RISK*e*Learning

Nanotechnology – Applications and Implications for Superfund

May 31, 2007
Session 5:
"Nanotechnology – Environmental Sensors"
Paul Gilman, ORCAS
Desmond Stubbs, ORCAS
Ian Kennedy, UC - Davis

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Vanderbilt University

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Oak Ridge Center for Advanced Studies

Nanotechnology Applications in Environmental Health: Big Plans for Little Particles

May 31, 2007
Desmond Stubbs
Paul Gilman

Where did ORCAS come from?

UT + Battelle + ORAU OAK RIDGE ASSOCIATED UNIVERSITIES = UT-BATTELLE

ORAU OAK RIDGE ASSOCIATED UNIVERSITIES = Virginia Tech

Florida State University

Georgia Tech

Vanderbilt

Duke University

NC State University

University of Virginia

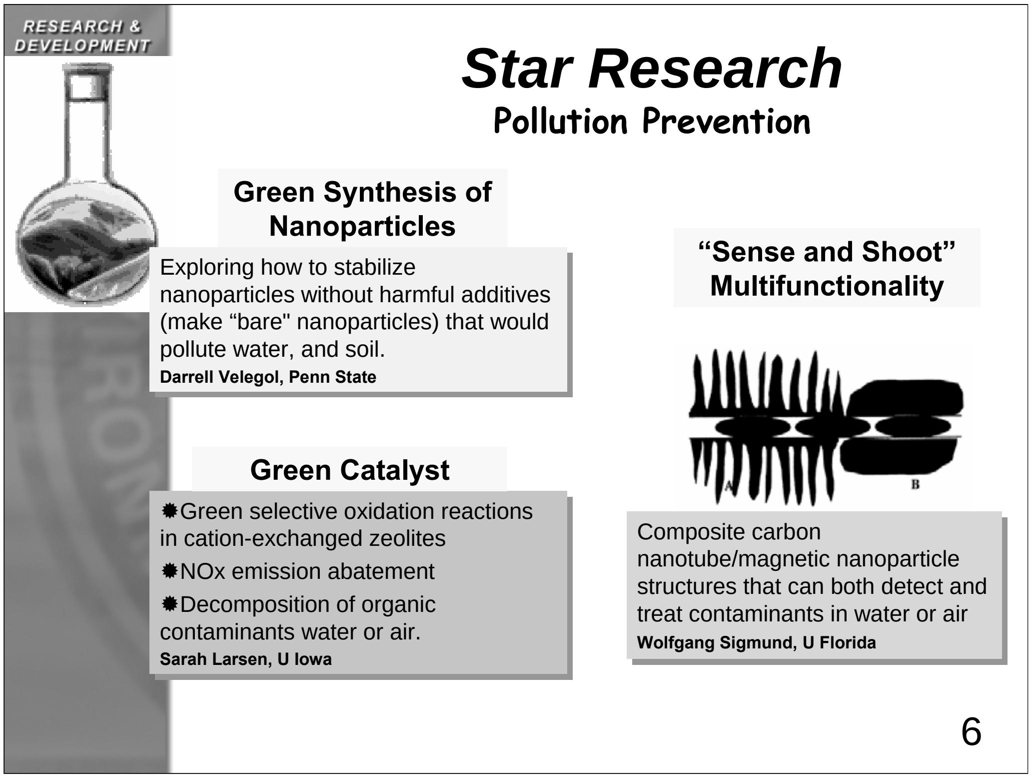
The Mission…

- The ORCAS is a think and do consortium of research universities, government, industry, and non-governmental organizations.
- It is focused on critical issues with strong science and technology content.
- Problems are framed broadly, taking into account their scientific, technical, economic, social, and policy dimensions to develop research and integrated strategies for addressing those challenges.
- We attempt to ensure that our ideas and research are translated into action.

Bricks and Mortar – but …

ORCAS operates in a virtual mode-using staff with project management expertise and experts drawn from wherever appropriate…

- Lecture room with 4 cameras for video conferencing
- Distance Learning Center
- Multiple Conference Rooms with state-of-the-art technology

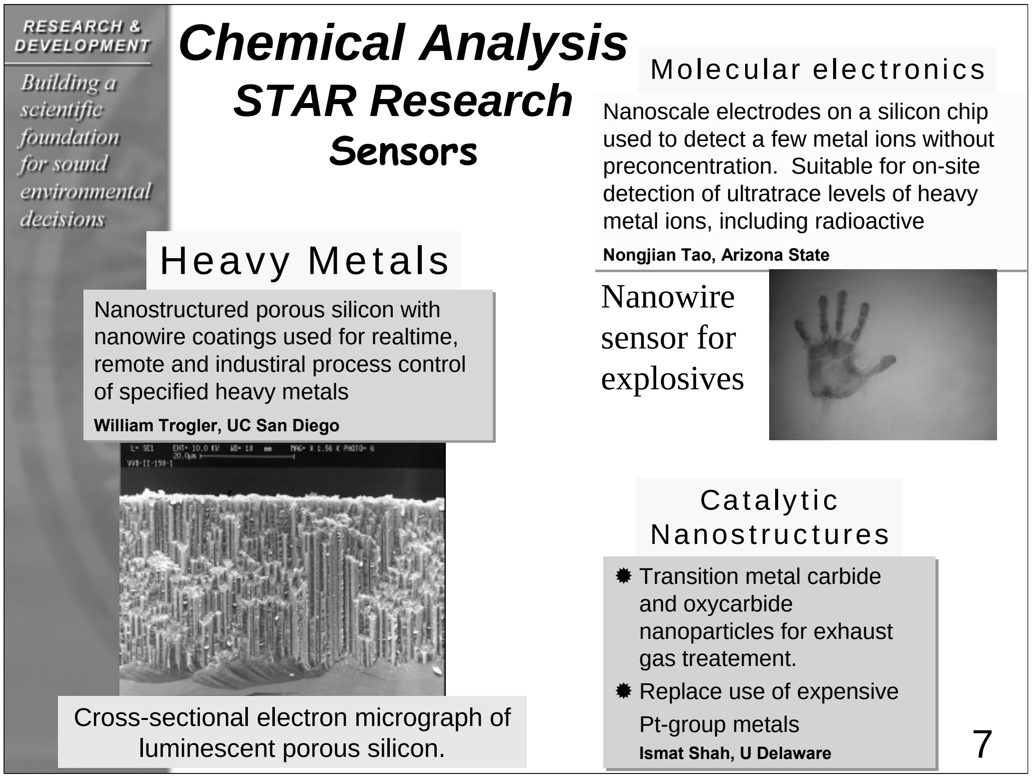


If polluting chemicals are not made in the first place, there will be no danger from them. Green synthesis of chemicals, more selective and efficient catalysts, and a combined analytical and decontamination technology aid the goals of homeland security.

Green Synthesis of Nanoparticles

Nanotechnology promises tremendous advances in electronic circuits, superstrong ceramics, optical imaging, and gene vector materials. However, the transition from the laboratory to the storeshelf has a critical barrier: nanoparticles spontaneously aggregate, negating their beneficial properties. Various methods have been used to stabilize particles, but all have involved dispersant molecules such as surfactants or polyelectrolytes. Not only do these dispersants alter the chemistry and physics of the nanoparticle systems, they also produce a tremendous waste stream during burnoff because they occupy a significant (>50 percent) mass fraction of a nanoparticle system. This research is to identify whether solvation or depletion forces can be manipulated to produce dispersed suspensions of "bare" nanoparticles (i.e., without adsorbed additives). Findings indicate that solvation forces could play an important role in determining the stability of colloidal nanoparticle suspensions. By engineering the solvent-nanoparticle interaction and by carefully choosing the nanoparticle shape, it may be possible to achieve stable suspensions or assemblies of bare nanoparticles. This would considerably reduce the waste associated with the common practice of adsorbing molecules on nanoparticle surfaces to prevent them from aggregating or to achieve their selective assembly (necessary to stabilize nanoparticle systems).

Green Catalyst



Heavy Metals

There is tremendous interest in the development of inexpensive portable electronic devices for the specific detection of toxic chemicals, such as nerve agents. We have been exploring the use of photoluminescent and electroluminescent silole polymers (Figure 2) as sensors for detecting electron deficient organics. Analogues and other luminescent copolymers have been prepared and explored for their behavior as luminescent sensors for explosives. There has been wide interest in our research, and especially recently in the context of homeland security. The main objective of this research project is to develop new selective solid state sensors for chromium(VI) and arsenic(V) based on redox quenching of the luminescence from nanostructured porous silicon and polysiloles.

Molecular electronics

The threat of heavy metal pollution is a serious environmental concern because of the toxicity of such metals for a broad range of living organisms including humans the fact that these pollutants are non-biodegradable. Due to the difficulty in the remediation of sites contaminated with heavy metals, there is an urgent demand for an in situ sensor that is sensitive enough to monitor heavy metal ions before the concentration reaches a dangerous level. This project exploits the phenomena of conductance quantization and quantum tunneling to fabricate nanoelectrodes for in situ detection of metal ion pollution. Our objective is to develop a high-performance and low-cost sensor for the initial on-site screening test of surface and groundwater that will provide early warning and prevention of heavy metal ion pollution. Progress: We have developed an electrochemical method to routinely fabricate nanoelectrodes separated by an atomic-scale gap. We have found that electron flow across such a small gap can be approximately described by simple quantum tunneling theory, but also observed a local state effect due to water molecules. Using the nanoelectrodes, we have detected trace amounts of metal ions at the ppt level by forming quantum point contacts. We also have shown that the deposition and stripping potentials can be used to identify the ions, similar to anodic stripping

April 2006 Workshop…

Nanotechnology Applications in Environmental Health: Big Plans for Little Particles

- Introduction of two research communities
  - Nanomaterials/nanosensors
  - Environmental health/ecological health
- Exploration of the "art of the doable" on the nano-side
- Discussion of the possible environmental health effects, exposure assessment and ecological health applications
- Better informed communities with likelihood of beneficial interactions in the future

The Case for Nanotechnology – *Commentary* *by Michael Strano (Asst. Professor, University of Illinois- Urbana)*

- It has been pointed out that generally the detection limit of a sensor scale approximates the cube of its characteristic length. So smaller sensor elements mean lower detection limits generally.

- The case varies both with the type of material used in its design and the physical and chemical properties of that material.

- Fluorescence-based techniques are some of the most powerful molecular detection methods available. Single molecule fluorescence analysis is a now routine. For optical fluorescence-based sensors, there are classes of nanoparticles that exhibit extremely enhanced photostability in fluorescent emission. This means that for the first time, new types of sensors can be devised with extremely long operational lifetimes. This is not possible with conventional fluorophores (e.g., single-walled carbon nanotubes are infinitely photostable at moderate light fluxes).

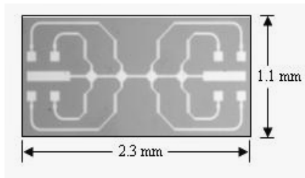
- Some nanosystems emit light at longer wavelengths where few conventional materials operate whereas few conventional materials do so. The human body is particularly transparent to near-infrared light in a narrow region of the electromagnetic spectrum. These systems will form the basis of novel detection technologies that can operate in strongly scattering media where fluorescent spectroscopy is limited.

- Nanoparticles can also possess features that are commensurate with biomolecules and other important macromolecular analytes. Electrodes that are narrow enough to fit or conform to biological structures should be capable of transducing subtle changes in these structures, as several pioneering efforts are already demonstrating.

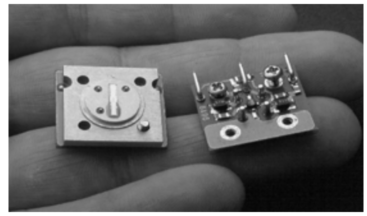
The Case for Nanotechnology – Sensor shelf-life, Real-time detection, Useful life

- Shelf Life – varies as a function of the sensing layer. For example, bioreceptors (antibodies, enzymes, lipid layers) are limiting factors because of their inherent short life span under non physiological conditions. On the other hand, aptamer- and polymer-based sensing layers have been used in an effort to extend the lifetime of the device.
- Real-time Detection - is a common feature of nanosensing technology. The nanosensors described in the meeting all operated on a time scale ranging from seconds to minutes.
- Useful life - The binding mechanisms for the sensor platform can be described as reversible—requiring little or no surface treatment to return the sensor to its steady state—or irreversible where analyte binds with high affinity such that surface treatment is required to remove the bound substrate.

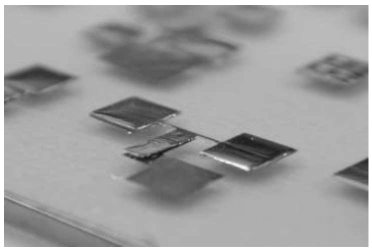
Emerging Technologies in Exposure Assessment



Passive RFID Tag



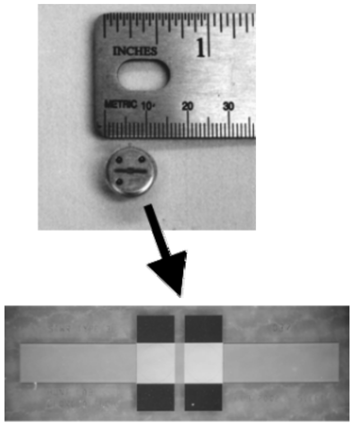
Electronic nose: “Dog-on-a-chip”



Microelectromagnetic Sensor

Interferometric Optical Sensor

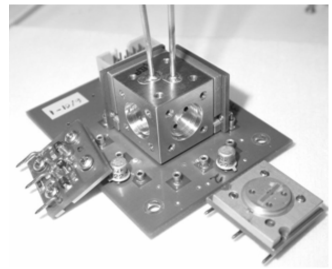
Vapor phase sensor system



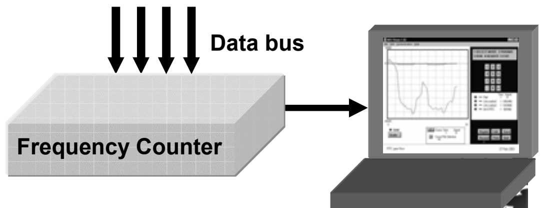
250MHz SAW resonator

Flow cell and oscillator circuit

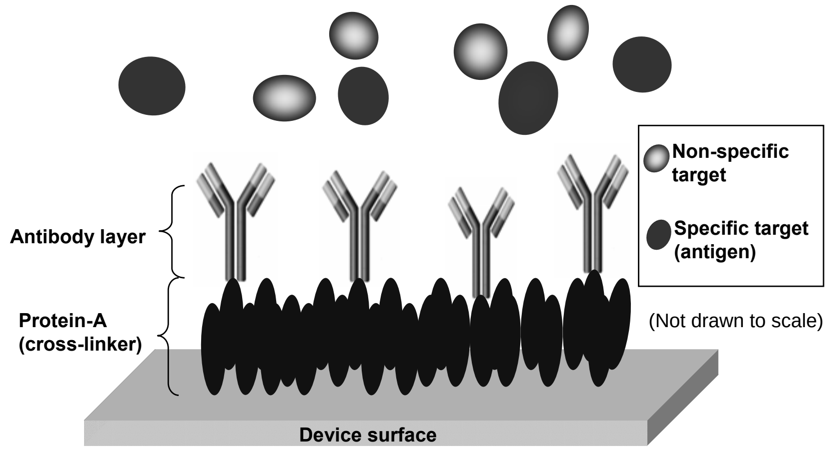
Gas in Gas out



PC

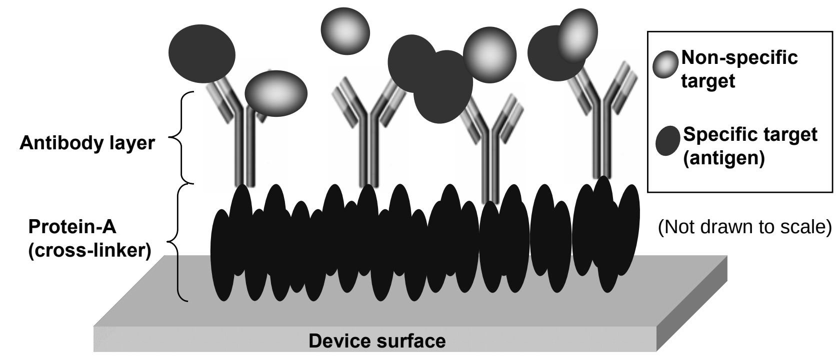


Antibody Immobilization on Au Electrodes

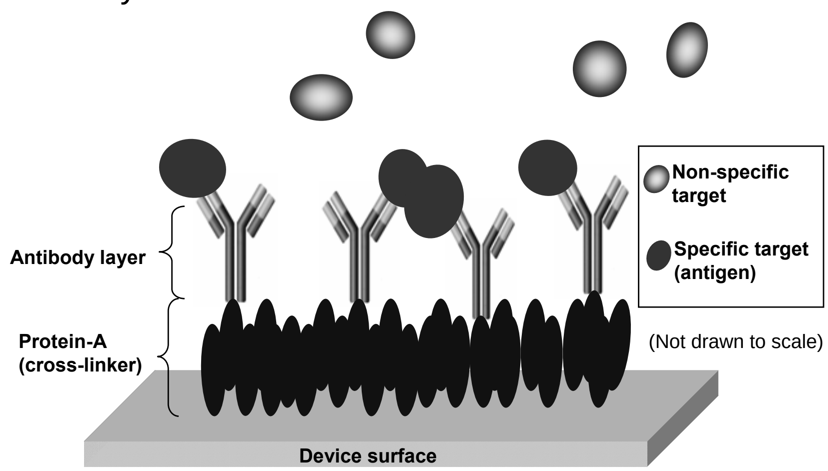


Courtesy Sang Hun Lee

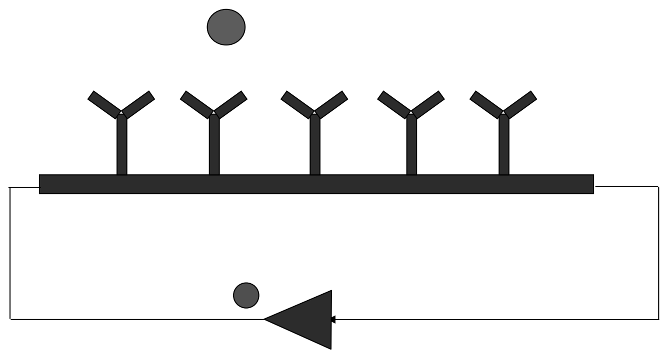
Antibody Immobilization on Au Electrodes



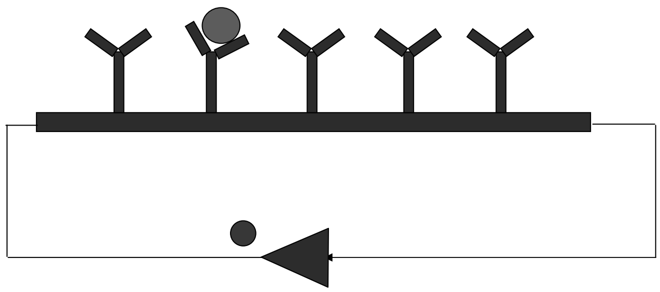
Antibody Immobilization on Au Electrodes



Principle of Operation



Principle of Operation



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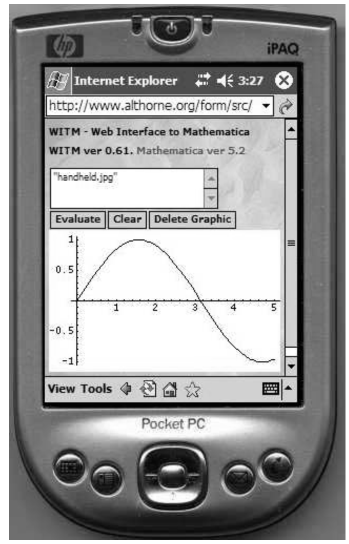
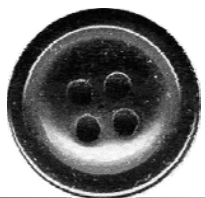
Technology Description	Technology Platform	Value to Exposure Scientist	Advantages	Limitations
Location and activity sensors	GIS, GPS, and Accelerom-eters	Information on potential sources	Location and activity information for participants	Steel and concrete structures block GPS
Electronic Diary	PDA devices	Link between personal exposure and activity	Real-time personal diaries and questionaires	Subject must be technoliterate
Wearable Sensors	Microelectronic arrays, cantilever arrays, acoustic sensors, radio frequency tags, microelectromagnetic sensors	Less intrusive and multiple compounds measured in real time	Real-time measurements Small size Sensitive (ppb/ppt) Low cost ($3/chip)	Design issues Still in R&D phase
Portable Sensors	Fluorometric biosensor, optical sensor	Link between personal exposure and activity	Near real-time Links compound and health effect Sensitive (ppb/ppt)	Not commercially available

Environmental Sensors for Personal Exposure Assessment

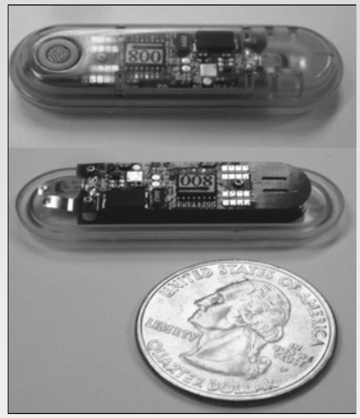
- Multiple Compounds
- Real-time continuous data acquisition
- GPS location data
- Health indicators (e.g.





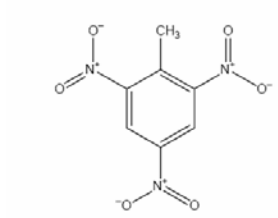
Implantable Sensors With Telemetry:*Batteries Included – testing in WiStar Rats in the Lab of Dr. William McBride at Indiana University*

- 8 Piezoresistive cantilevers
- Integrated electronic readout
- Telemetry
- Low power consumption
- 3 cm X 1 cm (diameter)
- No pump



Implanted in rats for in-vivo detection of alcohol
Ferrell (UT)

Microlelectronic Array – an example



Why TNT?

2,4,6-Trinitrotoluene (TNT)

Low vapor pressure ~ 1.99 x 10^{-4} Torr

Ability to detect trace levels of TNT is key to:

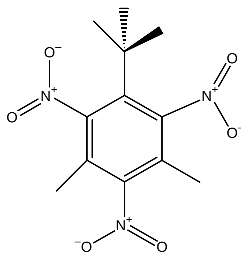
•Reducing fatalities from land mines (TNT constitutes 80% of all land mines -there are over 100 million scattered across the planet)

•Tracking explosives materials (Anti-terrorism)

•Environmental concerns (water and soil contamination)

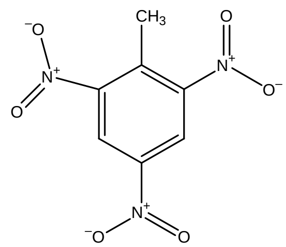
TNT Analogs

Musk Oil (Musk Xylene)



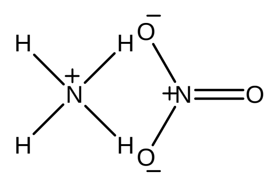
1-*tert*. -Butyl-3,5-dimethyl -2,4,6-trinitrobenzene

TNT

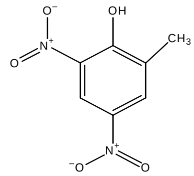


2-Methyl-1,3,5-trinitro-benzene

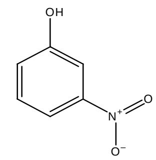
Ammonium nitrate



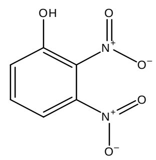
TNT Analogs



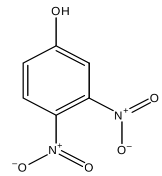
2-Methyl-4,6-dinitro-phenol



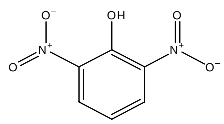
3-Nitro-phenol



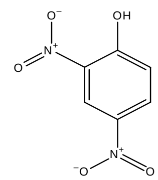
2,3-Dinitro-phenol



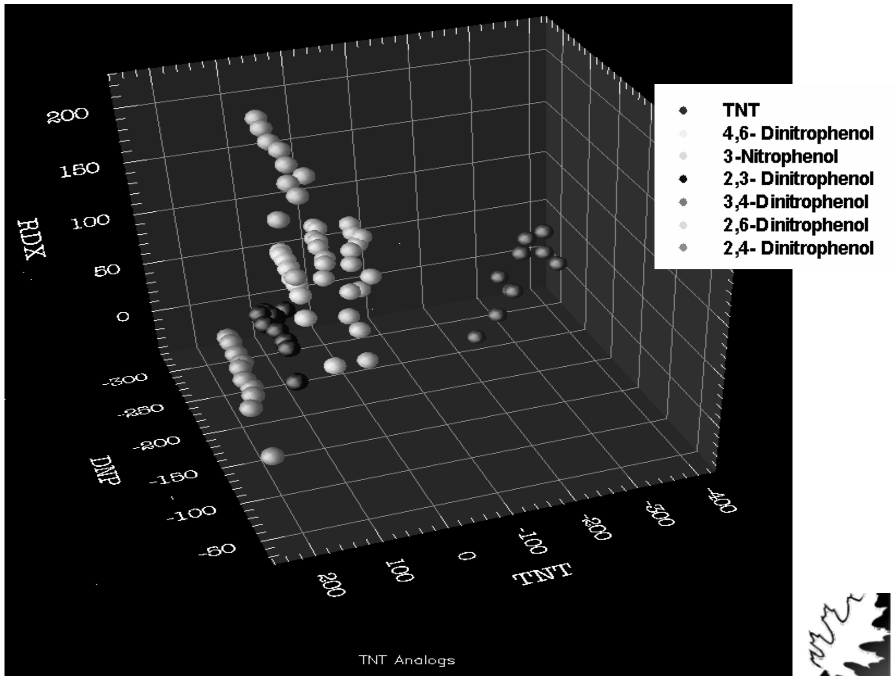
3,4-Dinitro-phenol



2,6-Dinitro-phenol



2,4-Dinitro-phenol


TNT
4,6- Dinitrophenol
3-Nitrophenol
2,3- Dinitrophenol
3,4-Dinitrophenol
2,6-Dinitrophenol
2,4- Dinitrophenol
RDX
200
150
100
50
0
DNP
-300
-250
-200
-150
-100
-50
TNT
200
100
0
-100
-200
-300
-400
TNT Analogs

Questions/Comments

Nanoscale Materials for Sensors

Ian M. Kennedy
and
Dosi Dosev, Mikaela Nichkova, Bruce Hammock, Shirley Gee, R. Dumas, Kai Liu, Zhiya Ma, Ahjeong Son, Krassi Hristova

Department of Mechanical and Aeronautical Engineering,
Department of Entomology,
Department of Physics
Land Air Water Resources

University of California Davis

Introduction

- Magnetic particles are widely used in biochemistry for a variety of biochemical and biomedical applications such as magnetic separation, MRI imaging, drug delivery etc

- Lanthanide oxides as phosphors
  - Narrow emission spectra due to electronic transitions
  - Single nanoparticle of lanthanide oxide can contain thousands of luminescent ions
  - Large Stokes shift, and can be up-converting
  - Long lifetime
  - Excellent photostability
  - Possibility of low price synthesis (good for environmental application, large amounts of samples)

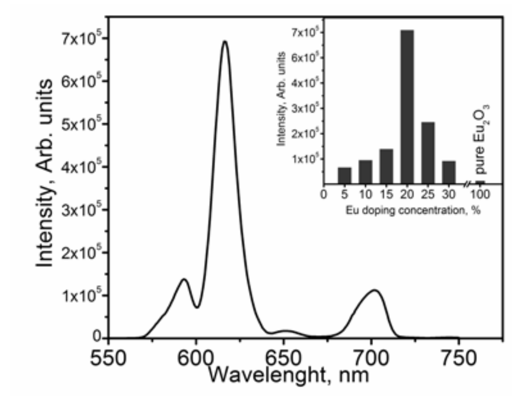
Spectral properties of Eu:Gd_2O_3

Exc: UV<280 nm, 395 nm, 466 nm

Em: 610 - 615 nm
($^5D_0 \rightarrow {}^7F_2$ transition of the Eu^{3+} ion)

Lifetime: 1 - 2 msec

Optimal doping concentration achieved – 20 at.%
Greater doping concentration than achieved with other synthesis methods



The high optimal doping concentration (20%) may be attributed to the fast temperature quenching which does not allow the ions to migrate and phase segregation to occur. Other methods achieve up to 5% doping before concentration quenching. This can be underlined as an advantage ot the spray pyr.

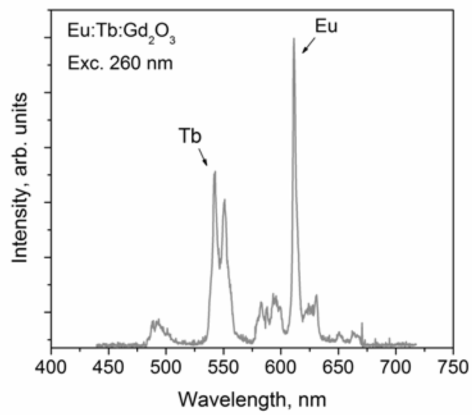
Other comment: For example, B. tissue measures at very low temp to get a spectra. We measure at RT and in water solutions!

Spectral properties – mixed doping

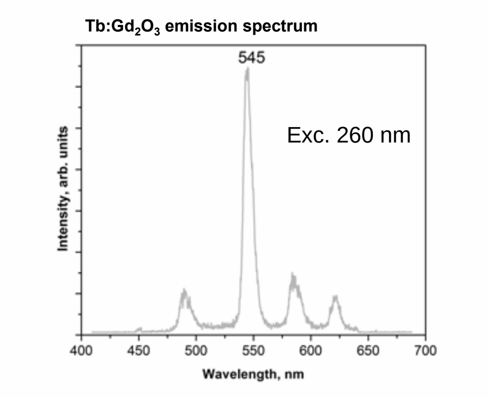
Doping of Y_2O_3 with more than one dopant.

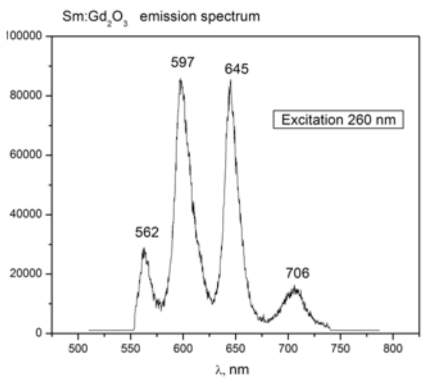
- same excitation wavelength – simultaneous emission
Example - UV

- different excitation wavelength – different emissions from one type of particles
Example – 466 nm



Spectral properties of $Tb:Gd_2O_3$ and $Sm:Gd_2O_3$





$^5D_4 \rightarrow {}^7F_5$ (545 nm)

$^4G_{5/2} \rightarrow {}^6H_{7/2}$ (597 nm)
$^4G_{5/2} \rightarrow {}^6H_{9/2}$ (645 nm)

$LaPO_4$ Nano phosphors

Ce/Tb

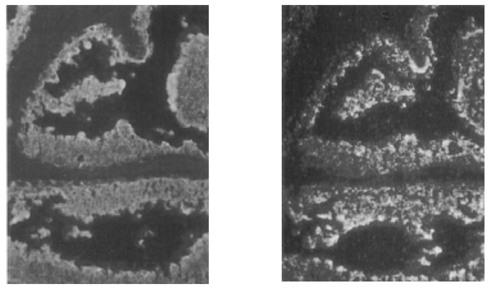
Ce/Dy

Eu

Schuetz, P., and Caruso, F. (2002) *Chemistry of Materials* **14,** 4509-4516

Imaging PSA

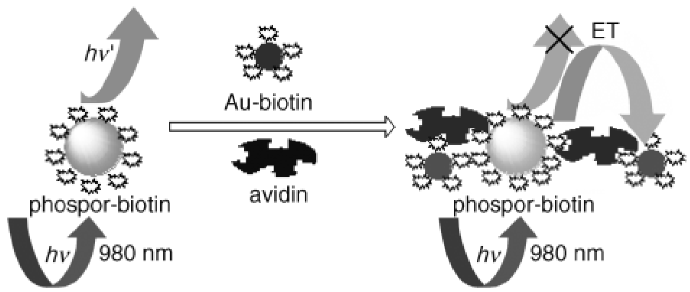
A



B

Detection of prostate-specific antigen (PSA) in paraffin-embedded sections of human prostate tissue with biotinylated antibodies: (A) avidin-FITC (B) Neutravidin-green phosphor.

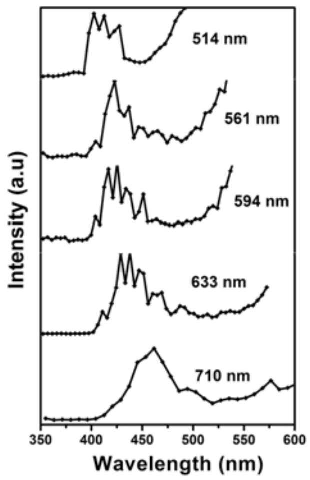
From Zijlmans, H. et al. (1999) *Analytical Biochemistry* **267,** 30-36.



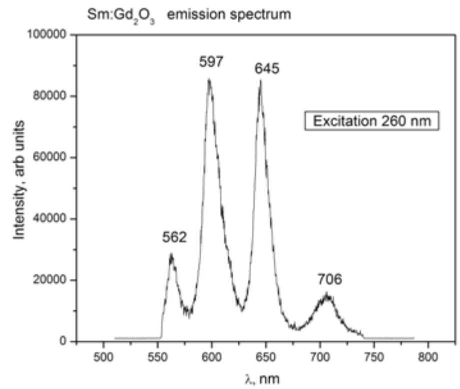
Scheme of the FRET system, with phosphor-biotin nanoparticles as energy donors and Au-biotin nanoparticles as energy acceptors, in the analysis of avidin

From Wang et al., *Advanced Materials* **17,** 2506, 2005

Sm:Gd2O3 nanoparticles produced by spray pyrolysis – fluorescent properties



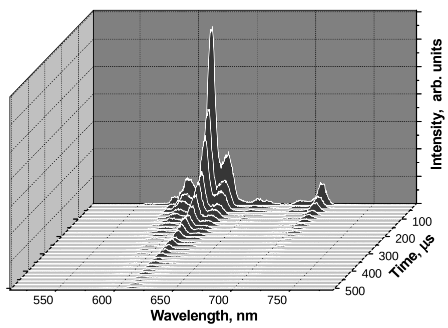
Up-converting emission under various excitation wavelengths



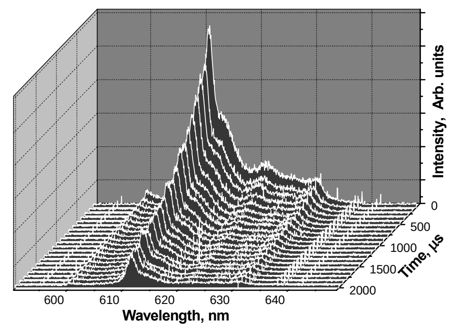
Emission under UV excitation

D. Dosev, I. M. Kennedy , M. Godlewski , K. Tomsia , I. Gryczynski , E.M. Goldys; **Fluorescence upconversion in Sm doped Gd_2O_3, Applied Physics Letters 2006**

$Eu:Y_2O_3$ lifetime

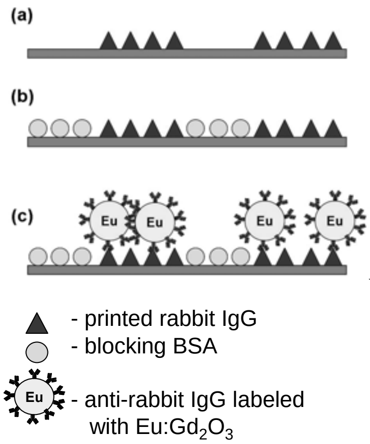


Pure Eu_2O_3

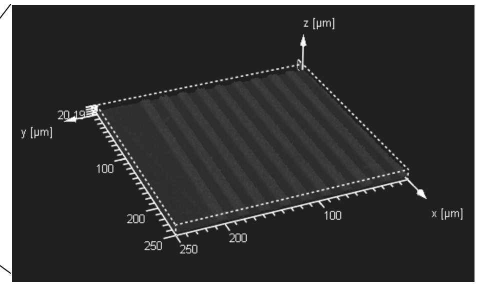


$Eu:Y_2O_3$

Specific recognition using IgGs, IgG particle coating, control of binding sites, qualitative immunoassay, SEM evaluation

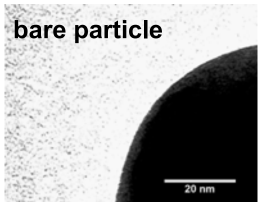


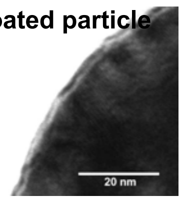
Confocal microscope image

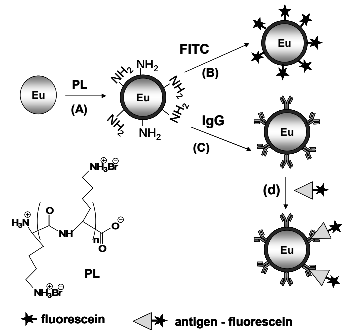


Mikaela Nichkova, Dosi Dosev, Richard Perron,
Shirley J. Gee, Bruce D. Hammock, Ian M. Kennedy
Eu^{3+} - doped Gd_2O_3 nanoparticles as reporters for optical detection and visualization of antibodies patterned by microcontact printing, Analytical Biochemistry 2006

Introducing internal fluorescent standard, new polymer coating of the $Eu:Gd_2O_3$ nanoparticles, quantitative immunoassay for detection of PBA.



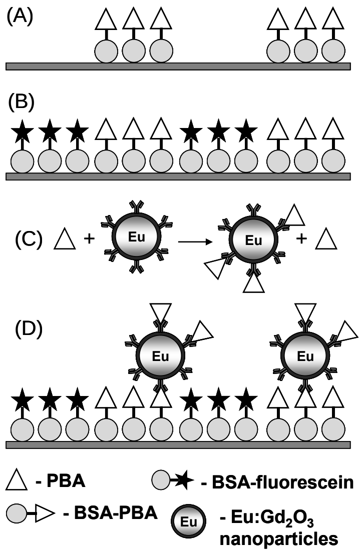




A- PL coating;
B- Evaluation of surface amino groups
C- Covalent binding of antibodies
D- Evaluation of active binding sites

Mikaela Nichkova, Dosi Dosev, Shirley J. Gee, Bruce D. Hammock, Ian M. Kennedy; **Microarray immunoassay for phenoxybenzoic acid using polymer encapsulated $Eu:Gd_2O_3$ nanoparticles as fluorescent labels, Analytical Chemistry**

Stage 3 – Introducing internal fluorescent standard, new polymer coating of the Eu:Gd_2O_3 nanoparticles, quantitative immunoassay for detection of PBA.



A- microcontact printing of the coating antigen (BSA-PBA)

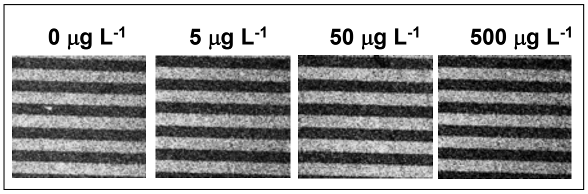
B- immobilization of the internal standard (BSA-fluorescein)

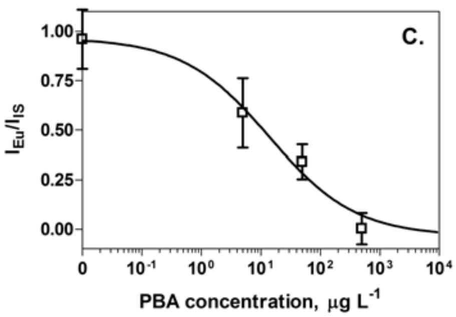
C- preincubation of the labeled antibody with the analyte (PBA)

D- incubation of the micropatterns with the labeled antibody

Mikaela Nichkova, Dosi Dosev, Shirley J. Gee, Bruce D. Hammock, Ian M. Kennedy; **Microarray immunoassay for phenoxybenzoic acid using polymer encapsulated Eu:Gd_2O_3 nanoparticles as fluorescent labels, Analytical Chemistry**

Introducing internal fluorescent standard, new polymer coating of the $Eu:Gd_2O_3$ nanoparticles, quantitative immunoassay for detection of PBA.

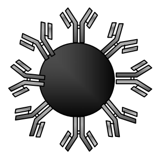




The detection limit of the PBA is in the low ppb range.

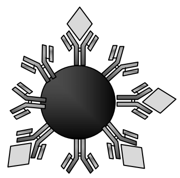
Mikaela Nichkova, Dosi Dosev, Shirley J. Gee, Bruce D. Hammock, Ian M. Kennedy; **Microarray immunoassay for phenoxybenzoic acid using polymer encapsulated $Eu:Gd_2O_3$ nanoparticles as fluorescent labels, Analytical Chemistry**

Novel format for immunoassay using magnetic/luminescent particles



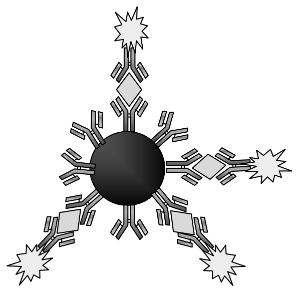
Primary antibodies are immobilized on the particle’s surface.

Novel format for immunoassay using magnetic/luminescent particles



Primary antibodies are immobilized on the particle's surface.

Novel format for immunoassay using magnetic/luminescent particles



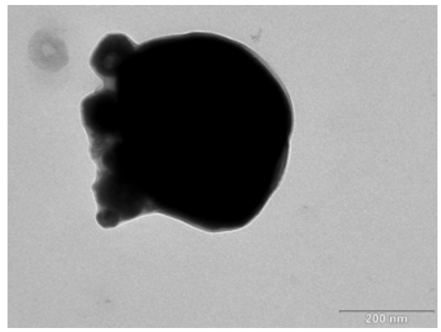
Primary antibodies are immobilized on the particle's surface.

Measurements rely on relative (ratiometric) fluorescence intensity reading.

Characteristics of magnetic/luminescent core/shell particles

From one to several primary $Co:Nd:Fe_2O_3$ particles can be embedded in a single composite particle.

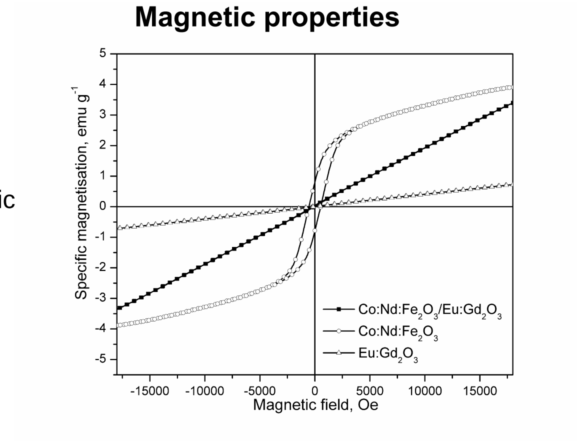
Shell with thickness of 10 to 50 nm can be distinguished.



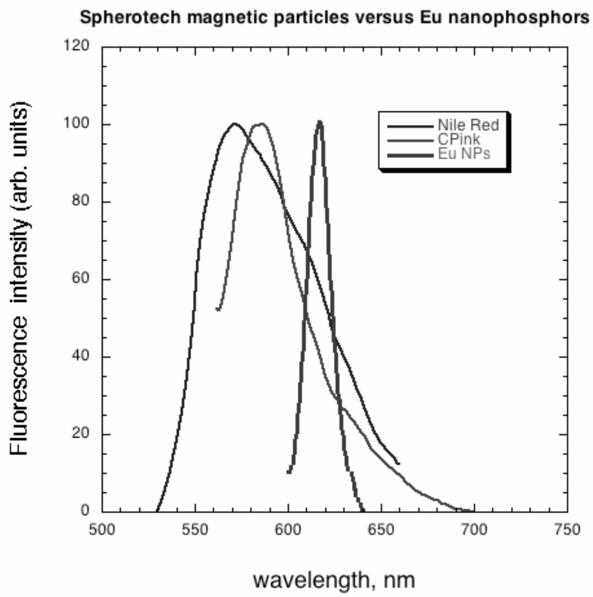
Characteristics of magnetic/luminescent core/shell particles

In contrast to the ferromagnetic core characteristics, the composite particles showed paramagnetic behavior with magnetization at comparable levels to that of primary magnetic cores.

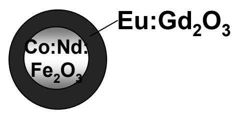
Possible phase transformation during the second spray pyrolysis.



Luminescent properties were identical with that of pure $Eu:Gd_2O_3$ particles

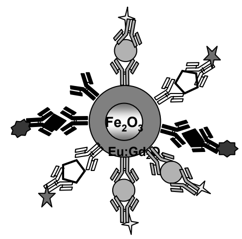

Spherotech magnetic particles versus Eu nanophosphors
Fluorescence intensity (arb. units)
wavelength, nm
Nile Red
CPink
Eu NPs
0
20
40
60
80
100
120
500
550
600
650
700
750

Multianalyate magnetic fluorescent immunoassay for toxins – model analytes



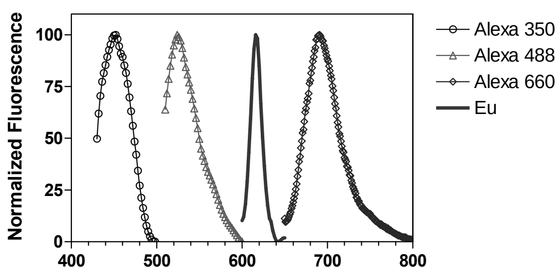
Fe/Co/Nd=80/20/5

Low-cost synthesis:
flame spray pyrolysis

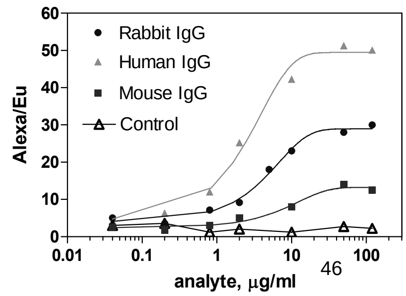


Immunocomplex after magnetic separation

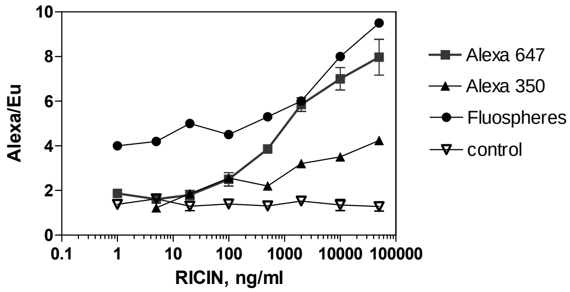
Multiplex assay

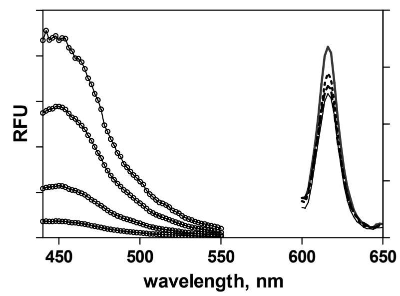


In mix



RICIN magnetic fluorescent immunoassay





- Amount of nanoparticles per assay
  (1 mg NPs in 0.5 ml sample)
- Amount of capture Ab per mg NPs
  100 ug/mg
- Choice of label:

Alexa 350, Alexa 647, Fluospheres (505/515)

- Direct labeling/biotin amplification

Limit of detection: ~ 20 ng/ml Ricin

4 ug/g food for children
20 ng/ml * 10 ml/g food = 0.2 ug/g

Ian, I think that this assay is limited by the detectability of Alexa 350 on the plate reader.

I will play a litle bit more with concentration range and time of incubation to see if I could improve it.

However, I believe that we have to think about other label such as fluospheres. I will try them (I have them in the fridge)

Regarding speed: we could speed it in the channel.

DNA assays

- MTBE (Methyl Tertiary-Butyl Ether)

: Fuel additive, widespread contaminant in soil and groundwater environment

- *Methylibium petroleiphilum* PM1

: capable of MTBE biodegradation, major microorganism in many gasoline-contaminated aquifer in the US, Mexico, and Europe

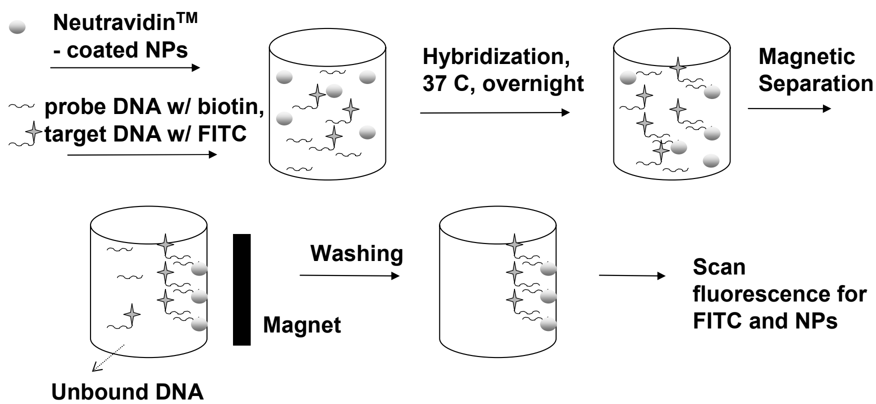
- Target DNA

: PM1 16S rDNA bacterial DNA

Future work on breast cancer P53

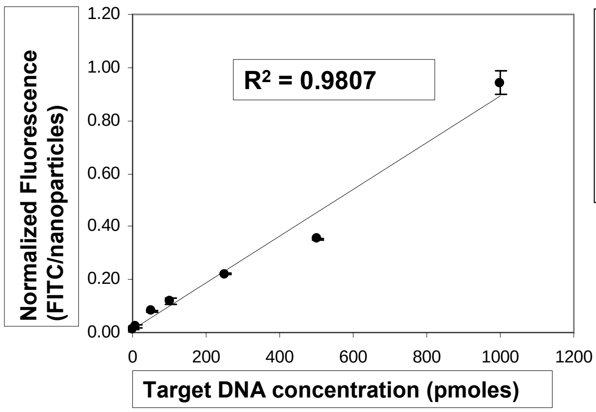
Chronic kidney disease

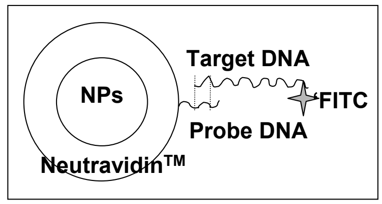
Hybridization in Solution



Hybridization in Solution

- **Result** – DNA quantification



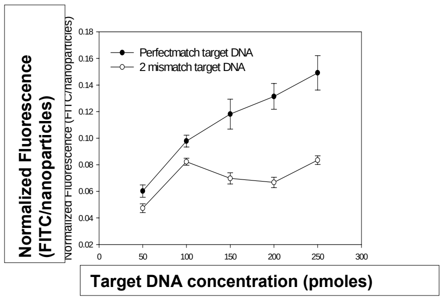


• Detection Limit

: 20 pM/mL water

Hybridization in Solution

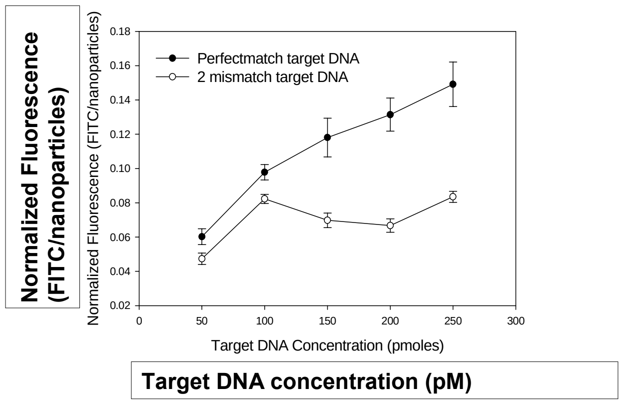
- **Result -** Single Nucleotide Polymorphism (SNP)



• 2 mismatch target DNA was successfully discriminated in this assay

Hybridization in Solution

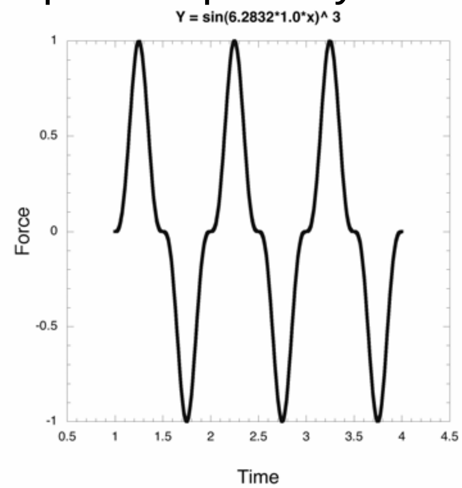
- **Result -** Single Nucleotide Polymorphism (SNP)

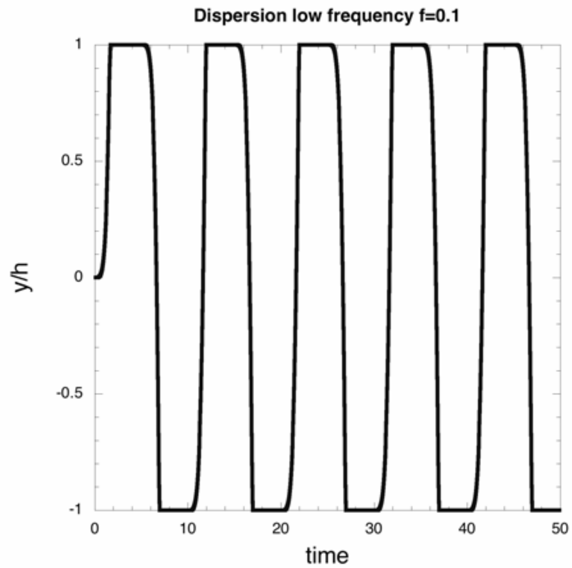


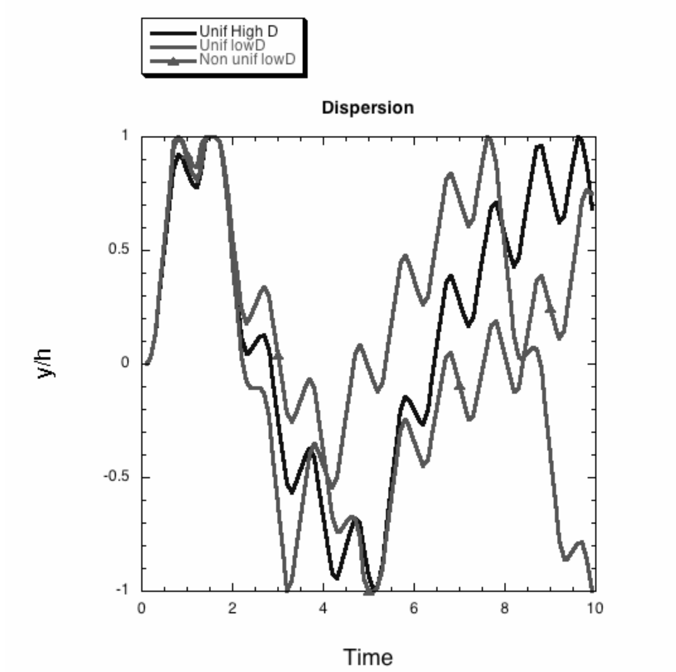
•Target DNA with 2 bp mismatch was successfully discriminated in this assay

Magnetics in a microchannel

Oscillating magnetic field across channel speeds up assay x10

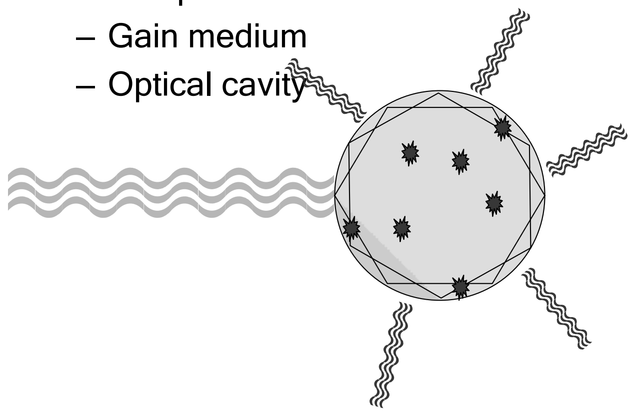



Dispersion low frequency f=0.1
y/h
1
0.5
0
-0.5
-1
0
10
20
30
40
50
time


Unif High D
Unif lowD
Non unif lowD
Dispersion
y/h
1
0.5
0
-0.5
-1
0
2
4
6
8
10
Time

Optical Resonances

- For lasing:
  - Pump source
  - Gain medium
  - Optical cavity



$$x = \frac{2\pi r}{\lambda}$$

We can make an analogy between the regular lasing mediums and our microcavity lasing system. For lasing to occur we have three basic requirements We need a pump source which is usually a pump laser or a flash lamp. And a gain medium can be a gas medium or a solid state medium. Here our pumping source is second harmonic NdYAG laser. Our gain medium is Rhodamine 6G which has emission ranging from 550 to 610 nm. The lasing peaks are based on the R6G emission. The optical cavity would be our microdroplet. The Rhodamine 6G emission obeying the resonance condition goes through Total Internal Reflection at the droplet air interface.

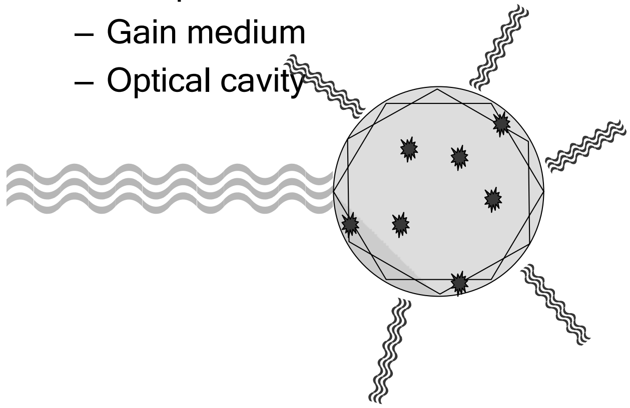
This phenomenon can be understood in two ways: the classic view and the quantum view. In the classic view, the light is trapped within the droplet through total internal reflection. In the quantum view, the light inside cavity forms standing waves. Only the allowed wavelengths are amplified and the other wavelengths are prohibited. The modes can be described by quantum numbers n,l,m similar to the wavefunctions of an electron in a hydrogen atom.

Optical Resonances

Droplet lasing:

- Laser (Nd:YAG, Argon ion)
- Fluorescent dye, QD
- Microdroplet

- For lasing:
  - Pump source
  - Gain medium
  - Optical cavity



$$X = \frac{2\pi r}{\lambda}$$

57

We can make an analogy between the regular lasing mediums and our microcavity lasing system. For lasing to occur we have three basic requirements We need a pump source which is usually a pump laser or a flash lamp. And a gain medium can be a gas medium or a solid state medium. Here our pumping source is second harmonic NdYAG laser. Our gain medium is Rhodamine 6G which has emission ranging from 550 to 610 nm. The lasing peaks are based on the R6G emission. The optical cavity would be our microdroplet. The Rhodamine 6G emission obeying the resonance condition goes through Total Internal Reflection at the droplet air interface.

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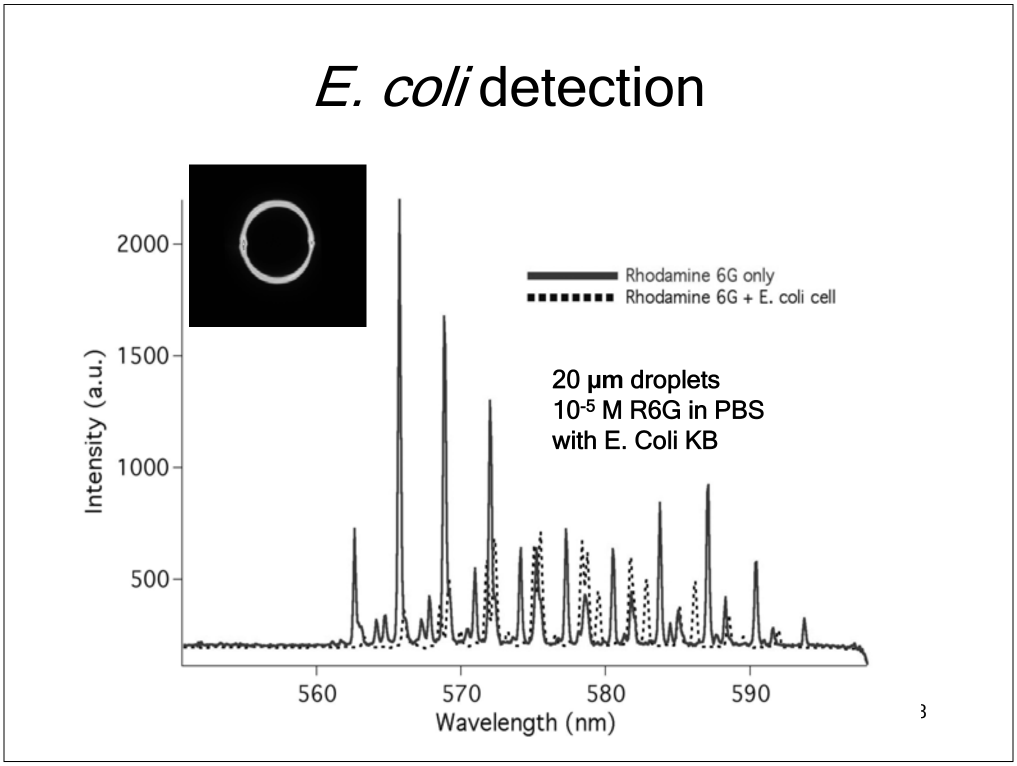
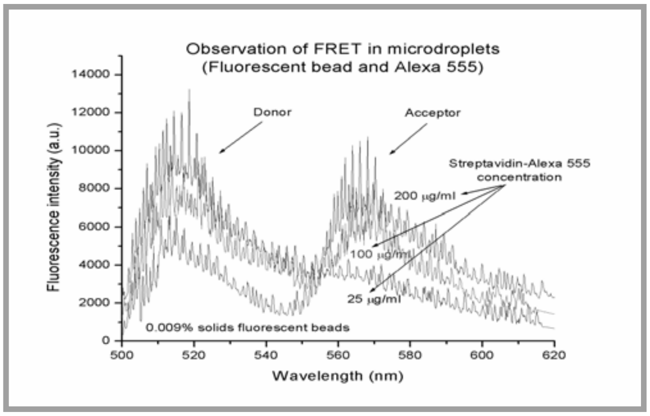
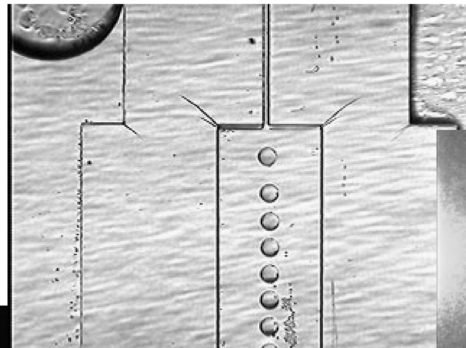


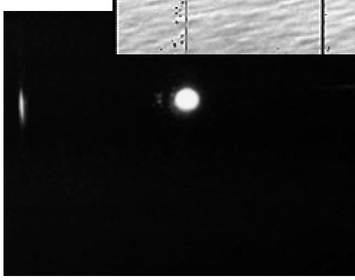
Here are some results from experiments done with cells. We prepared a roughly 1 billion cells/ml solution of E. Coli KB cells in 10 micro molar PBS/R6G solution. The red lines show the 10 micro molar R6G in PBS data. And the blue lines show when we run the cell solution through our experimental setup. Here we see the suppression of the lasing peaks when cells are present inside the microdroplets. This happens due to two effects: One of them is the local refractive index change due to the presence of cell inside the microcavity. The other effect is the scattering of the light due to the cell presence. Scattering inside the microcavity due to an inclusion degrades the Q factor resulting in the suppression of the lasing. But you can also see here that some blue modes have higher intensity compared to the original spectrum. This results from the leakage of the high order modes to the outside of the droplet due to scattering. This result in general also confirms previous experimental and theoretical studies of the effect of inclusions in microdroplets. Several groups including (Campillo in Naval Research Academy, Armstrong and Pinnick) has observed these effects for inclusions in microcavities.

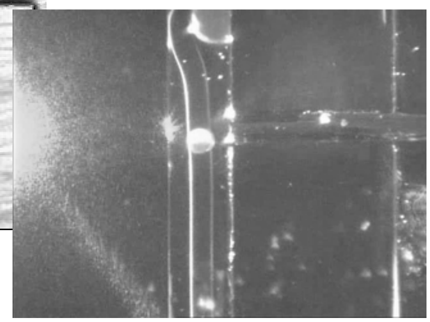
FRET experiments in microdroplets



Droplets in a microchannel







Conclusions

• The combination of magnetic and luminescent properties within the same particle enables a new format of immunoassay to be developed based on ratiometric measurements with internal luminescent standard.

• A non-optimized immunoassay was demonstrated for detection of rabbit IgG to demonstrate the novel immunoassay format. It is currently being applied to food borne toxin detection

•DNA assay on particles has been shown for use in bioremediation of MTBE, with potential for other targets including breast cancer and kidney disease

Acknowledgements

National Science Foundation, Grant DBI-0102662,

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U.S. Department of Agriculture, under Award No. 05-35603-16280

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