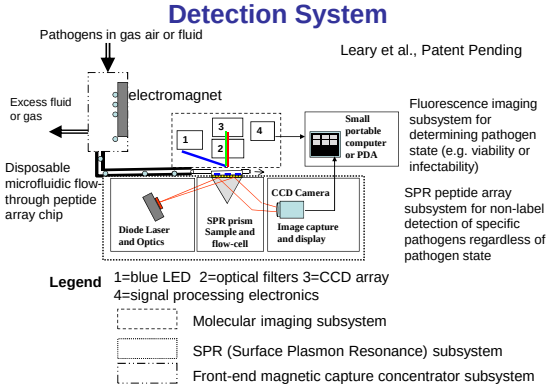
- Enzyme-linked immunosorption assays (ELISA)
- Fluorescence microscopy
- PCR approaches
- MEMS-based biosensor

## Required Attributes

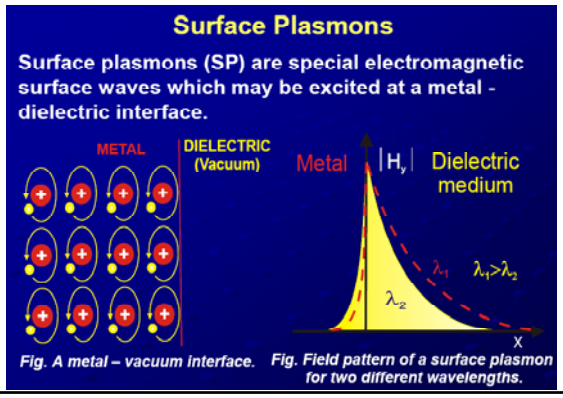
Despite the availability of several biosensor platforms, important challenges still remain:

- Reduction of sensor size
  For smaller sample volumes
- Shorter detection times
  For rapid detection
- Elimination of target labeling/signal amplification
  Time-consuming and can affect the target concentration in sample
- Simple and inexpensive fabrication protocols
  For commercially cheap device development

## Schematic of Portable Pathogen Detection System

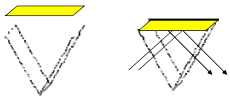



## What is Surface Plasmon Resonance (SPR)?

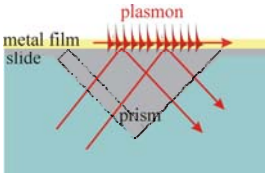



## Basic Principle of SPR

A plasmon can be defined as a ray of light bound onto a surface – propagating along the surface and presenting itself as an electromagnetic field.

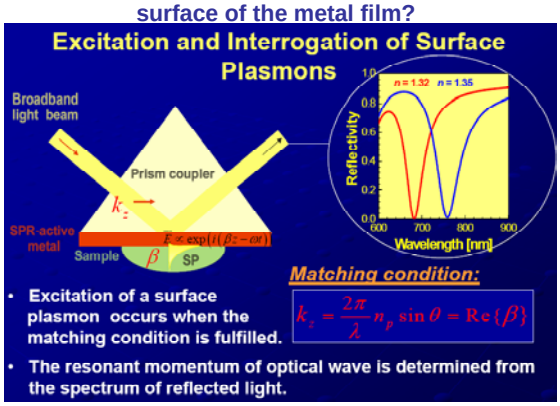


Kretschmann configuration uses a metal film thin enough to monitor the plasmon.

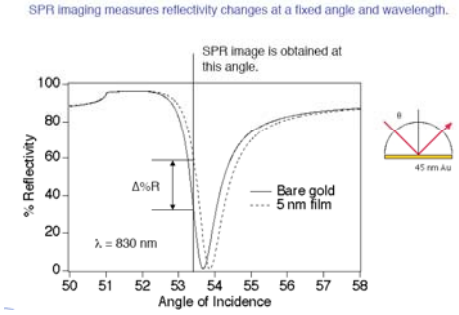



Thickness of the metal film is important!

## Why do we detect SPR at an angle to the surface of the metal film?

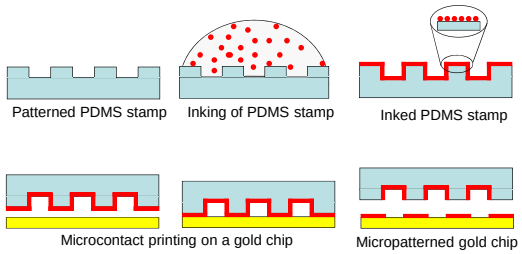



## Angle dependence of SPR signal

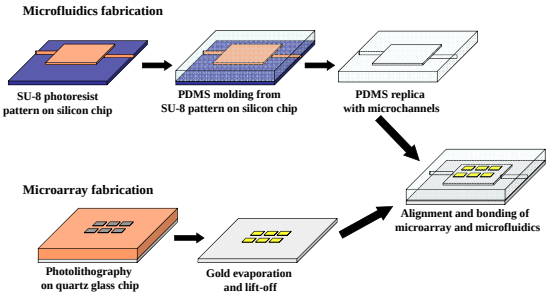



## Design and Manufacture of BioChips for Microbial Detection at Purdue University

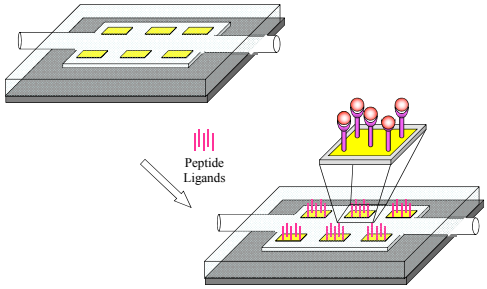
## Microcontact Printing




## Assembly of a Sensor Chip and Fluidic Cell

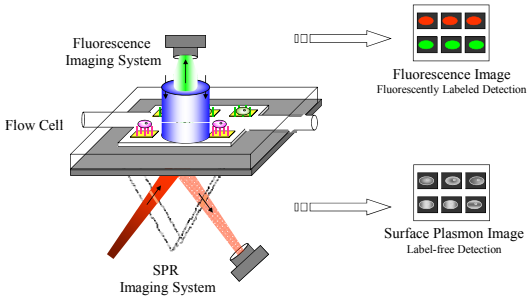



## Functionalization of the Sensor chip with Specific Peptide Ligands

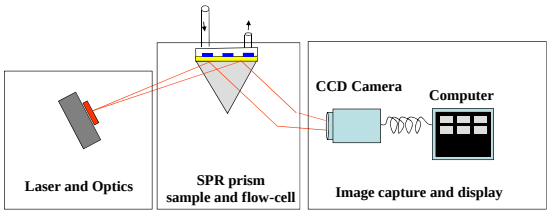



## A Hybrid Molecular Imaging/SPR Microdevice

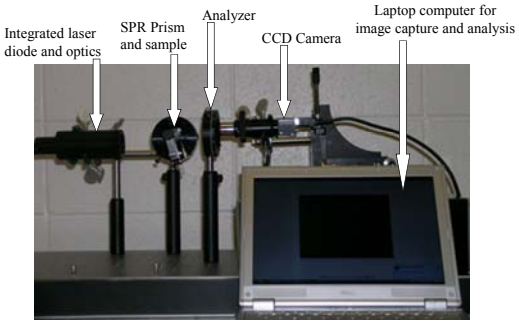
Fluorescence and SPR Imaging Integrated Detection System



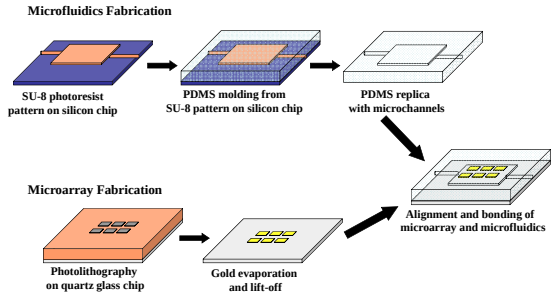

## Schematic of the SPR Imaging System



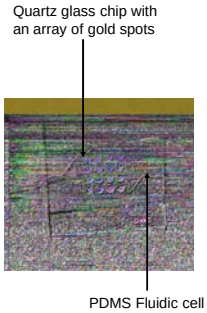

## Assembly of the original prototype SPR imaging device



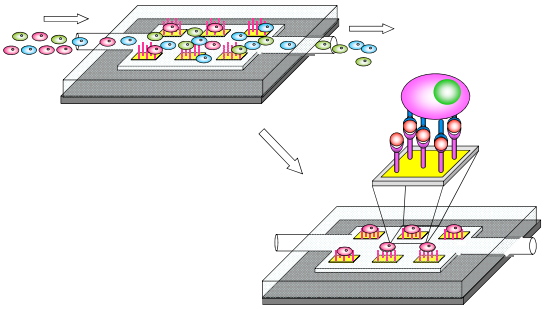
## Schematic of the Sensor Chip Fluidic Cell Assembly



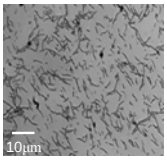

## Results: Sensor Chip Fluidic Cell Assembly



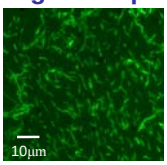

## Specific Peptide Capture of Pathogenic E. coli



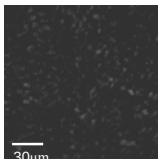
## Result: Capture of E. coli on antibody functionalized gold chip



Bright field Image



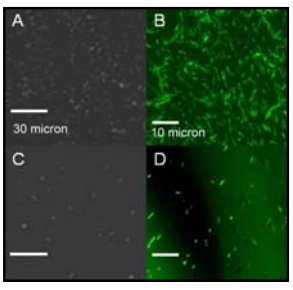
Fluorescence Image



SPR Image

## SPR/fluorescence images of bacteria

- Using the previous SPR imaging system we have imaged fields of *E. coli* O157:H7 of differenct densities using both SPR imaging (left) and fluorescence imaging (right).

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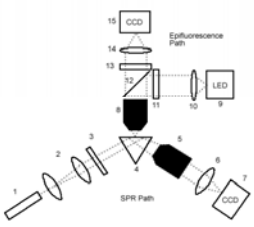
## Optical layout of hybrid device

- SPR path: The Kretschmann configuration* is used to perform SPR imaging. A 635 nm laser diode is used for illumination.
- Epifluorescence path: A blue (470 nm) LED is used for fluorescence excitation.




*Rothenhausler B, Knoll W. Surface–plasmon resonance microscopy. Nature 1988;332(6165):615-617.

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## New hybrid imaging device

- Built on rigid optical frame
- Uses laser diode and LED for SPR and epifluorescence excitation.
- Two USB CCD detectors.

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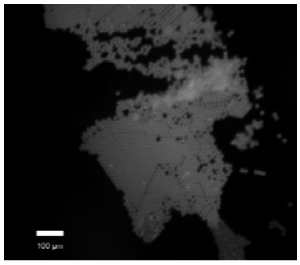
## Fluorescence images of beads from new prototype

- 10 micron green fluorescent beads were imaged on hybrid prototype.
- Can resolve individual beads



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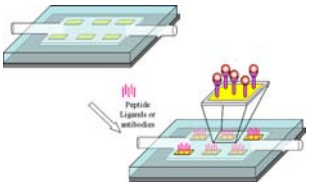
## Multiplexed array of microbial capture ligands

- The biosensor is built on a two-dimensional array of gold spots.
- A capture ligand specific to a pathogen is immobilized on each spot.
- Each spot in the array can have a different ligand.
- A SPR image can be taken of the entire array, and the spatial position of binding events will determine the identity of the captured pathogen.

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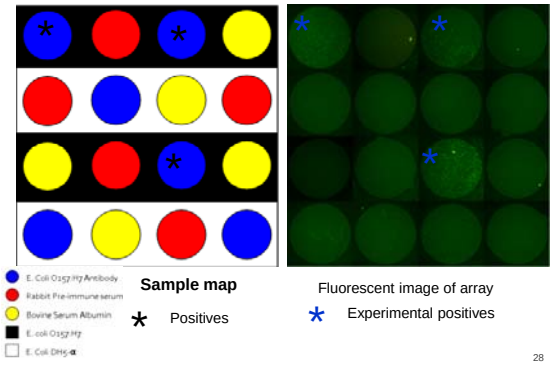
## Selective capture of pathogenic E. Coli O157:H7

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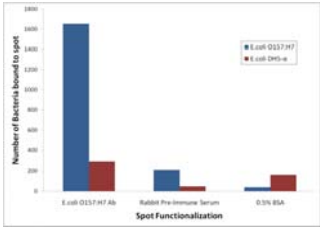
## Quantitative assessment of selective capture of E. Coli O157:H7

- Very high specific capture of pathogenic *E. coli* O157:H7.
- Low level of non-specific binding.
- ImageJ (NIH) software was used to count the number of bacteria bound to each spot.
- Captured pathogens found to be 97% viable using BacLight® kit.

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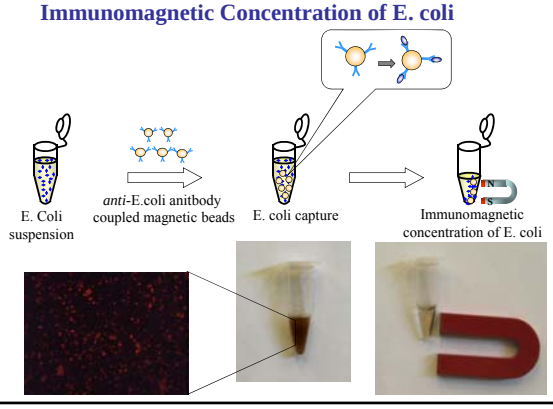
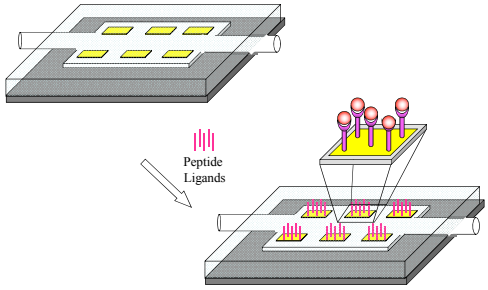
## How do we analyze macro fluids in a microfluidic device?

**Immunomagnetic Concentration of E. coli**



## Multiplexing the Assay

Functionalization of the Sensor Chip with Different Peptides for Simultaneous Multiplex Detection of Multiple Microbial Pathogens




## Recent Progress in Presentations and Publications

**Publications:**

Zordan, M.D., Grafton, M.M.G, Acharya, G., Reece, L.M., Cooper, C.L., Aronson, A.I., Park,K., Leary, J.F. Detection of Pathogenic E. coli O157:H7 by a Hybrid Microfluidic SPR and Molecular Imaging Cytometry Device. Cytometry Part A 75A: 155-162, 2009.

Zordan, M.D., Grafton, M.M., Acharya, G., Reece, L.M., Aronson, A.I., Park,K., Leary, J.F. A microfluidic-based hybrid SPR/molecular imaging biosensor for the multiplexed detection of food-borne pathogens. Proceedings of the SPIE, Volume 7167, pp. 716706-716706-10 (2009).

**Patents:**

**HYBRID MICROFLUIDIC SPR AND MOLECULAR IMAGING DEVICE**

James F. Leary, Kinam Park, Ghanashyam Acharya, Arthur Aronson, Michael D. Zordan Purdue University, PCT/US08/81571 (October 29, 2008)

## How effective is this concentration strategy?

- We have achieved 1000-fold reduction in volume from 10 ml initial sensing volume to 10 ul microfluidic analysis volume
- We retain >90 percent of the magnetic sensors within this 10 ul volume from this 10 ml initial volume
- We are working on designs for a 10-fold larger initial volume and think this is achievable.

## Next steps: Development of a very small, portable device

- The device for microbial pathogen detection in food needs to be taken to where food is produced and inspected
- It must be a very “smart device” meaning it does not need to be operated by very smart people!
- It needs to be relatively inexpensive and to have disposable microchips which are read in a small reader instrument module.

## Our MCF Team and Current Collaborators

**Nanochemistry**
Don Bergstrom (Purdue)
David Thompson (Purdue)

**Combinatorial chemistry/ Drug Discovery**
David Gorenstein (UTMB)
Xianbin Yang (UTMB)
Andy Ellington (UT-Austin)

**Nanoparticle technology**
Kinam Park (Purdue)
Joseph Irudayaraj (Purdue)
Jo Davisson (Purdue)

**Mathematics/Bioinformatics**
Bruce Luxon (UTMB)
Judah Rosenblatt (UTMB)
Seza Orcun (Purdue)
Jacob Smith (Texas A&M)

**Image/confocal cytometry**
Paul Robinson (Purdue)

**Biosensors**
Marshall Porterfield (Purdue)
Jenna Rickus (Purdue)

**Molecular Cytometry Facility**
Director: James Leary

Lisa Reece (SVM) – Lab Dir., flow cytometry/ BioMEMS; tissue culture

Christy Cooper (SVM) - bioanalytical chemistry, nanochemistry, XPS, AFM

Meggie Grafton (BME) - BioMEMS

Emily Haglund (BME) – multilayered Qdots for ex-vivo nanomedicine

Mary-Margaret Seale-Goldsmith (BME) – multi-layered magnetic nanomedical systems

Michael Zordan (BME) – prostate cancer, rare cell flow/image cytometry

Trisha Eustaquio (BME) – gene silencing/therapy; interactive imaging

Jaehong Key (BME)- 3D/MRI imaging

Ghanashyam Acharya–SPR imaging

**Nanomedicine studies**
Debbie Knapp (Purdue)
Deepika Dhawan (Purdue)
Sophie Lelievre (Purdue)
David Waters (Purdue)
Gerald Lutty (Johns Hopkins U)
Tarl Prow (U. Brisbane, Australia)

**X-ray Photon Spectroscopy**
Dmitry Zemlyanov (Purdue)

**High-Energy TEM**
Eric Stach (Purdue)
Dmitri Zakharov (Purdue)

**Atomic Force Microscopy**
Helen McNally (Purdue)

**Magnetic Cell Sorting**
Paul Todd (Techshot, Inc)
Dave Kennedy (IKOtech, Inc.)

**LEAP Interactive Imaging**
Fred Koller (Cyntellect, Inc.)

**BioMEMS/Microfluidics**
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Rashid Bashir (Univ. Illinois)
Huw Summers (Cardiff Univ, UK)

**Microelectronics**
Pedro Irazoqui (Purdue)
Byunghoo Jung (Purdue)

**Microbiology**
Arthur Aronson (Purdue)