Advanced Biophotonics Sensors for Environmental and Biomedical Applications

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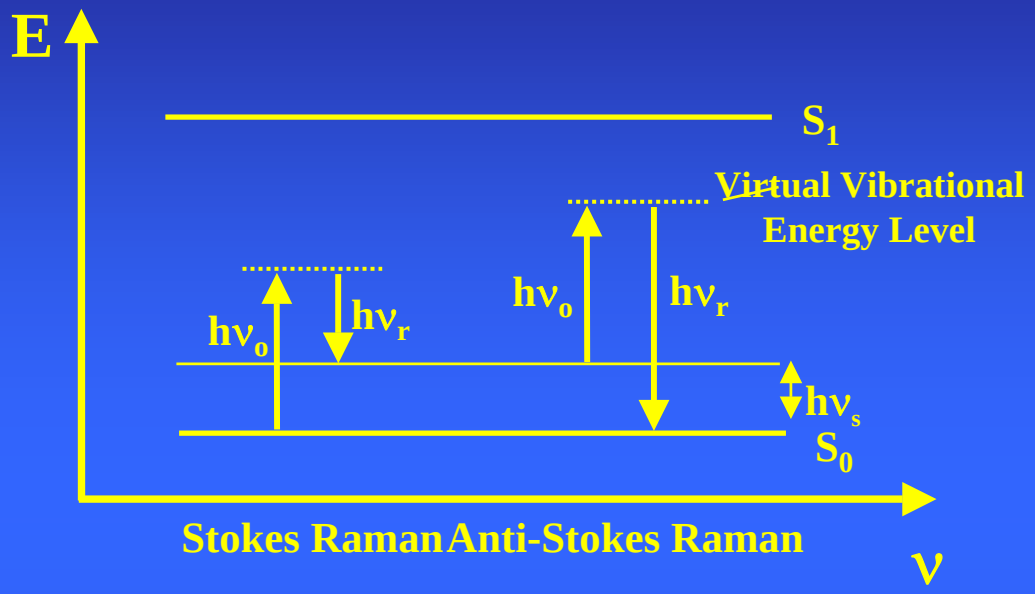
Outline

- Introduction to Raman spectroscopy
- Surface-enhanced Raman scattering gene probes
- Raman integrated tunable sensor
- Optical nanobiosensors for single cell analysis
- Multi-functional biochips
- “Optical biopsy” based on laser-induced fluorescence
- Conclusion

Raman scattering



- Based on vibrational transitions
- Offers structural information
- Narrow bands potentially afford spectral selectivity
- Widely considered as complement to IR spectroscopy
- Continuous, tunable excitation sources are not necessary
- Signals generally very low

Surface-enhanced Raman Scattering (SERS)

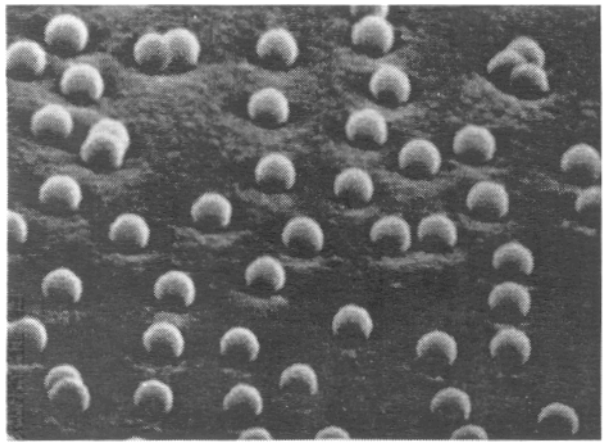
- Produces Raman scattering enhancement of generally 10^6-10^8
- Enhancements as high as 10^{15} observed with specific compounds in single-molecule detection studies
- Results from the adsorption of compounds on submicron-textured metal surfaces
- Has been explained in terms of electromagnetic and molecular enhancement models

$$\mu = E \cdot \alpha$$

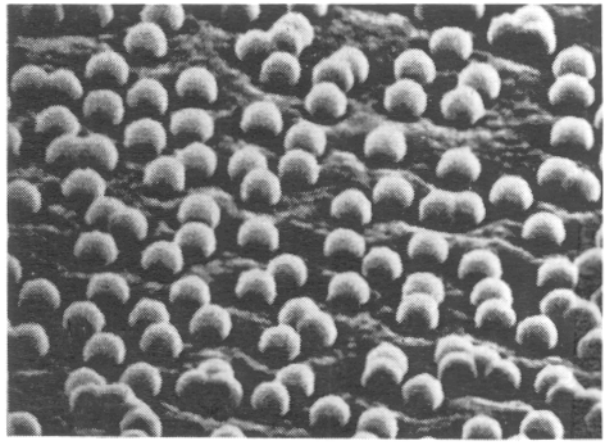
μ = Induced dipole moment
E = Incident electric field
α = Molecular transition polarizability

SEM Photographs of SERS-active nanoparticles

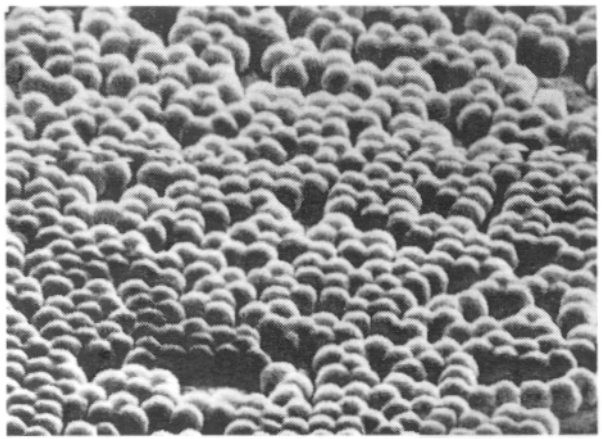
A) 0.33 %



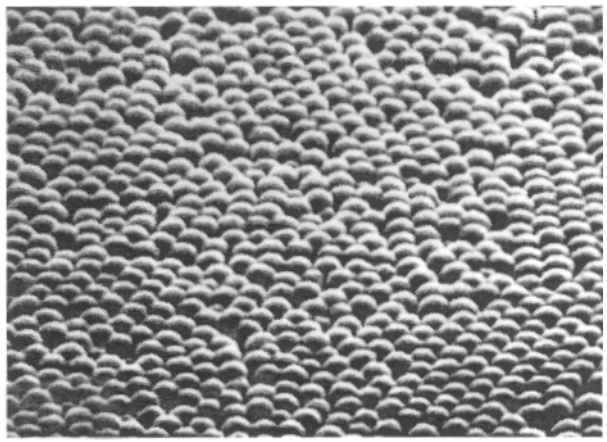
B) 1 %



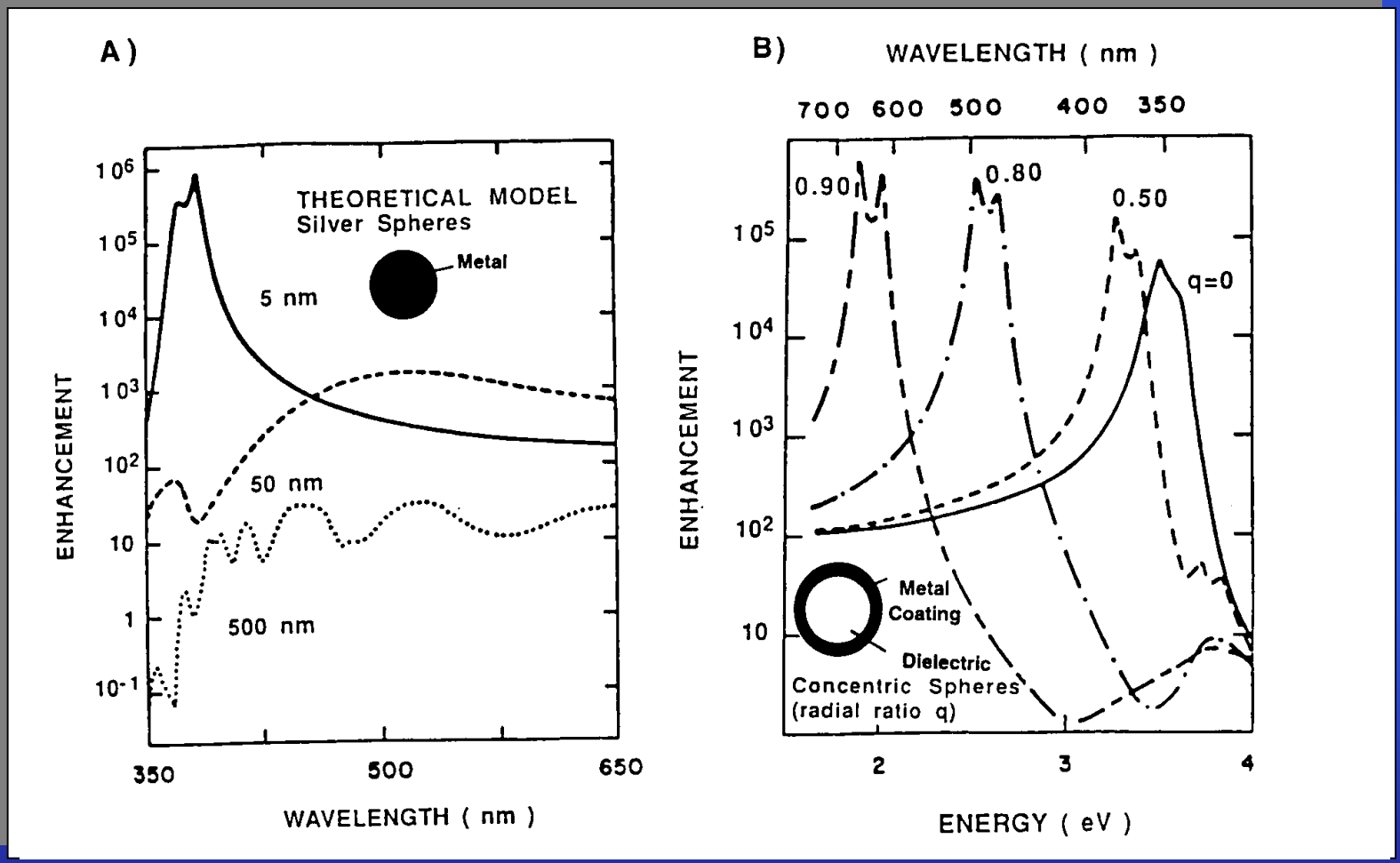
C) 3.3 %



D) 10 %

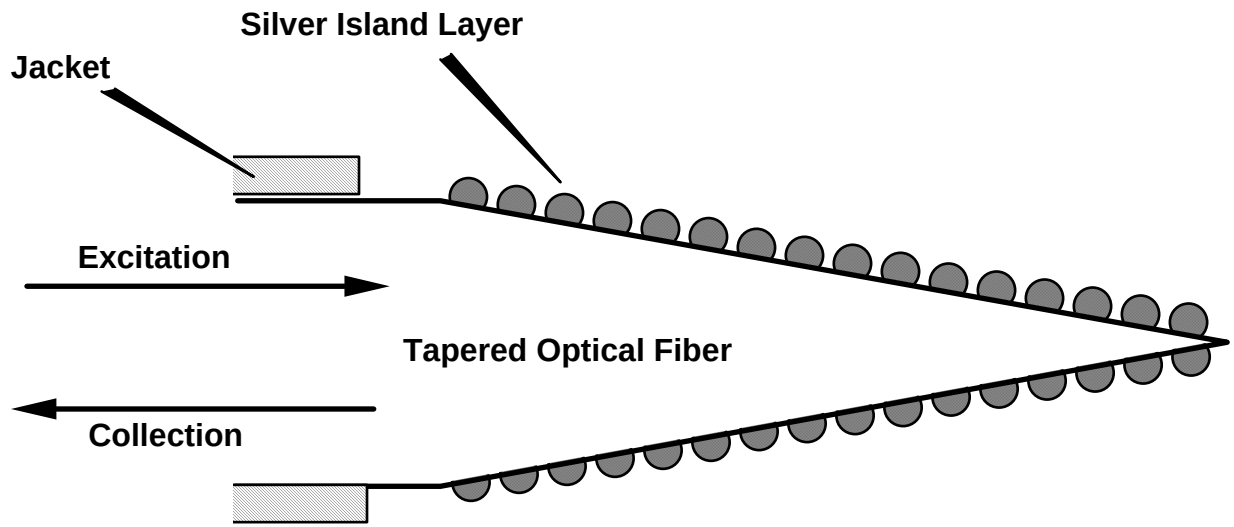


Calculated Raman enhancement and excitation profile using models for solid metal nanosphere and concentric dielectric/metallic nanospheres

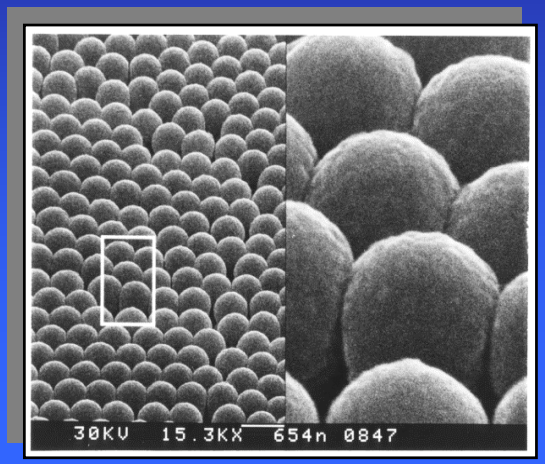


Source: M. Kerker, *Acc. Chem. Res.* 17 (1984) 271

Schematic diagram of a tapered fiberoptic SERS probe with silver island SERS-active layer



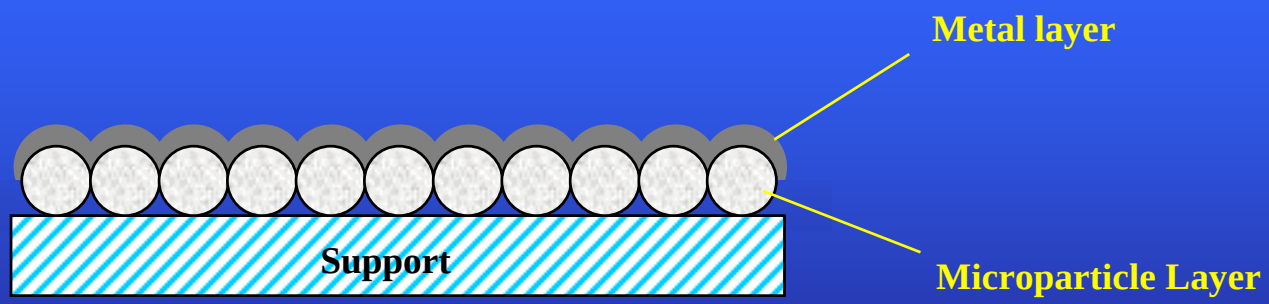
Microparticle-based SERS substrate



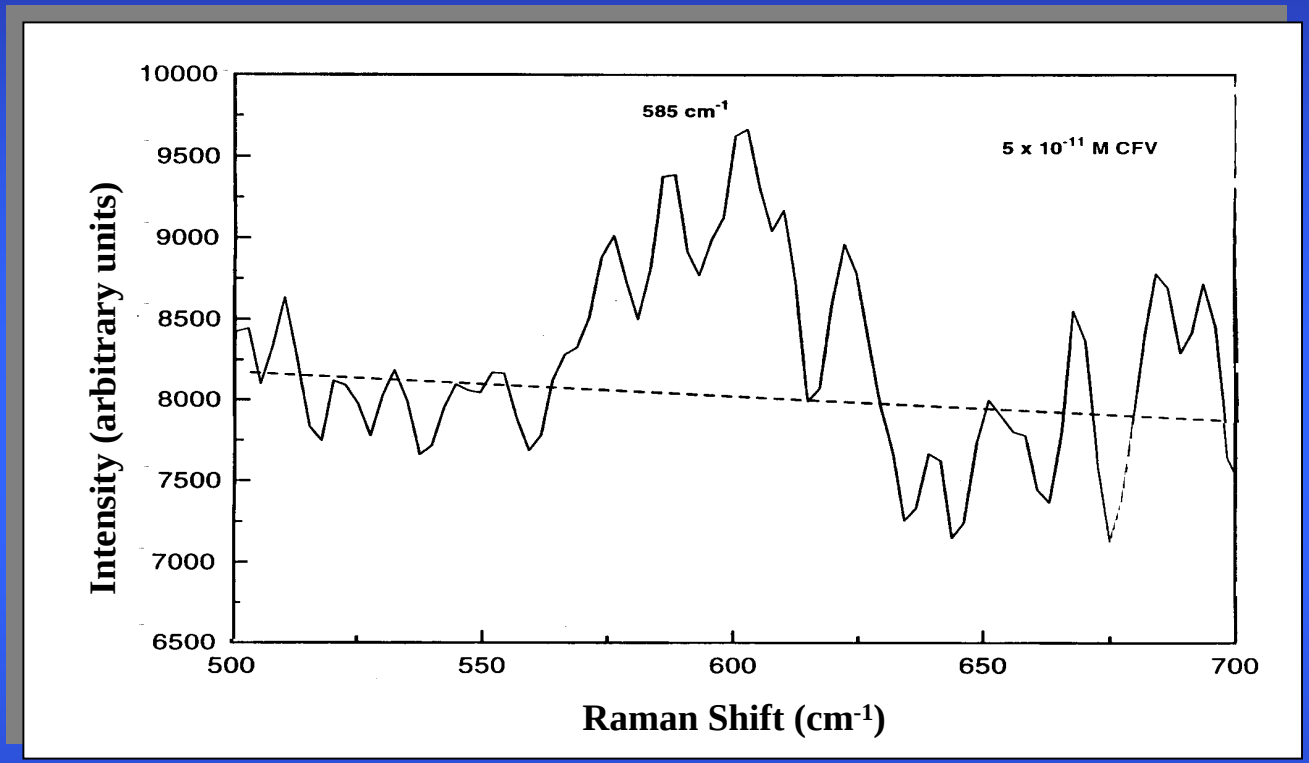
SEM micrograph of silver-coated polystyrene microspheres

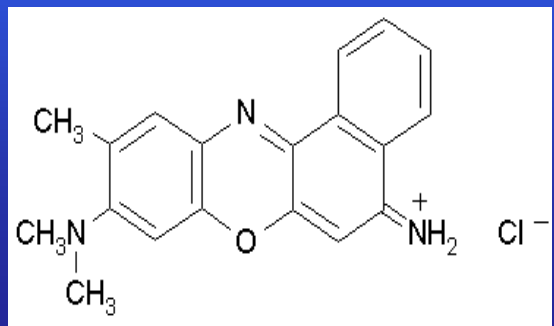
Microparticle-based Substrate Parameters:

- Microparticle material (e.g. alumina, titanium dioxide, polystyrene, fumed silica)
- Microparticle size (e.g. 0.05 - 0.5 μm)
- Metal (e.g. silver, gold, copper)
- Metal thickness (e.g. 750 - 1500 Å)

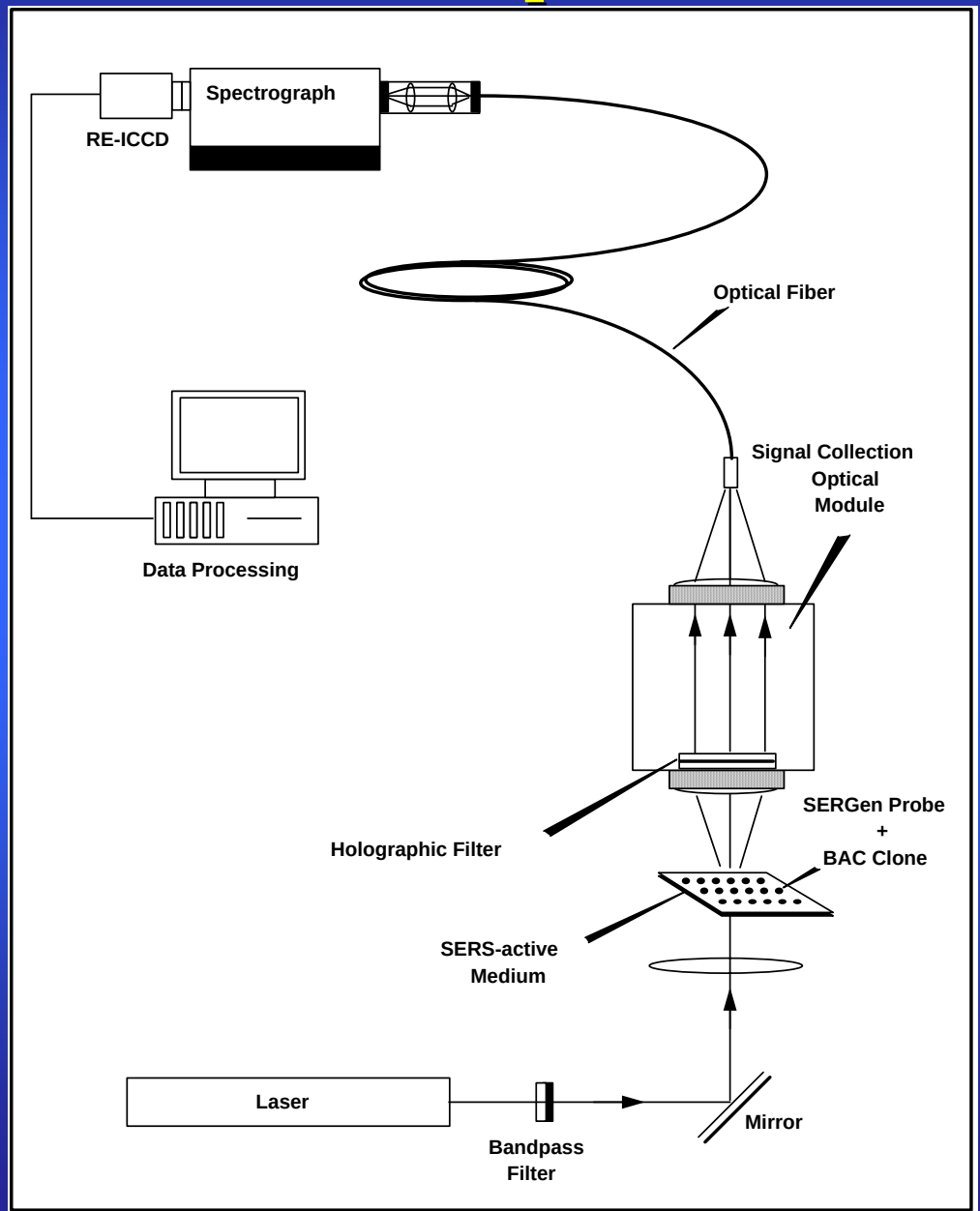


Single-molecule detection of cresyl fast violet using silver islands SERS substrate





Instrumental set-up for spectral recording of individual spots of SERGen probes

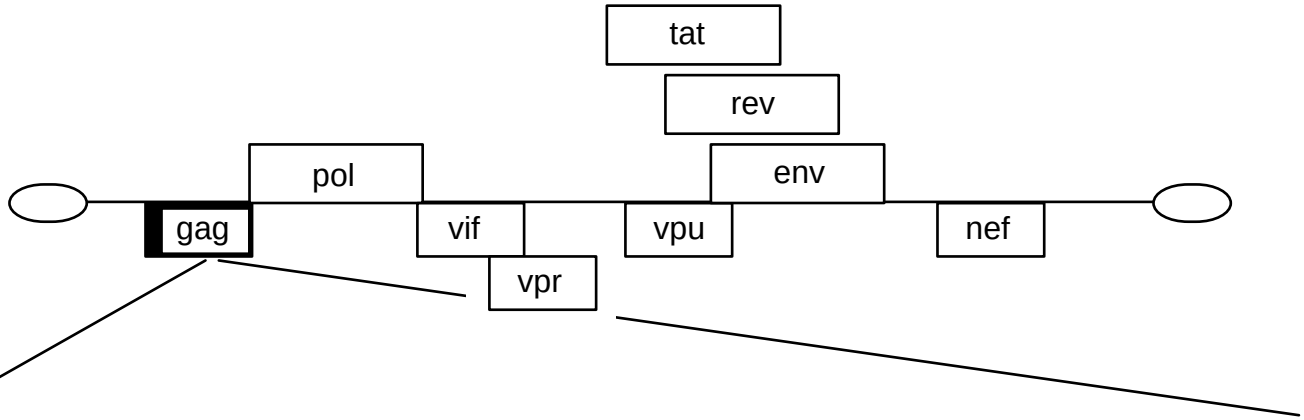


An individual spot corresponds to an individual microdot on a hybridization platform

Readily assembles using commercially available or off-the-shelf components (10K)

Bandpass filter isolates laser line prior to sample excitation. The collection module includes a Raman holographic filter which rejects the Rayleigh scattered radiation.

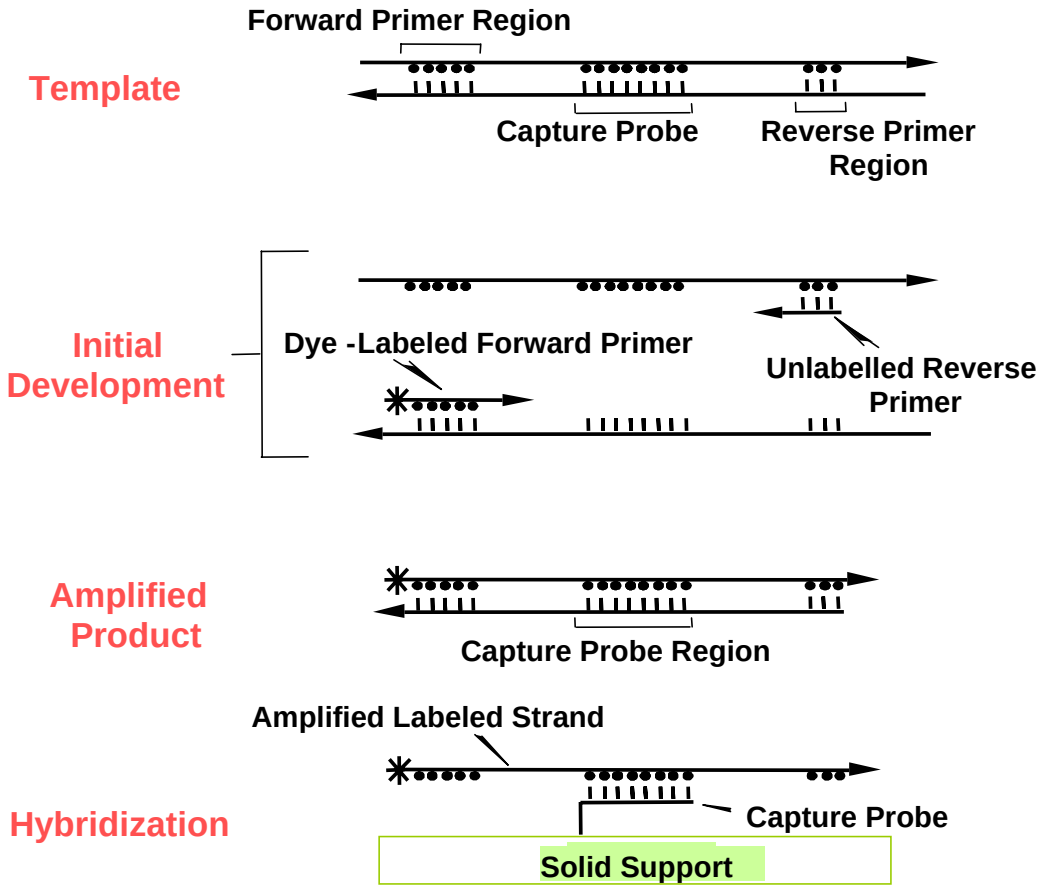
DNA sequence used with SERGen probe for HIV detection



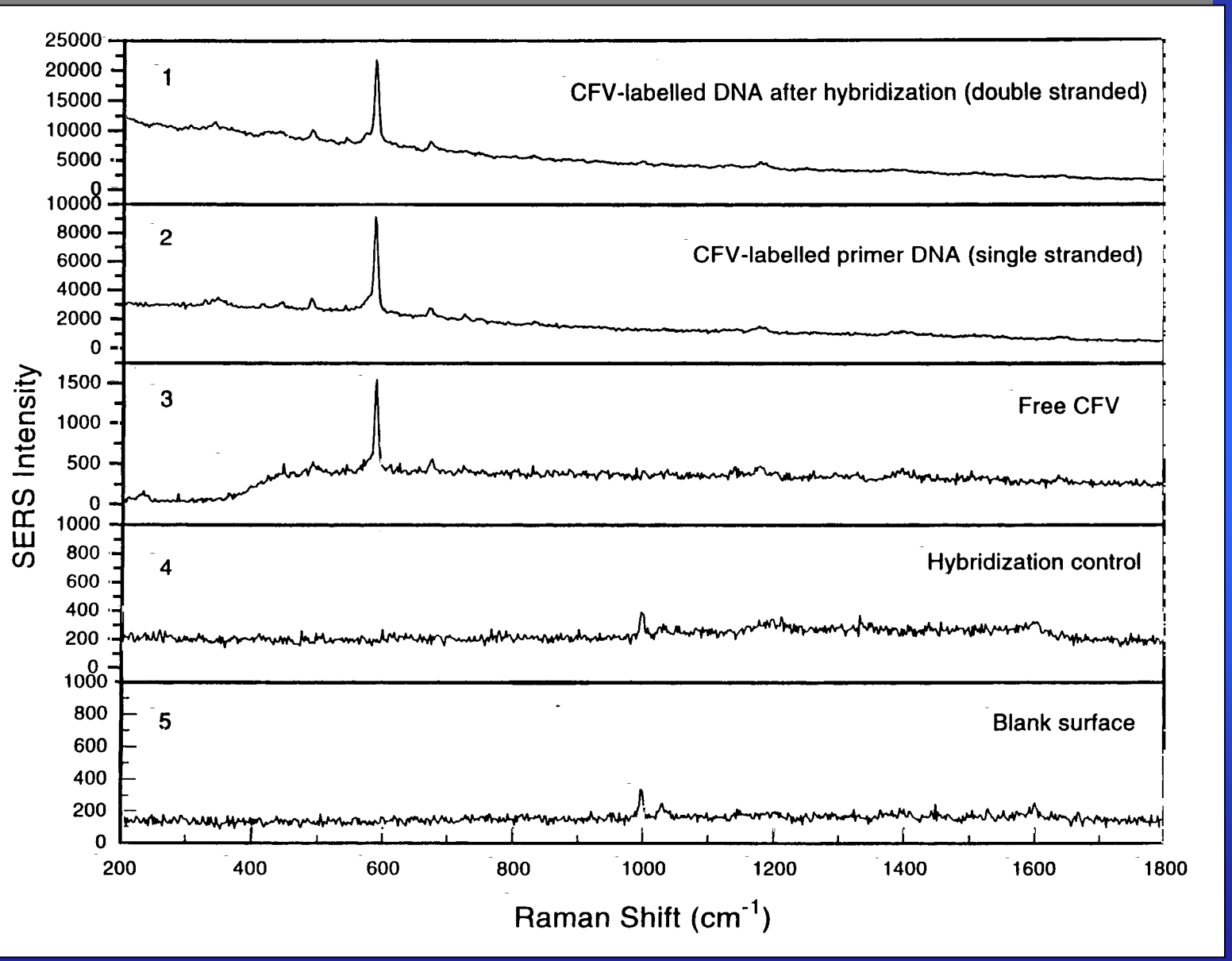
ATAGTGCAGA ACCTCCAGGG GCAAATGGTA CATCAGGCCA TATCACCTAG AACTTTAAAT
TATCACGTCT TGGAGGTCCC CGTTTACCAT GTAGTCCGGT ATAGTGGATC TTGAAA TTTA

GCATGGGTAA AAGTAGTAGA AGAGAAGGCT TTCAGCCCAG AAGTAATACC CAT
CGTACCCATT TTCATCAT CT TCTCTTCCGA AAGTCGGGTC TTCATTATGG GTA

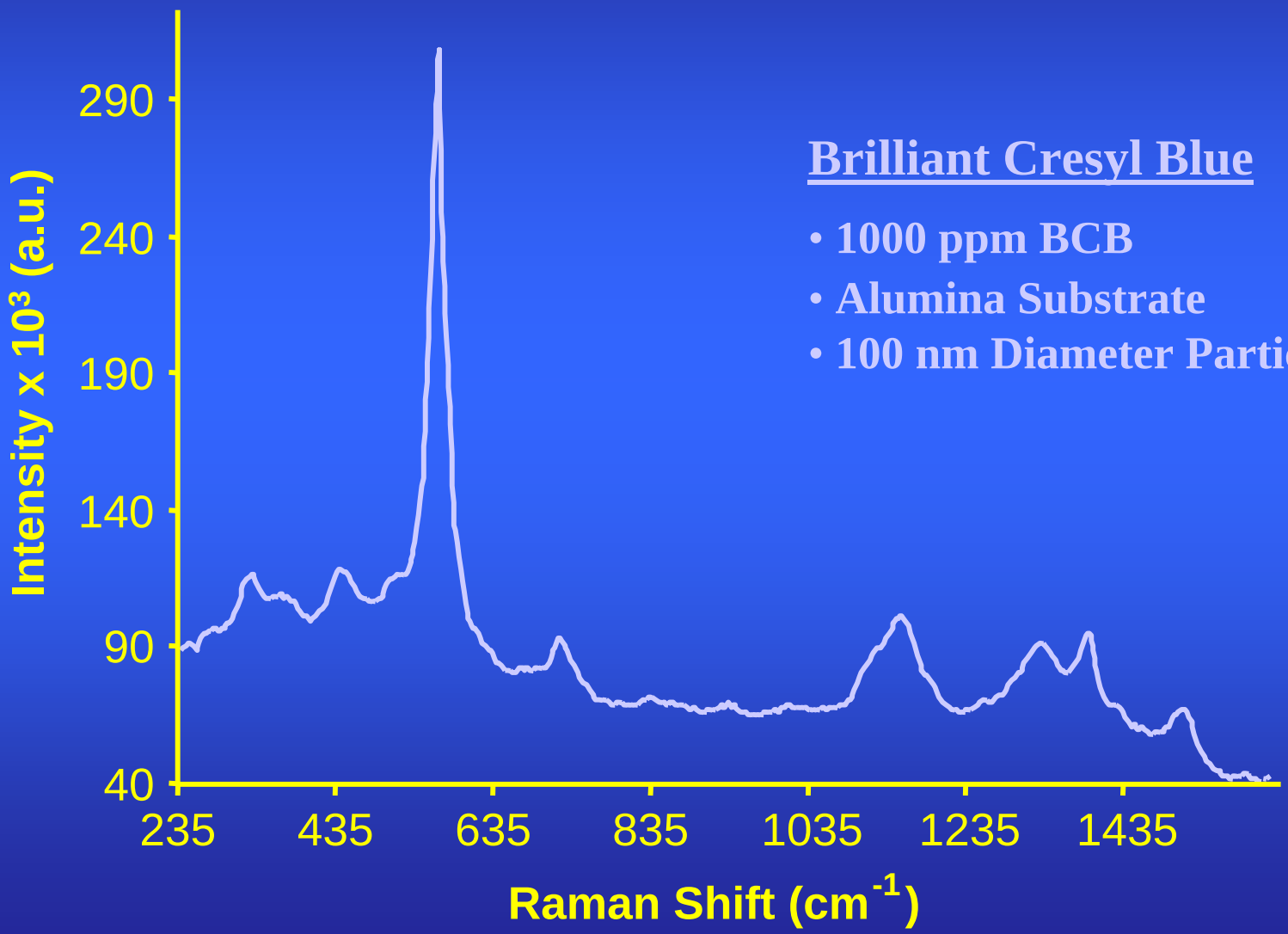
SERS gene probe development via PCR and hybridization for selective detection



Demonstration of the SERGen Technique for the Detection of HIV1 *gag* gene



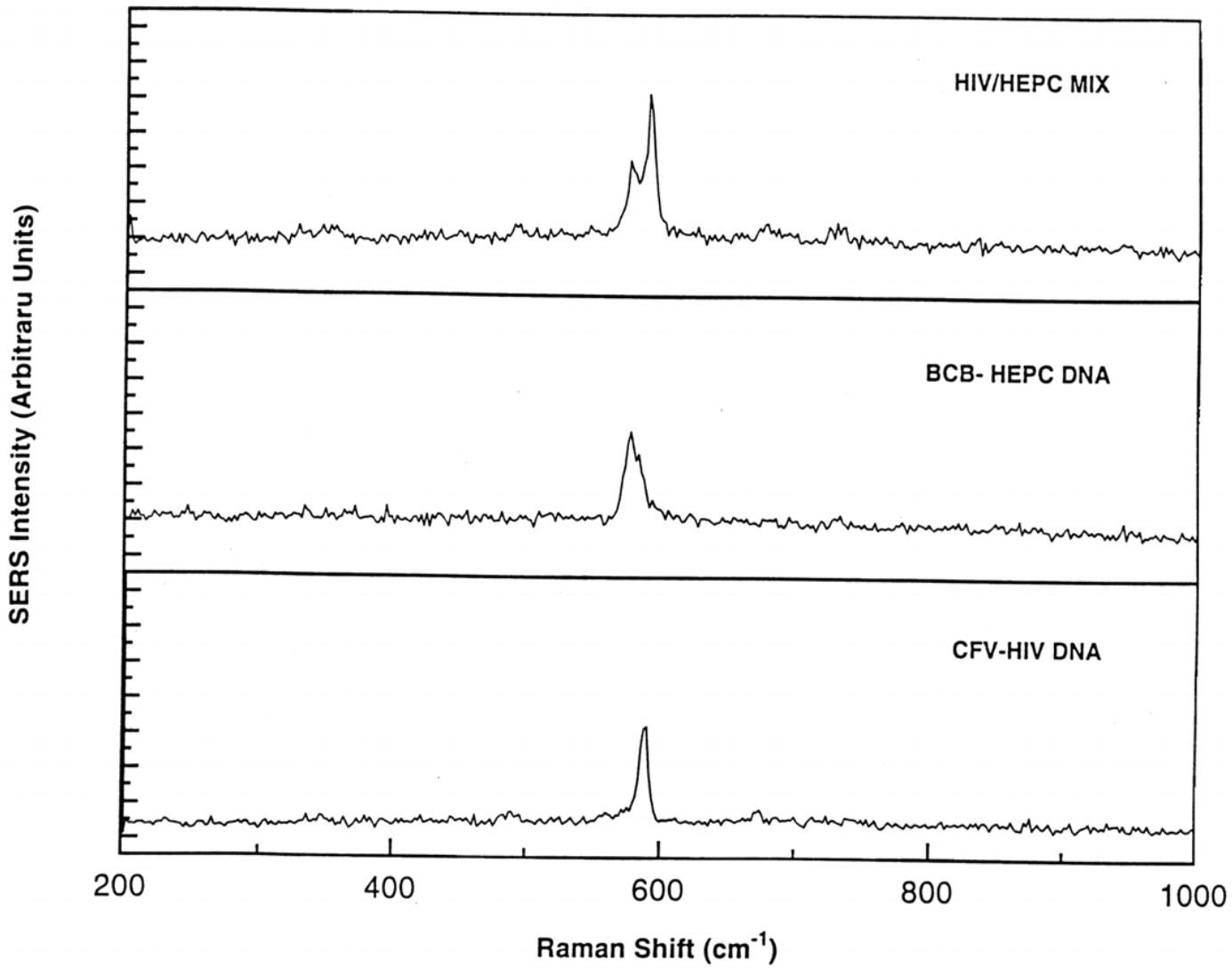
SERS Spectrum of BCB



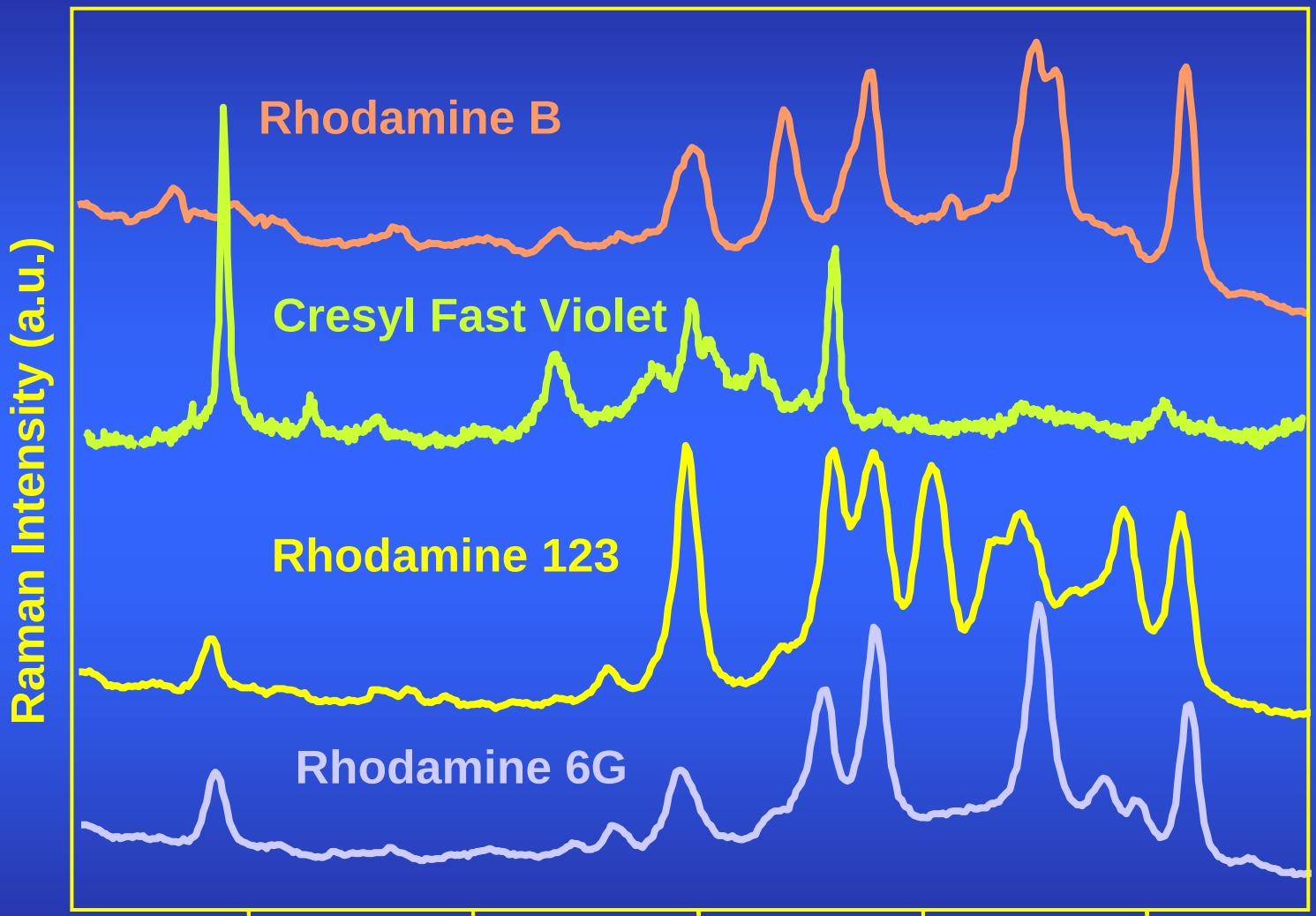
Brilliant Cresyl Blue

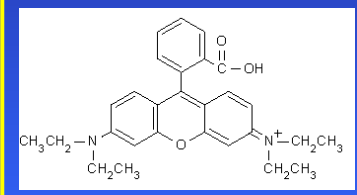
- 1000 ppm BCB
- Alumina Substrate
- 100 nm Diameter Particles

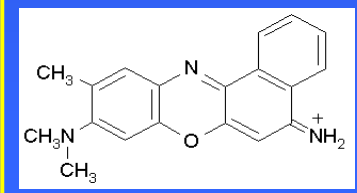
Simultaneous detection of HIV and HEPC SERS gene probe hybridizations

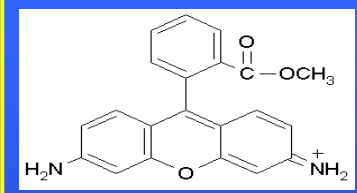


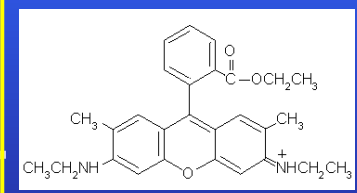
SERS spectra of Raman active dyes



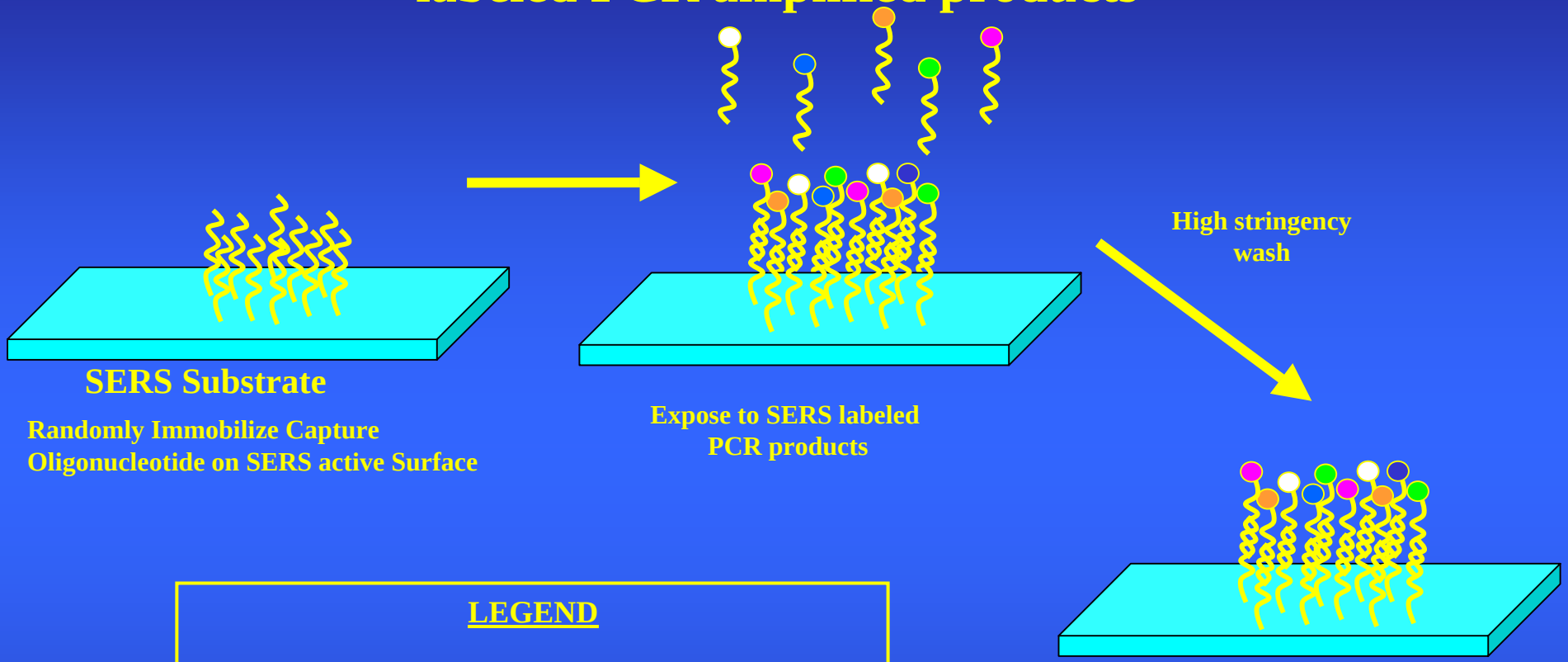








Multiplexed DNA assays using SERS-labeled PCR amplified products



Multiplexed Detection using SERS

SERS Challenges

Experimentally:

- Metallic nanostructures with nano-meter precision (nano-patterning)

Theoretically:

- Better understanding of Raman scattering enhancement mechanism

RAMITS

Raman Integrated Tunable Sensor

Raman instrument for field analysis

Requirements for field analysis:

- Provides *rapid*, *on-site*, *in situ* analysis,
- Improve the quality, reduce the costs, and enhance the safety of characterization and monitoring activities (i.e., hazardous waste sites).

Analytical advantages of Raman technique:

- Produces a unique, spectral "fingerprint" for each analyte
- Analyzes samples such as solids, liquids, and sludges directly, *without the need for isolation or cleanup*.

RAMiTS:

RAMan Integrated Tunable Sensor

System Specifications

Size:
8.0 x 9.0 x 21.0 in

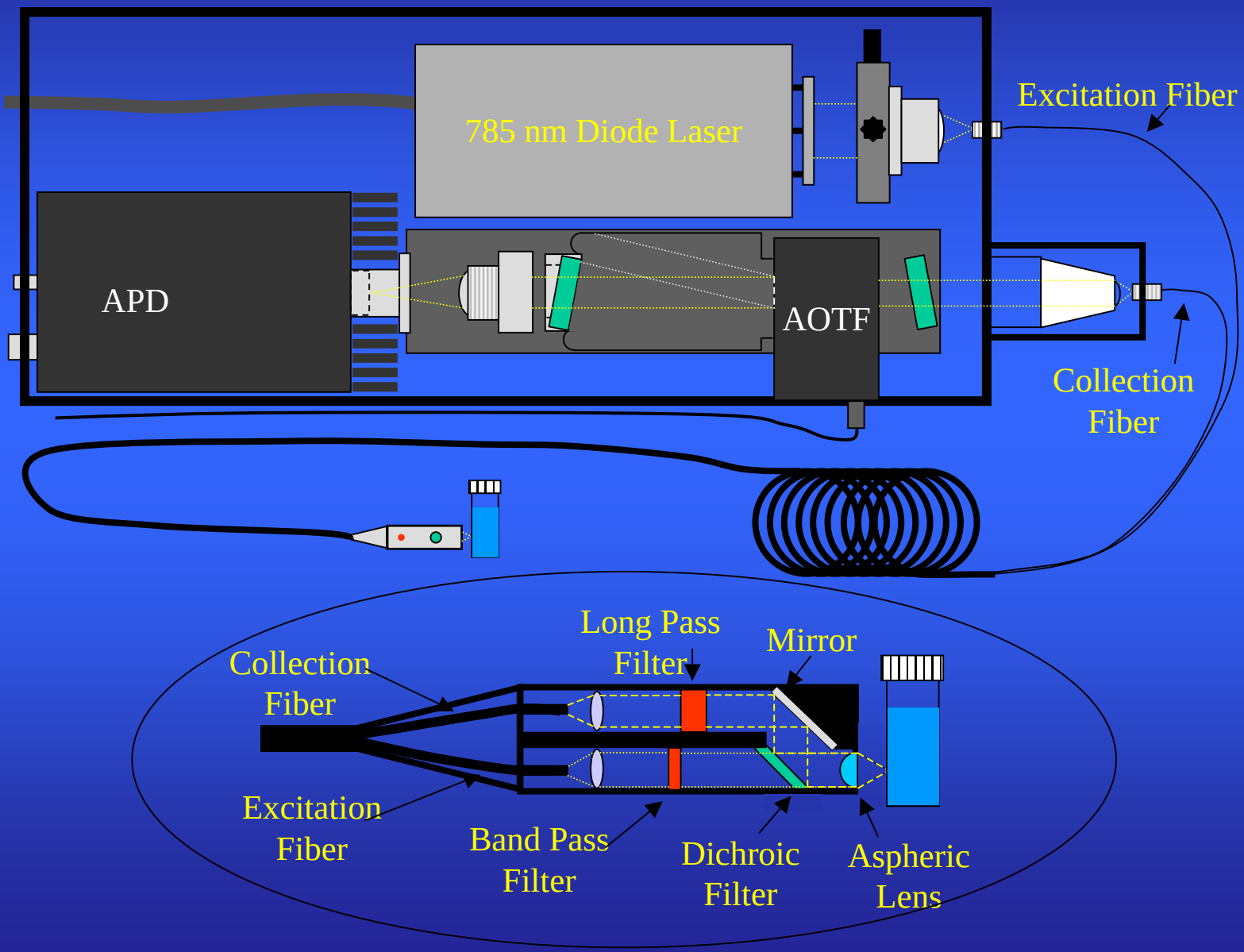
Weight:
~ 39 lbs

Resolution:
~ 7.5 cm^{-1}

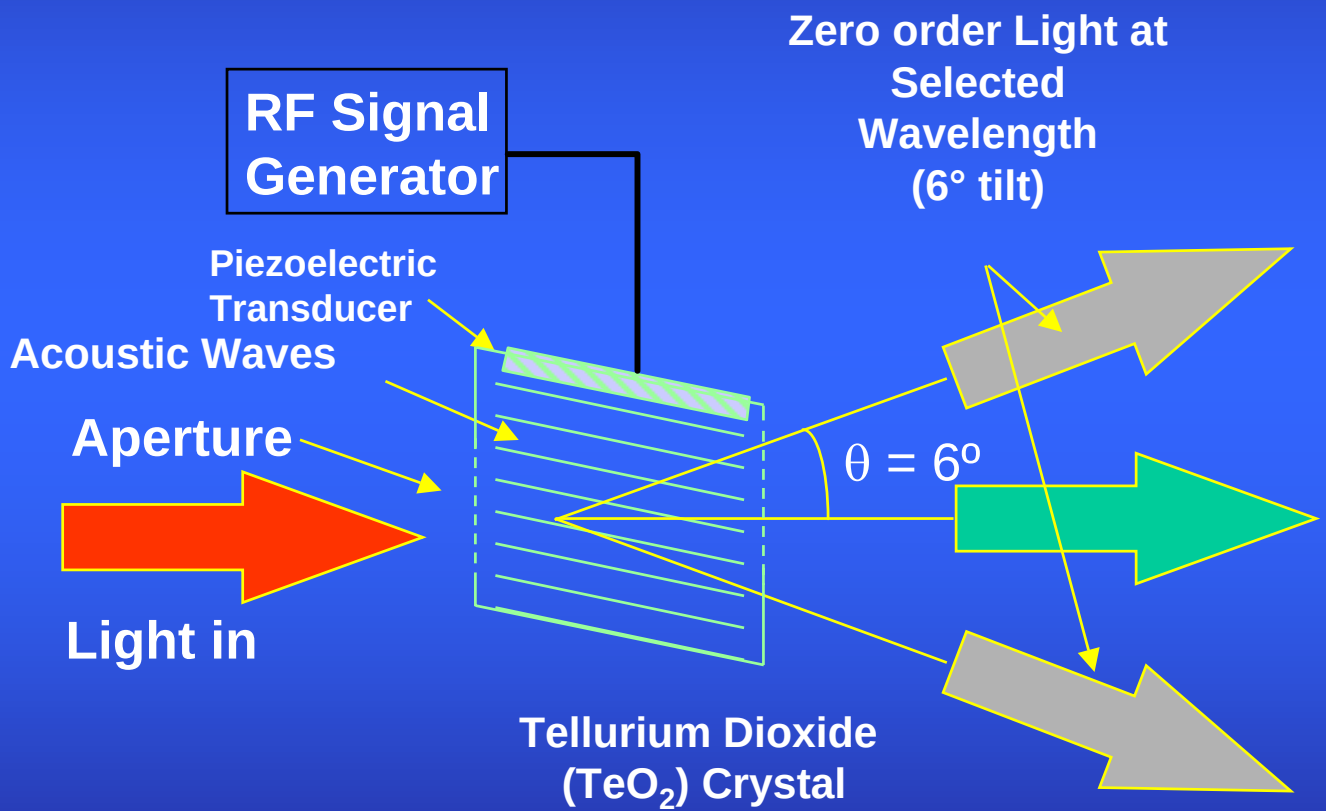
Battery Lifetime:
3 hours

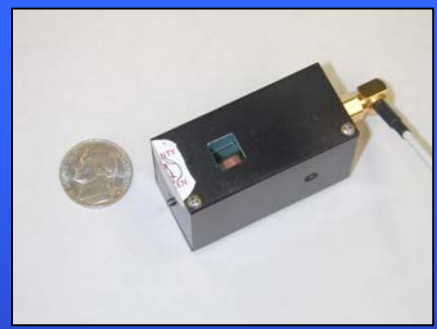
User Interface:
Touchscreen

Optical System

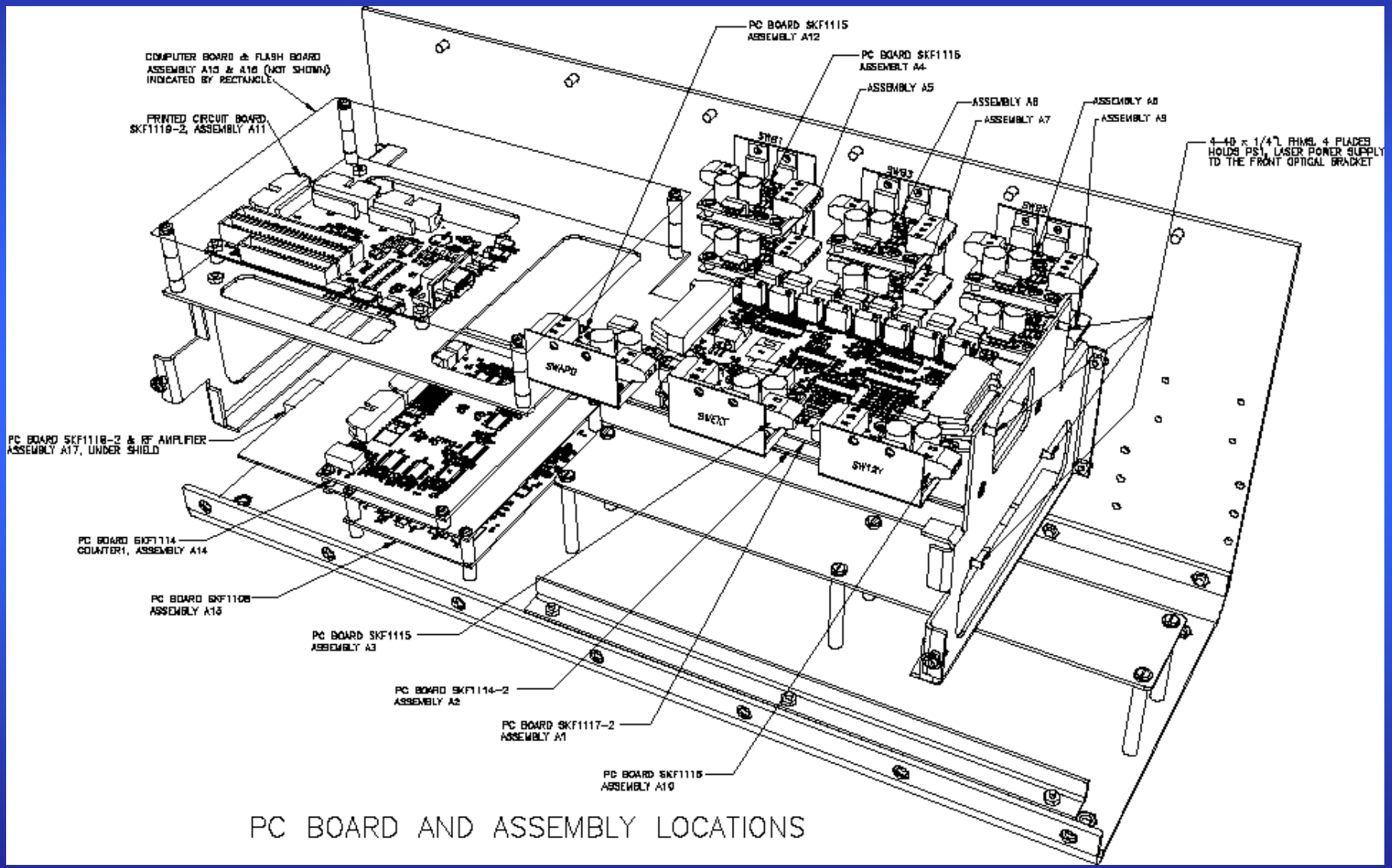


AOTF Operation



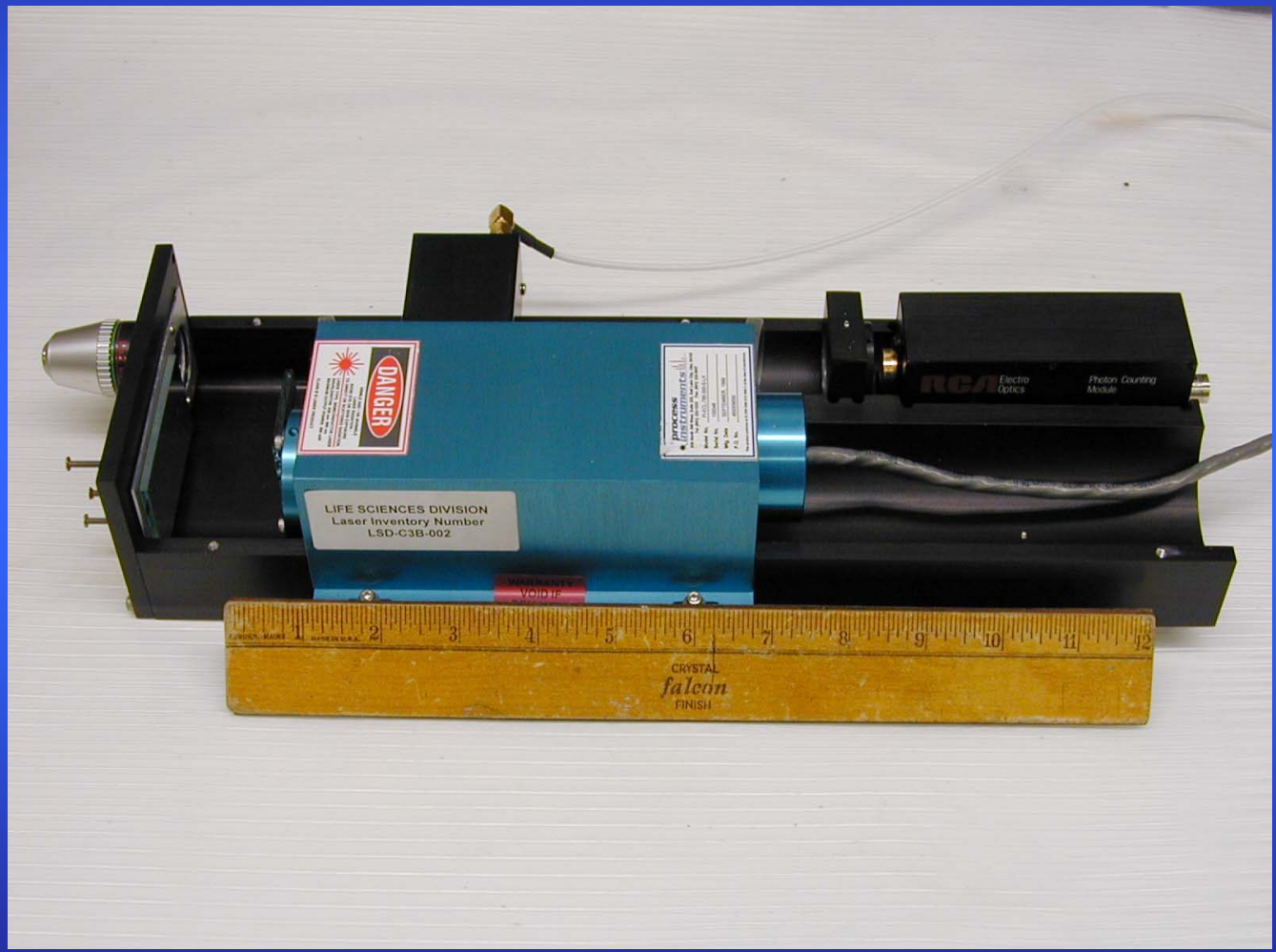


Electrocentric View

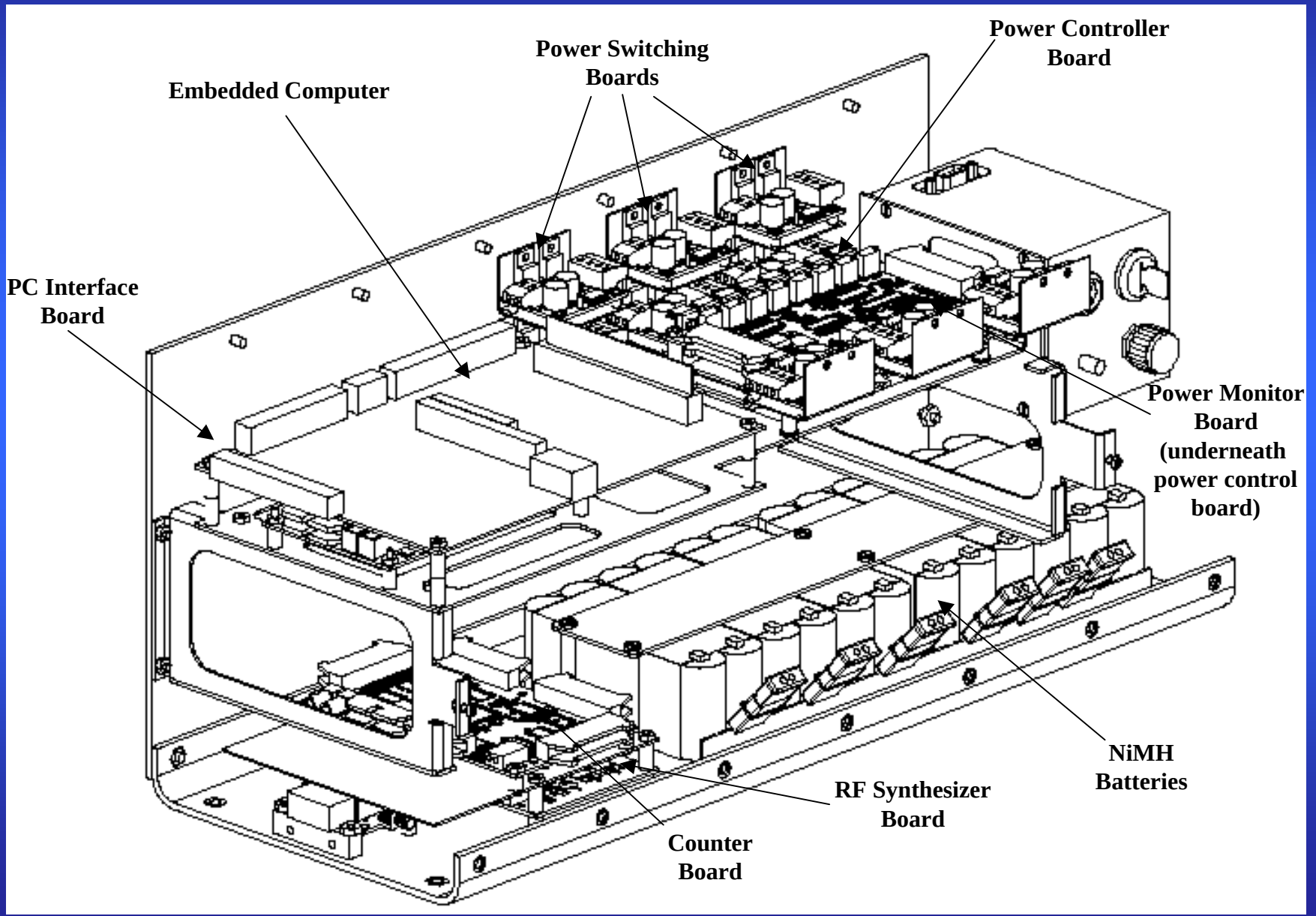


PC BOARD AND ASSEMBLY LOCATIONS

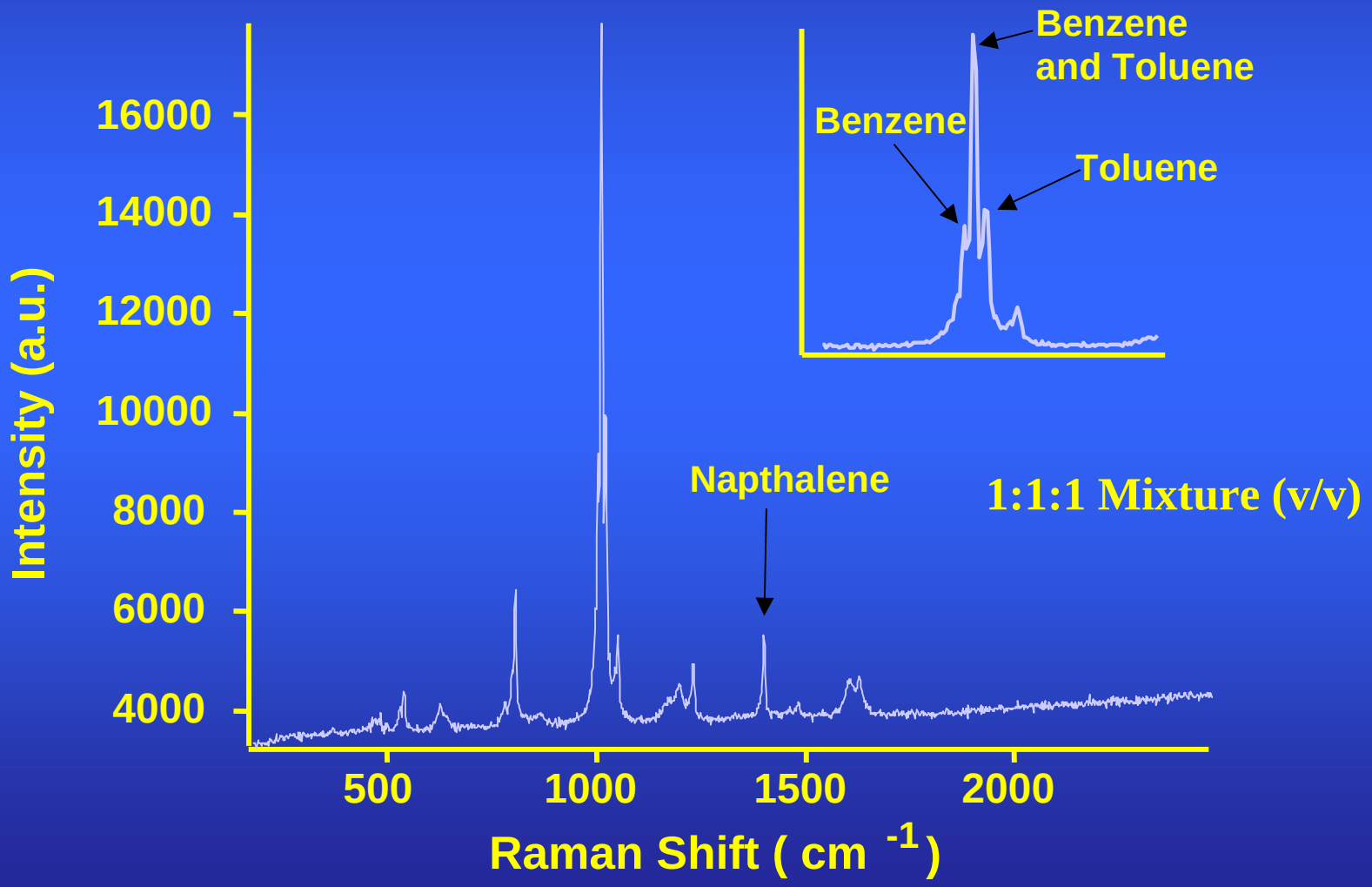
Image of the Optical Housing



Power Control Circuitry

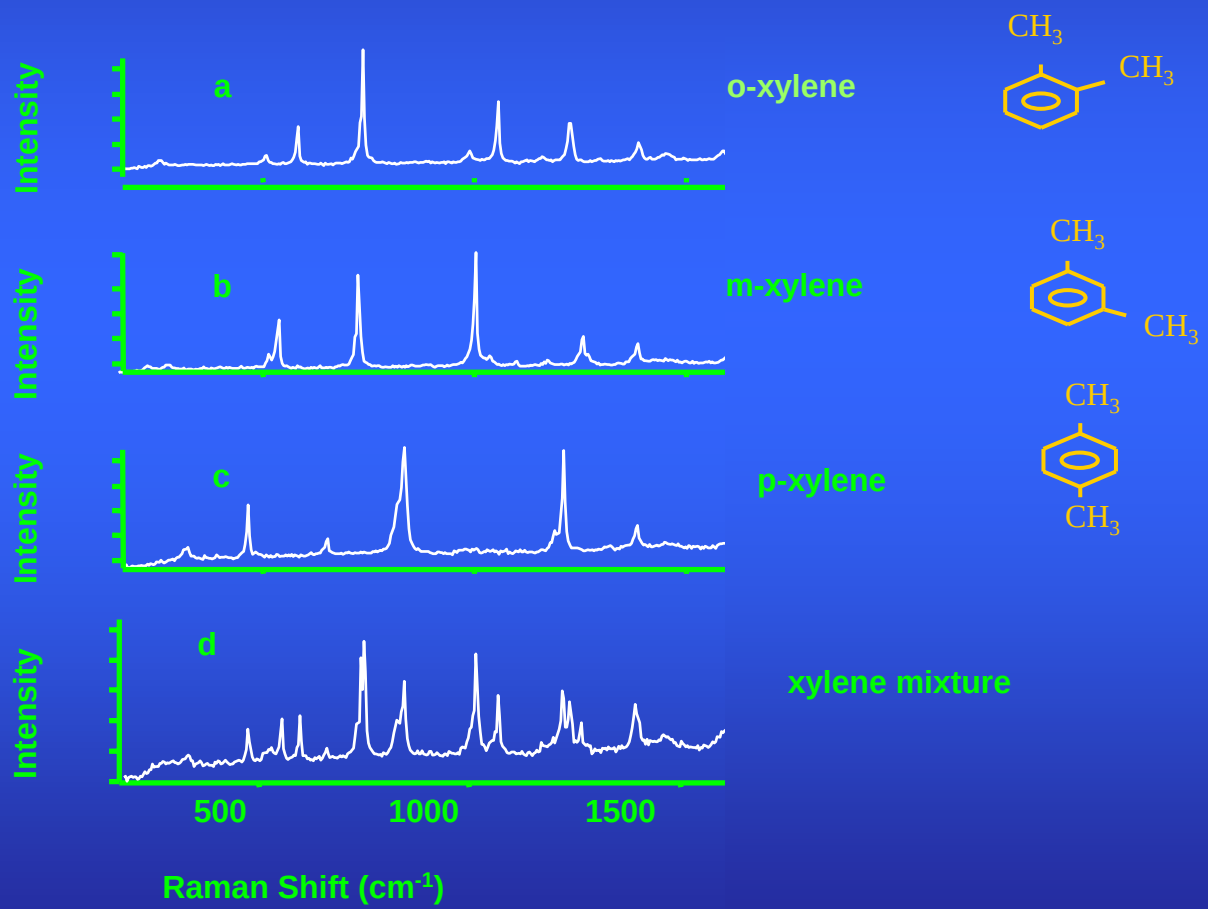


Raman Spectrum of Benzene, Toluene and Napthalene Mixture



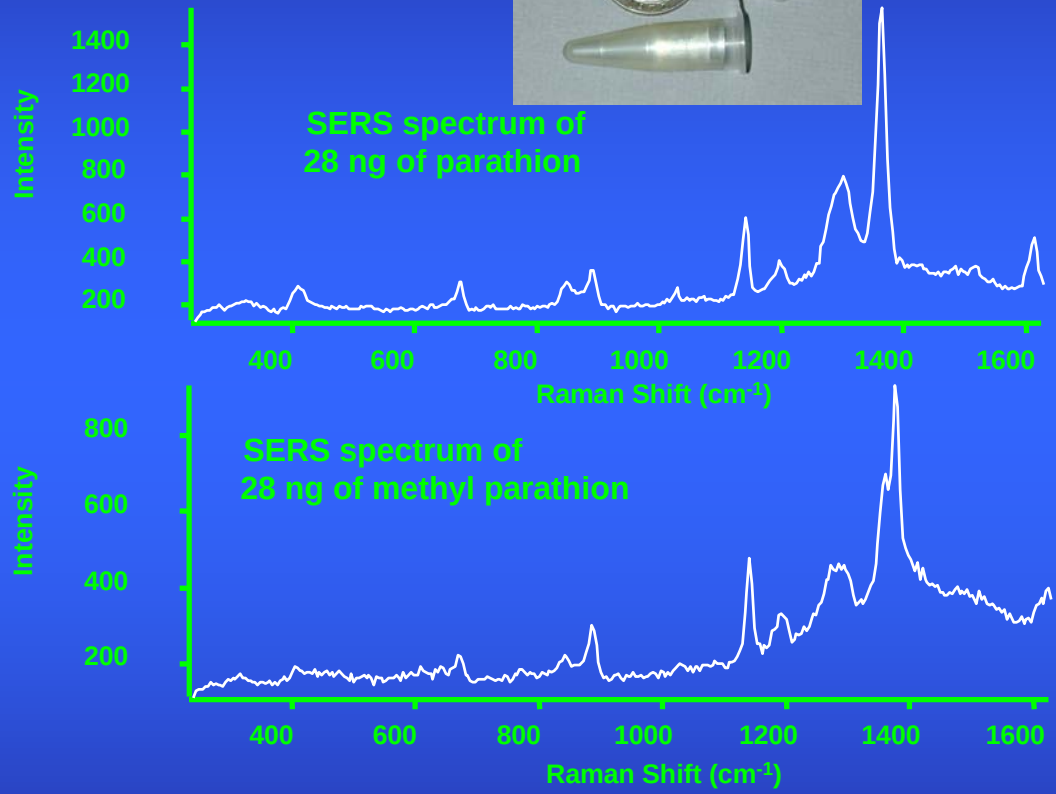
RAMiTS for the differentiation of isomers

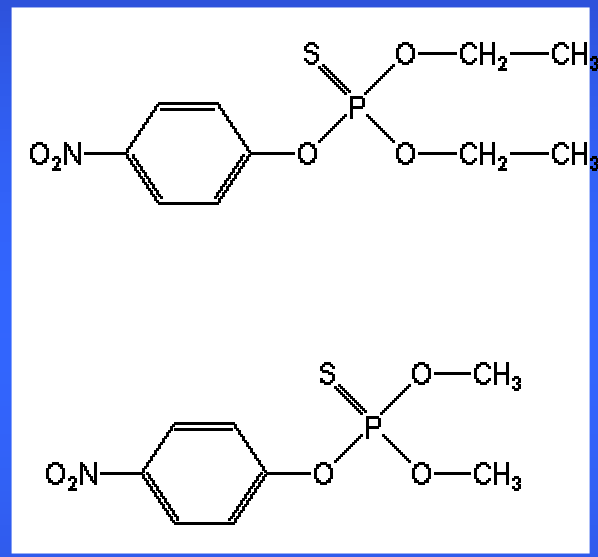
- Capable of rapid analyses, total acquisition for each of the spectra shown below is approximately 3 seconds or 16 ms per point.
- High resolution Raman spectra allow for the analysis of complex mixtures.



RAMiTS for the detection of pesticides

SERS vial





- Capable of trace detection of **chemical pesticides** using specially designed surface-enhanced Raman (SERS) snap-top vials.

RAMiTS
RAMan Integrated Tunable Sensor

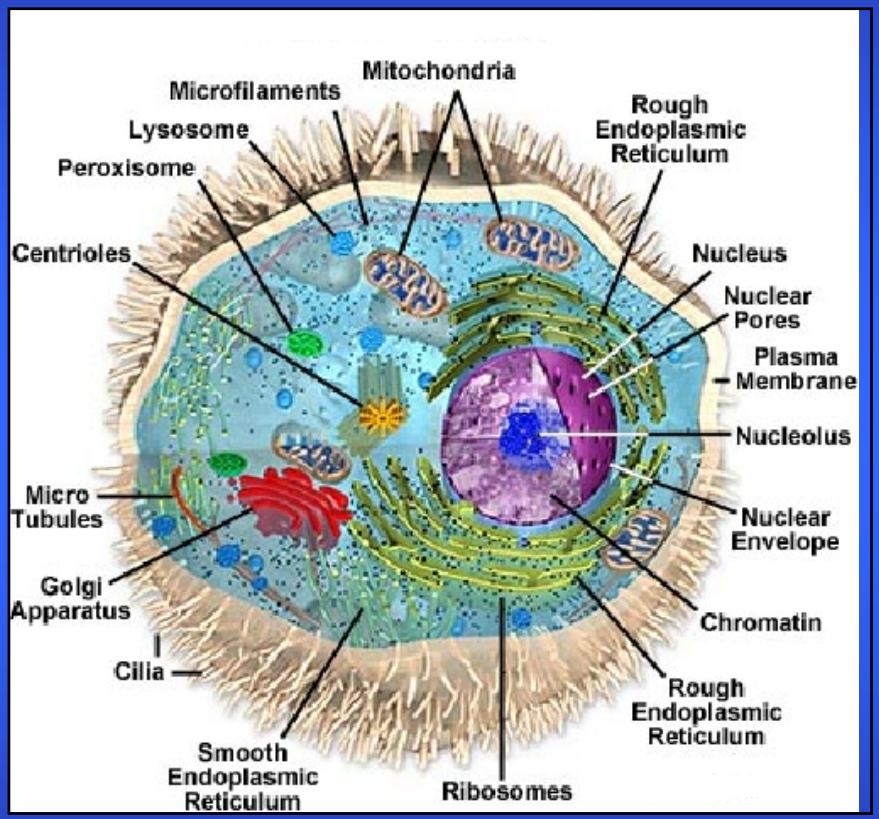
- Capable of performing analyses in seconds.
- Automated chemical identification (spectral library).
- Suitable for remote and non-invasive analyses.
- Capable of operating under typical room light conditions with no significant background signal
- Waterproof, complying with NEMA-4 standards

FAA and dozens of companies are actively pursuing license agreements for the use of or commercializing this device

Optical Nanobiosensors

An Enabling Technology for Single Cell Analysis

Cell –The Basic Unit of Life

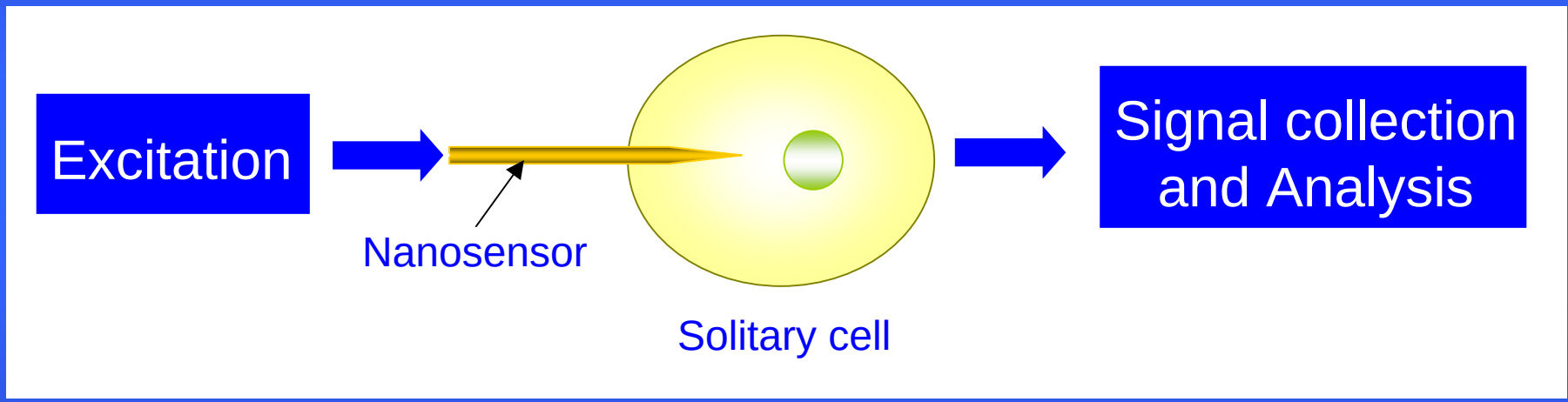


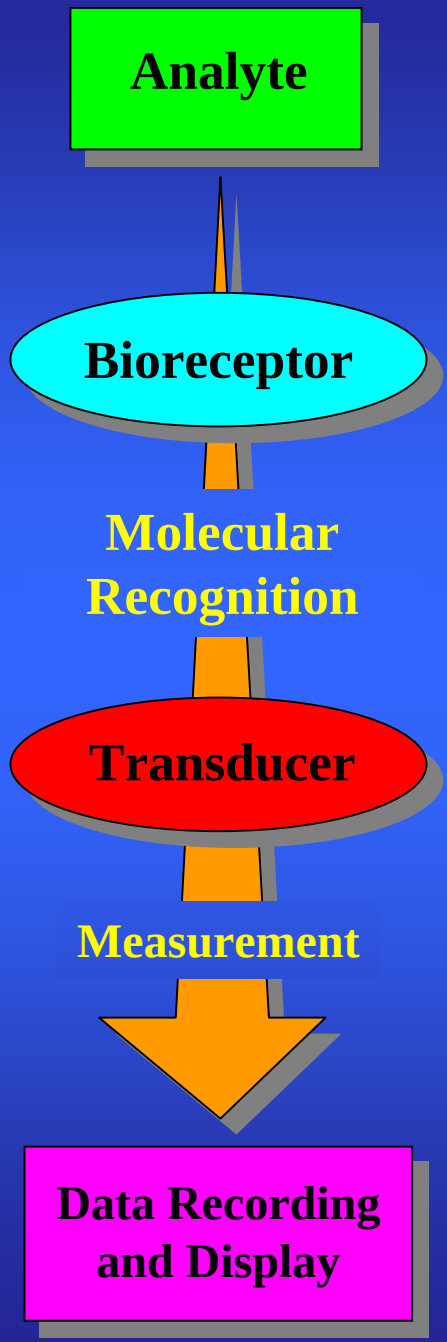
- Two ways to perform Single Cell Analysis:
  - (i) Extraction of its component parts from a whole cell, followed by separation and MS analysis
  - (ii) Examination of a whole cell its elements and their relationship

- ABST-ORNL: developed OPTICAL NANOSENSORS for single living cell analysis

• Source: http://www.fsu.edu/cells/animalcell.html

Concept of optical nanosensor for single cell analysis

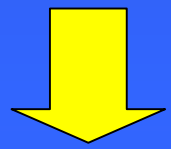



Analyte
Bioreceptor
Molecular Recognition
Transducer
Measurement
Data Recording and Display

Steps in nanosensor fabrication

Fabrication of Nanotips (600 μm → 50 nm)

Silver Coating (~ 100-200 nm)



Silanization of Fiber nanotips (GOPS)

Glycidoxypropyltrimethoxysilane

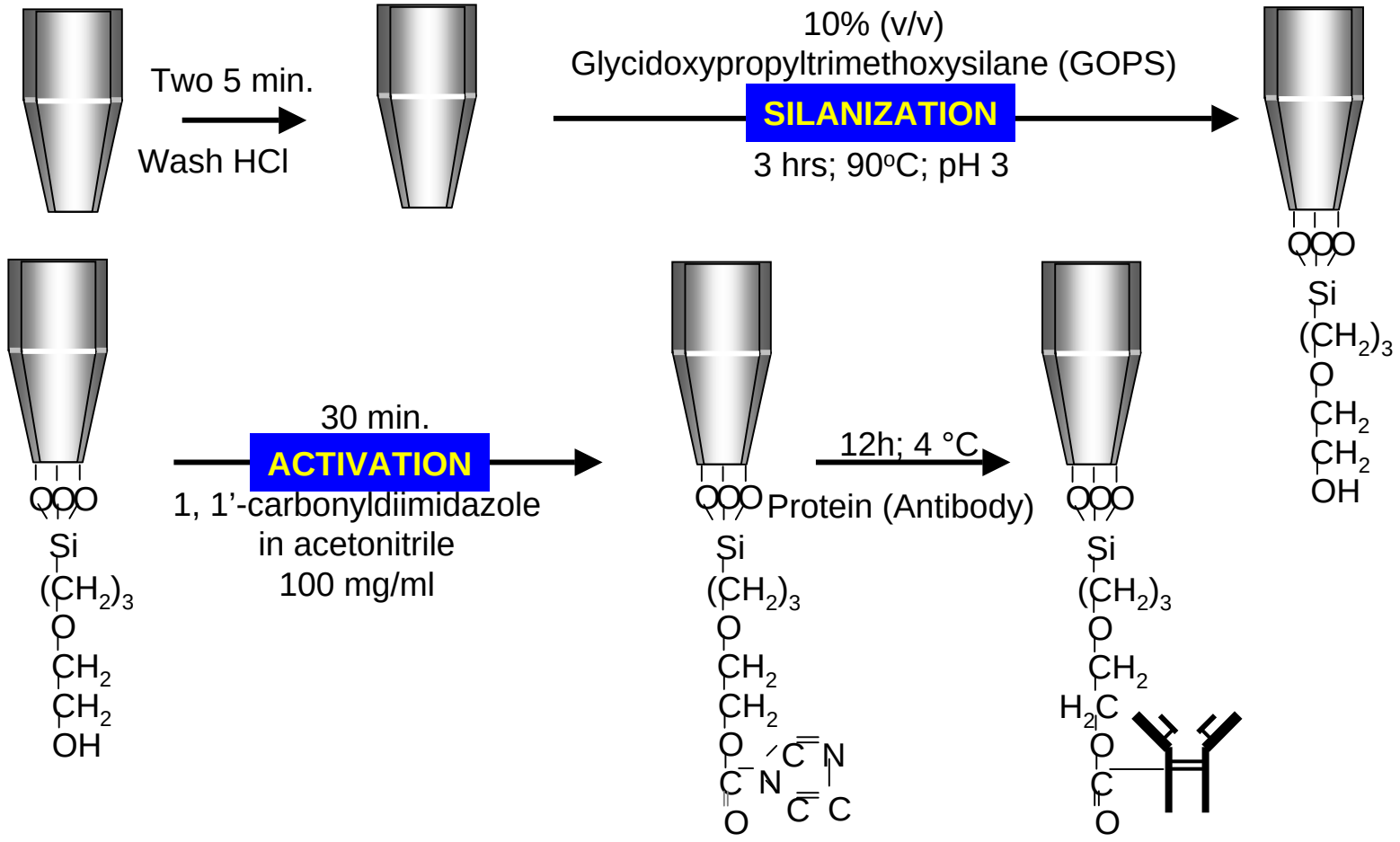
Activation of Fiber nanotips (CDI)

Carbonyldiimidazole

Protein Binding (e.g., mouse IgG for BaP)

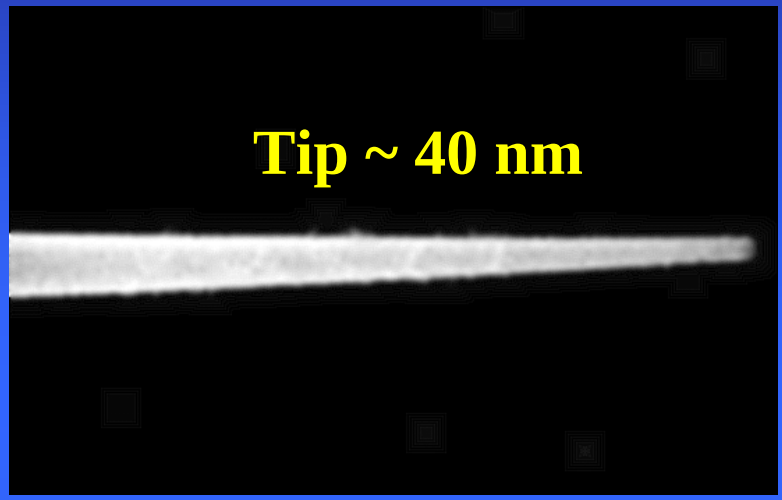
Benzo[a]pyrene

Protein Binding Chemistry



- Functionalize tips to facilitate protein immobilization

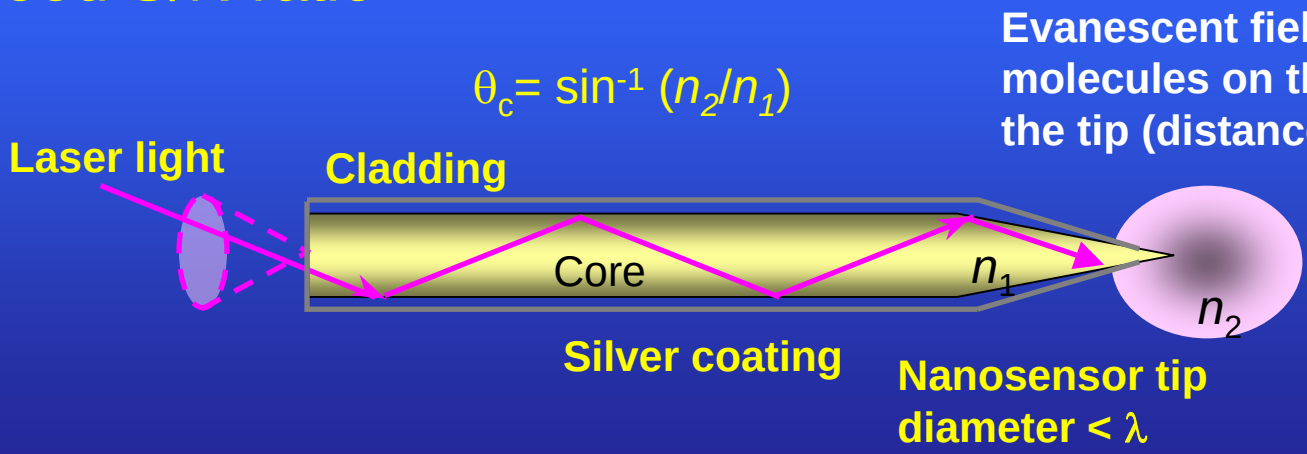
SEM Image of a Pulled Fiber



How the Optical Nanosensor Works?

- Total internal reflection (TIR) is used to generate an evanescent field in the near field of the optical nanosensor that is used to excite the analyte of interest bound onto the optical nanosensor

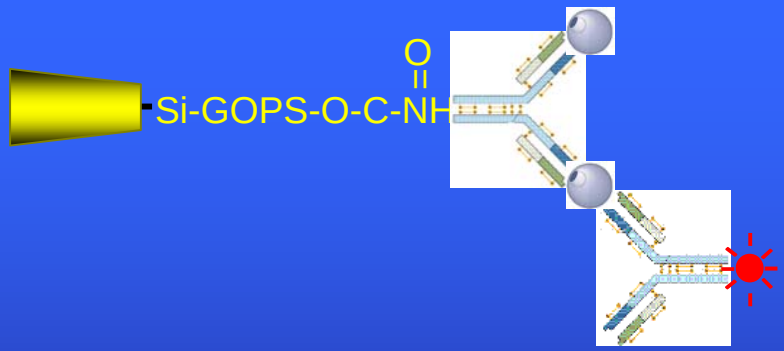
- The evanescent field provides a highly localized excitation area in the near field of the optical nanosensor, therefore good S/N ratio



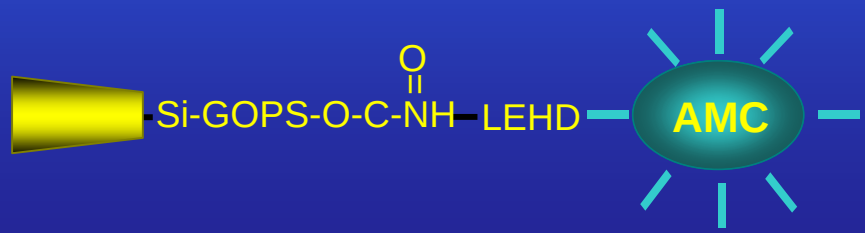
Various Assay Formats for Single Cell Analysis Using Optical Nanosensors

(I) Direct Antibody Binding: Chemical Analysis

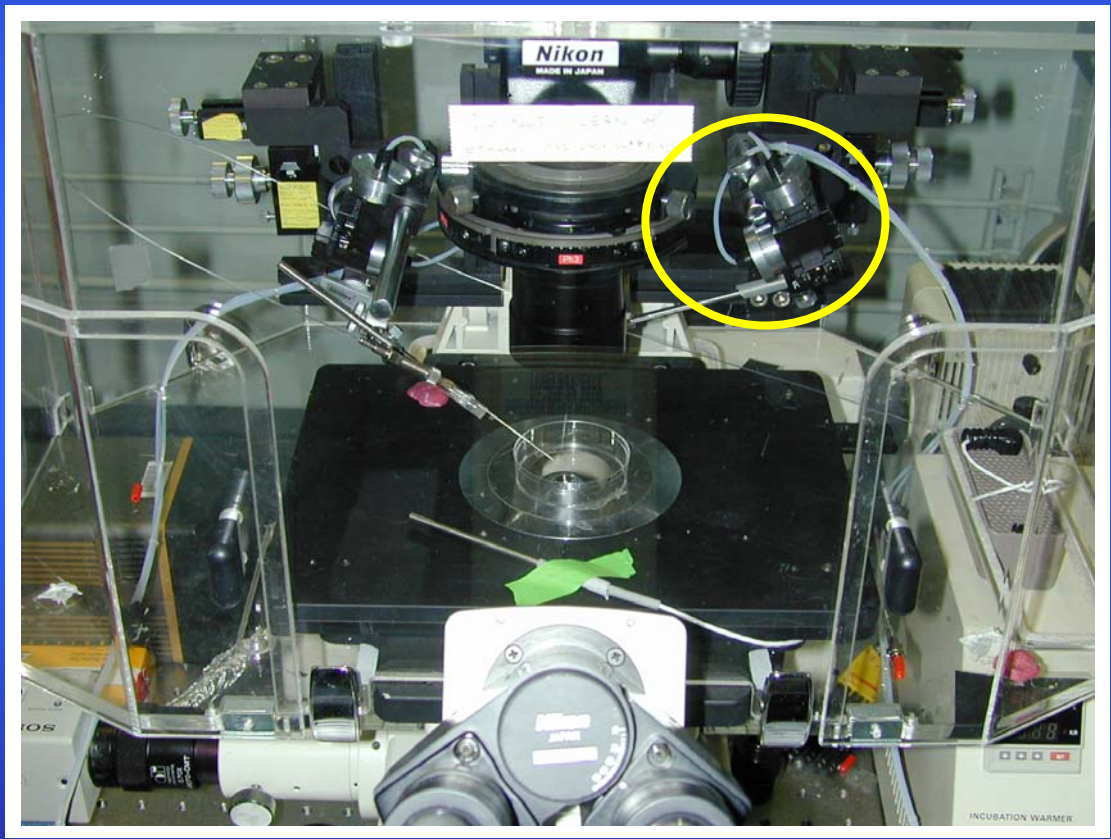
(II) Indirect Antibody Binding: Protein Analysis



(III) Peptide Substrate: Protein Analysis



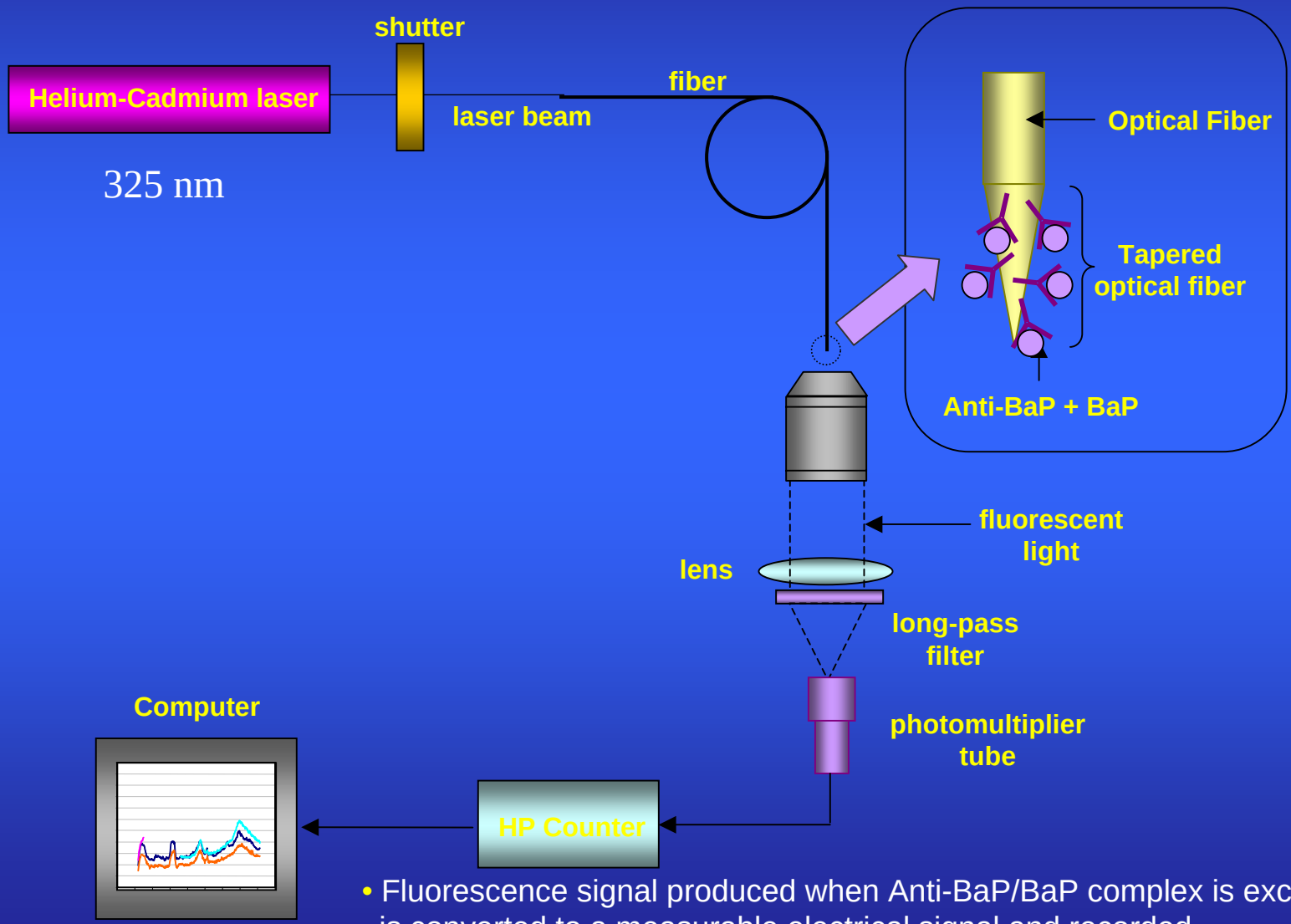
Intra-cell Workstation: Nikon Inverted Fluorescence Microscope



NIKON inverted fluorescence microscope

MMO-203 nanosensor holder 3-axis fine movement micromanipulator

Optical Nanosensor
In vivo measurement of Benzo[a]Pyrene



- Fluorescence signal produced when Anti-BaP/BaP complex is excited is converted to a measurable electrical signal and recorded

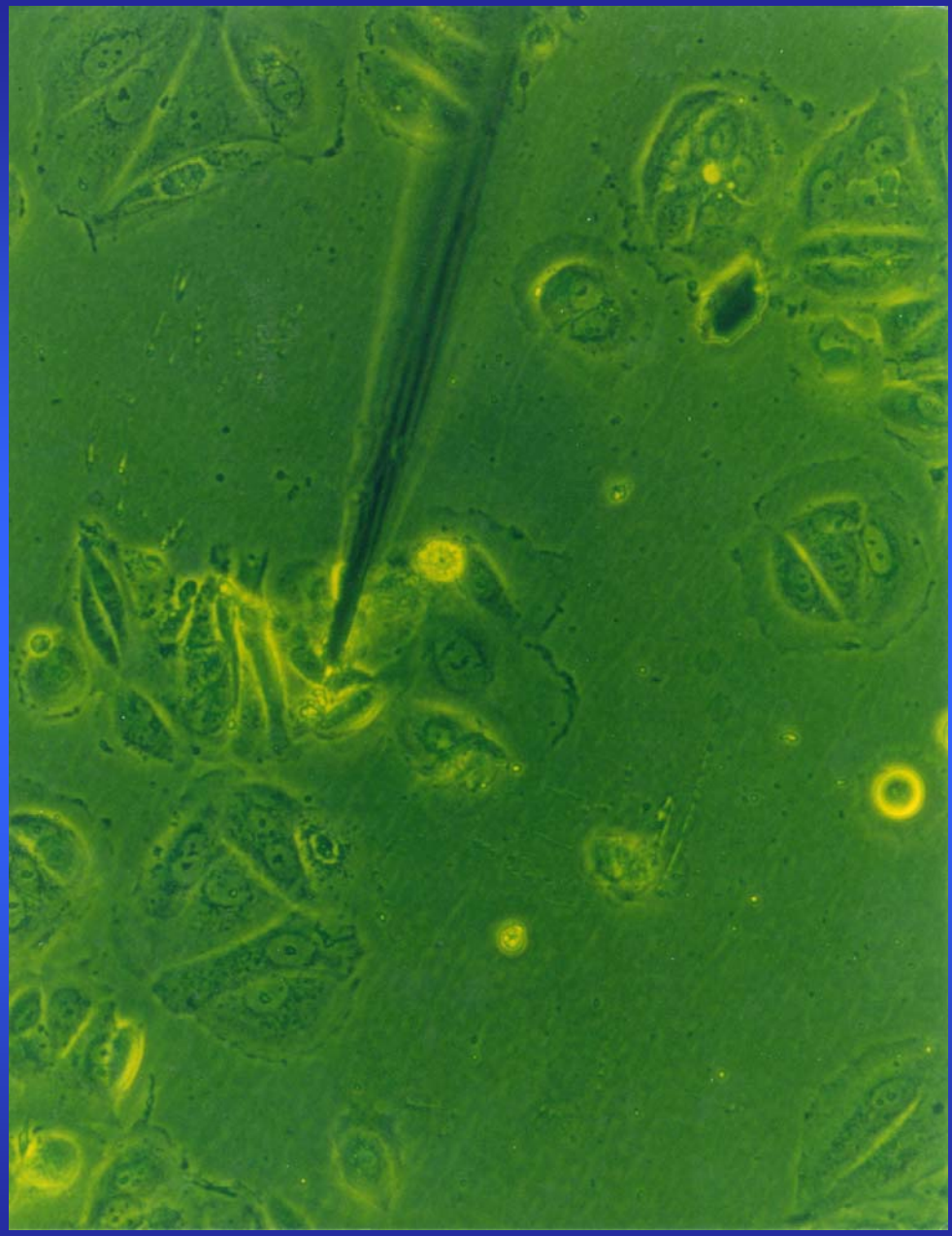
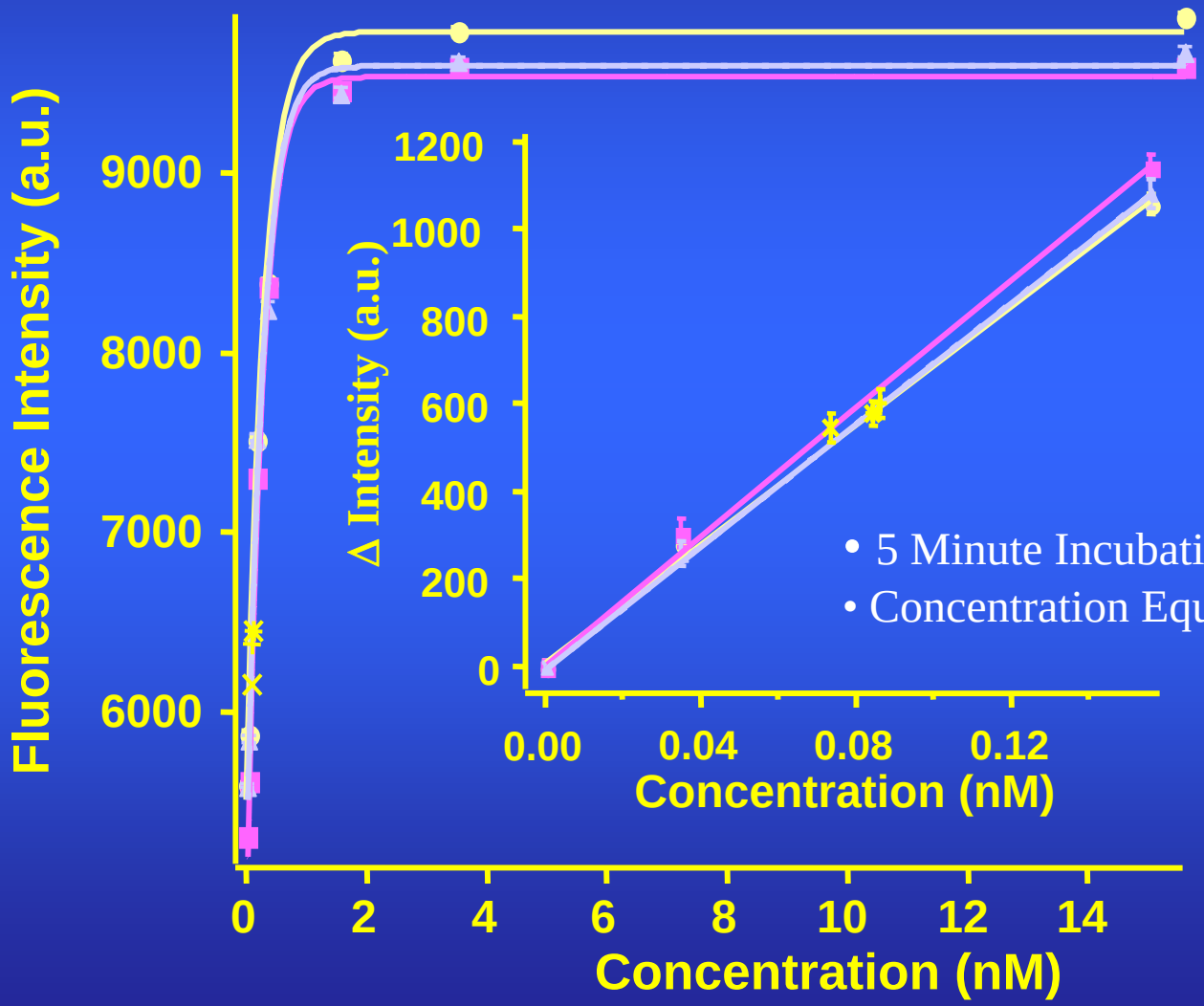


Fig 6

BaP Measurements of Human Mammary Carcinoma Cells

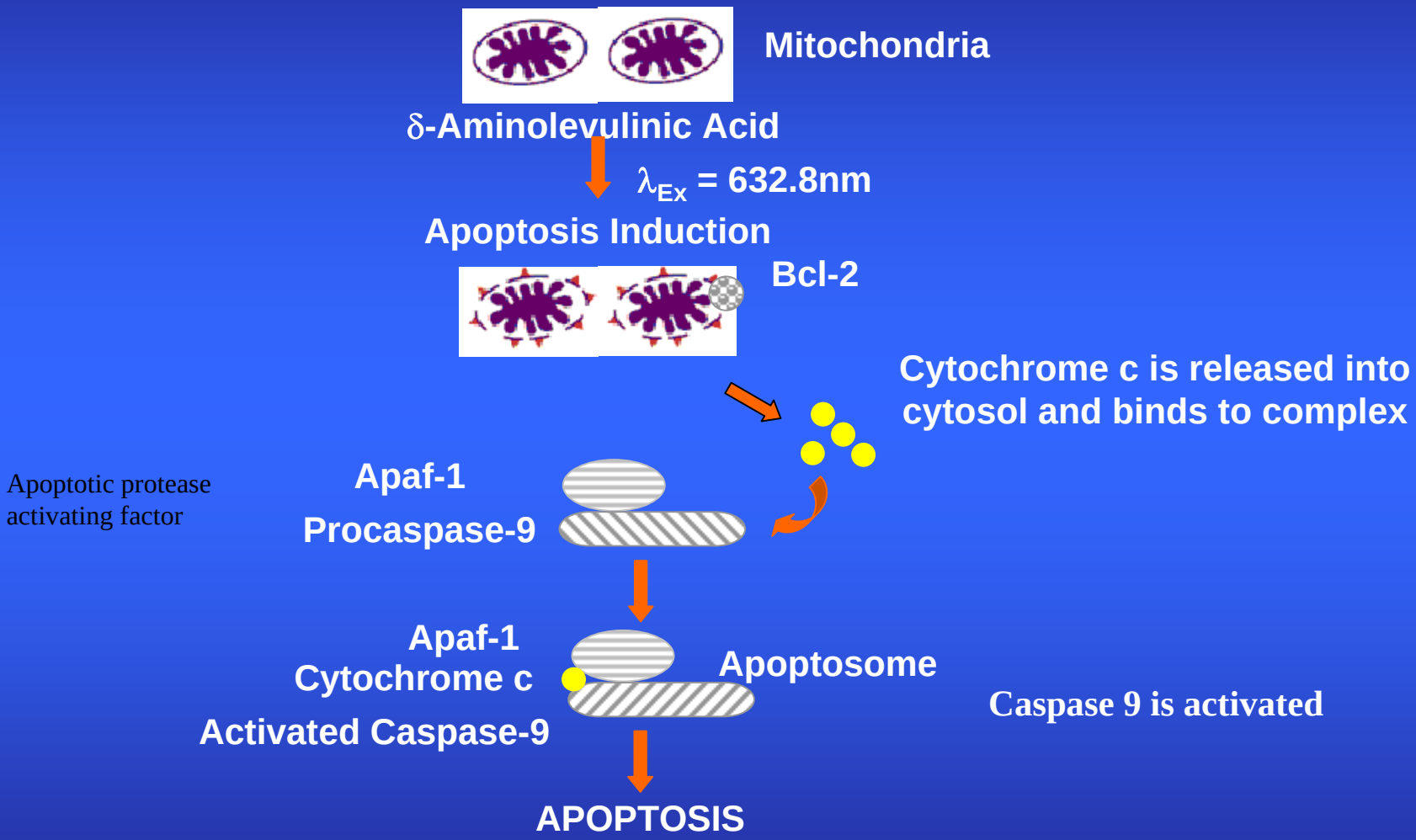


- 5 Minute Incubation
- Concentration Equivalent 81 ± 7 pM

Single Cell Analysis: Enzyme Peptide Substrate-based Nanosensor for Monitoring Apoptosis

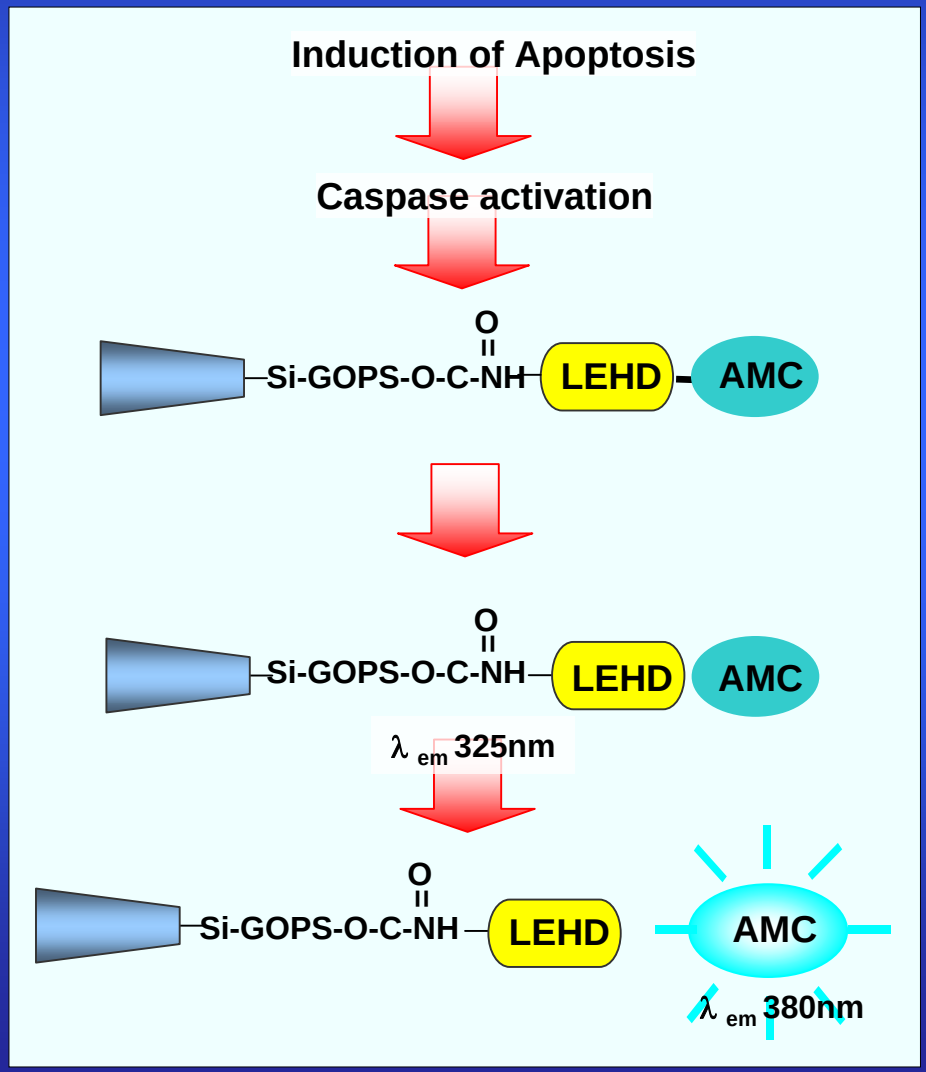
- Apoptosis or programmed cell death is induced in human mammary carcinoma (MCF-7) cells
- Aminolevulinic Acid (ALA), a photodynamic therapy drug is used to induce apoptosis
- Once apoptosis is induced, the near-field of optical nanosensor is used to probe the cytoplasm of individual human mammary carcinoma cells for caspase-9 activity

Aminolevulinic Acid-induced Apoptosis

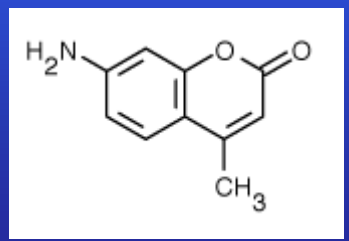


ALA photoactivation process in a MCF-7 cell, showing the activation of caspase-9 and ultimately leading to apoptosis.

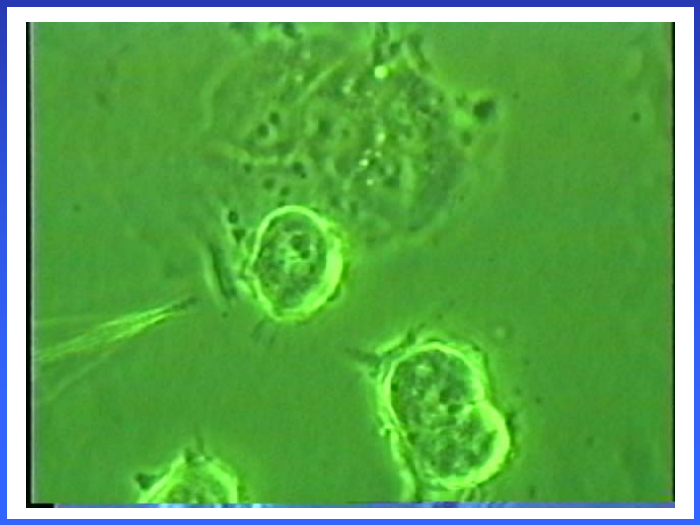
Application: Enzyme Substrate-based Optical Nanosensors for Caspase-9 Activity

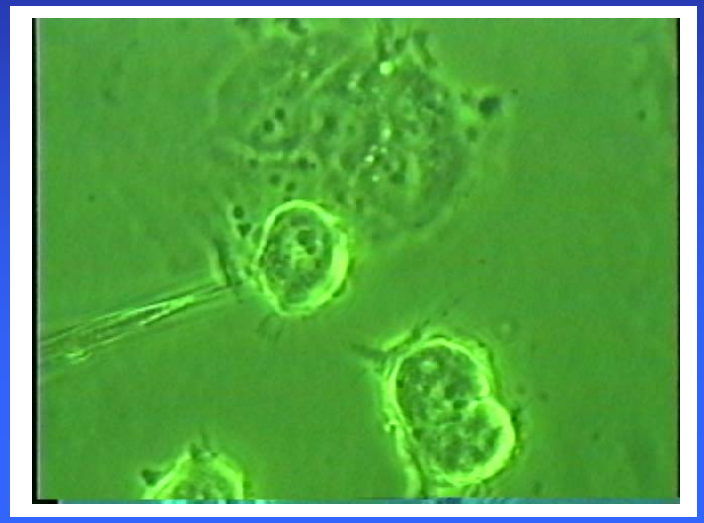


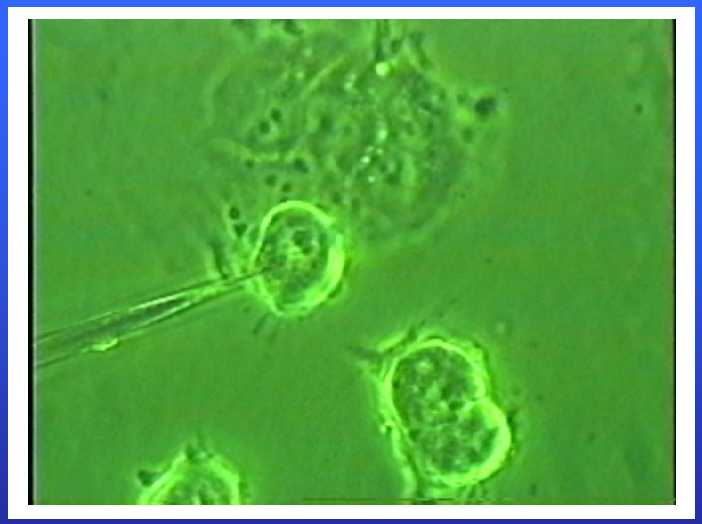
- Monitoring enzyme activity requires the use of specific fluorescent substrates such as:
  - Leu-Glu-His-Asp-7-amino-4-methyl-coumarin (LEHD-AMC) that is specific of Caspase- 9
  - LEHD-AMC is not fluorescent but the enzyme activity liberates the fluorescent compound, AMC (λ_{ex} 341nm, λ_{em}441nm)

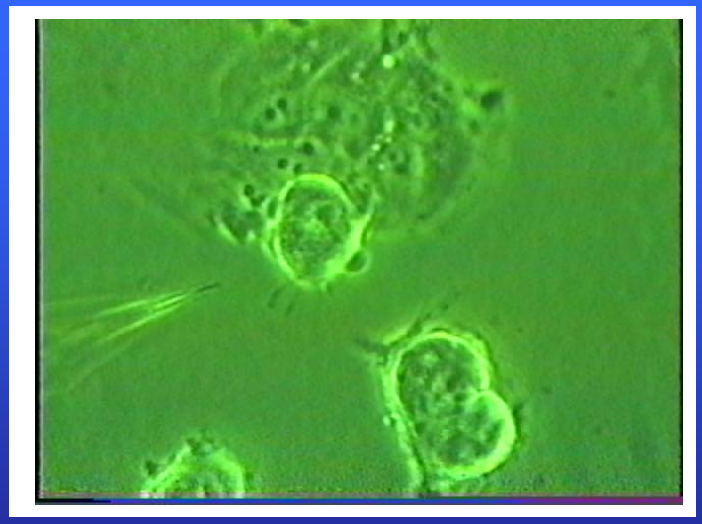


Intracellular Measurement



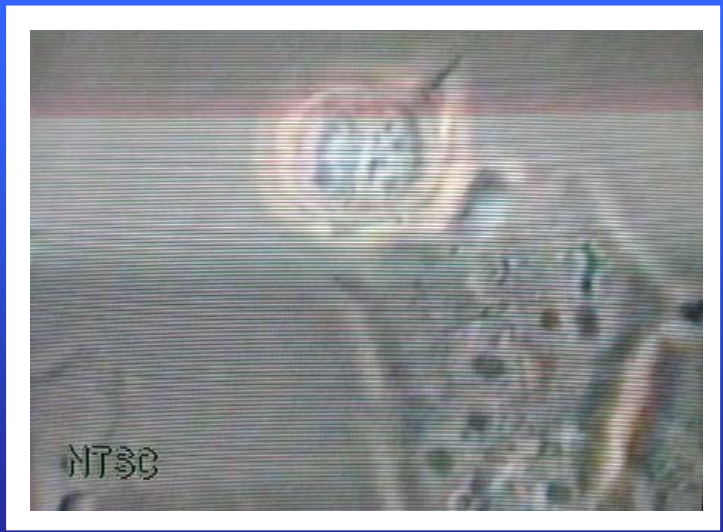


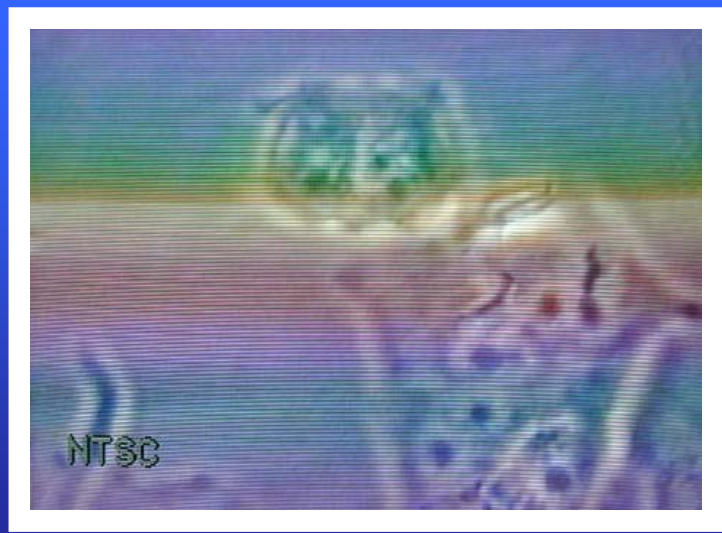




LEFT TO RIGHT, single cell analysis, optical nanosensor outside MCF-7 cell (ii) right before insertion into MCF-7 cell (iii) inserted into MCF-7 cell and (iv) extracted from MCF-7 cell

Mitosis





After probing the cytoplasm of the cell with a nanoprobe…

Mitosis

The cell carried out normal cellular activities, specifically mitosis

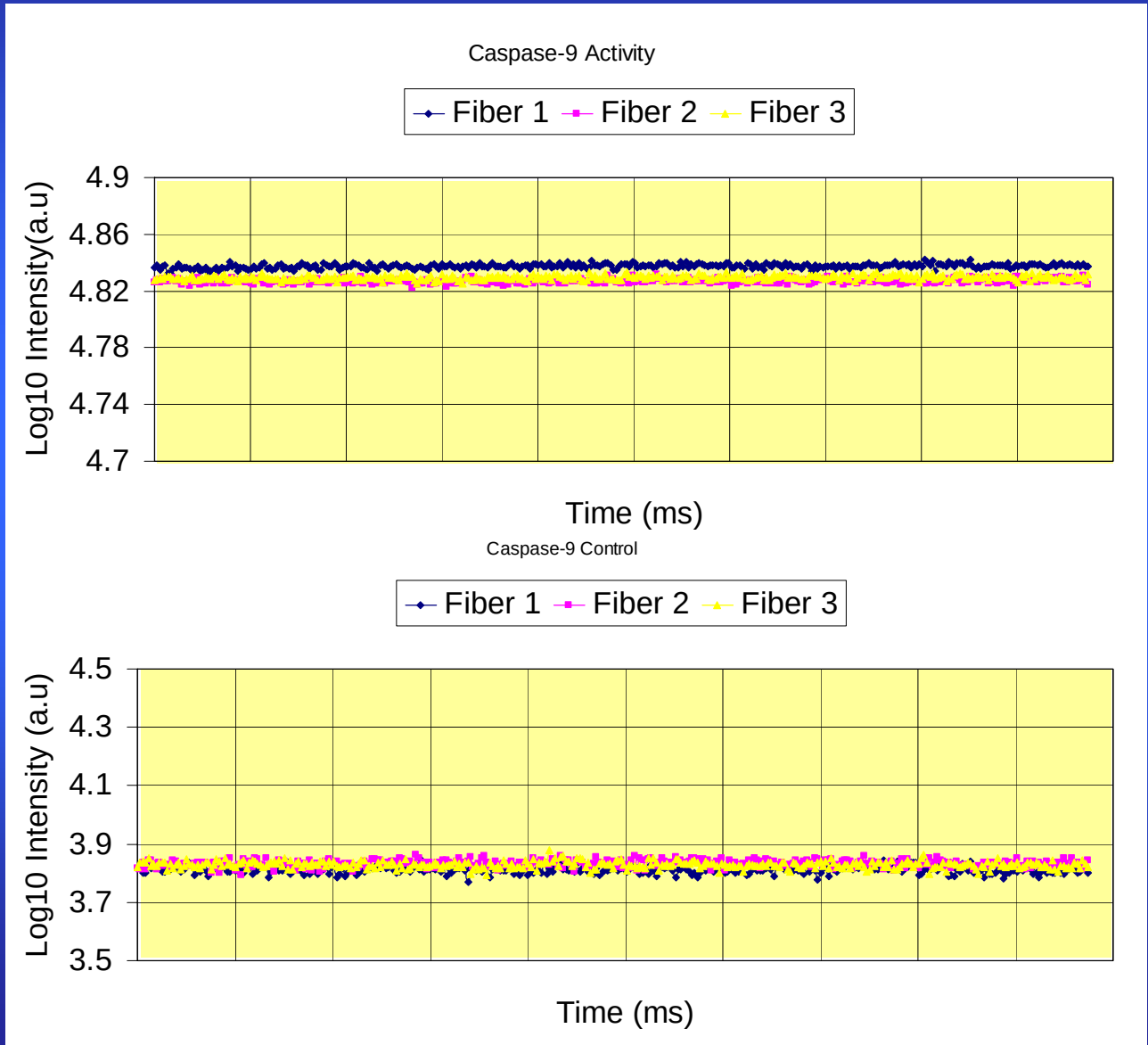
In vivo measurement of apoptosis using tetrapeptide fluorogenic substrate

- 2 groups:
  - Control: Functionalized optical nanosensors with peptide substrate attached are incubated in cells in which apoptosis has not been induced
  - Experimental: Functionalized optical nanosensors are incubated in cells undergoing apoptosis

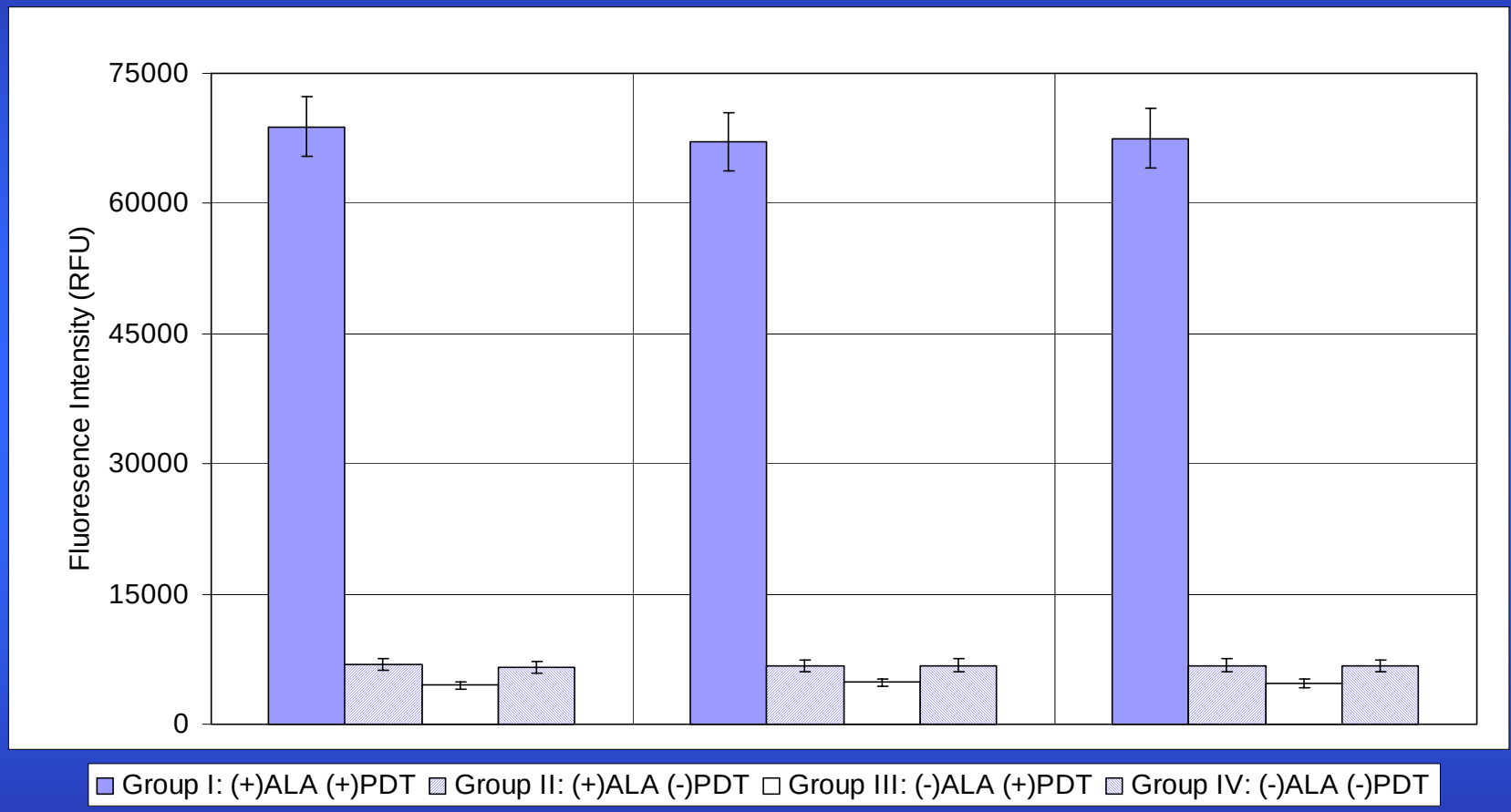
- ALA was used to induce apoptosis in MCF-7 cells
- Cells were placed on the stage of the microscope and intracellular measurements were made

Measurement of Caspase-9 activity

[Detection of 7-amino-4-methoxy coumarin (AMC)]



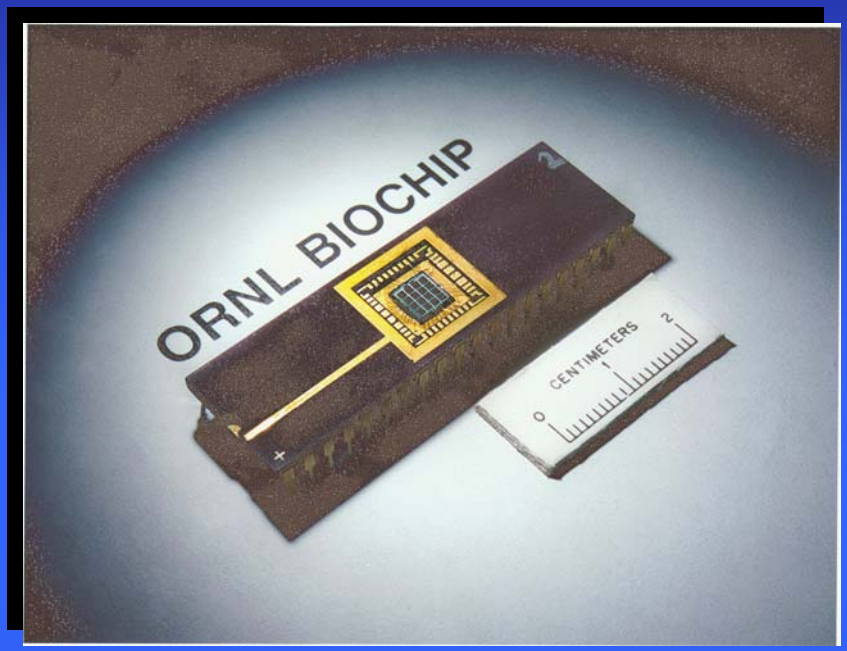
In vivo measurement of Caspase-9 activity



For the first time, we identified the onset of the mitochondrial pathway of apoptosis in a single live cell by detecting the enzymatic activity of caspase-9.

The Multifunctional Biochip

The Biochip Technology

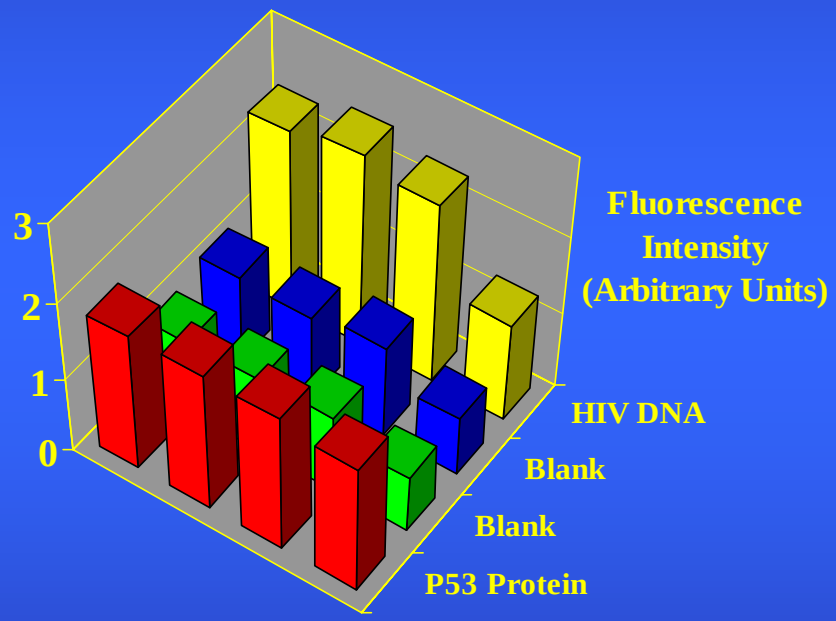


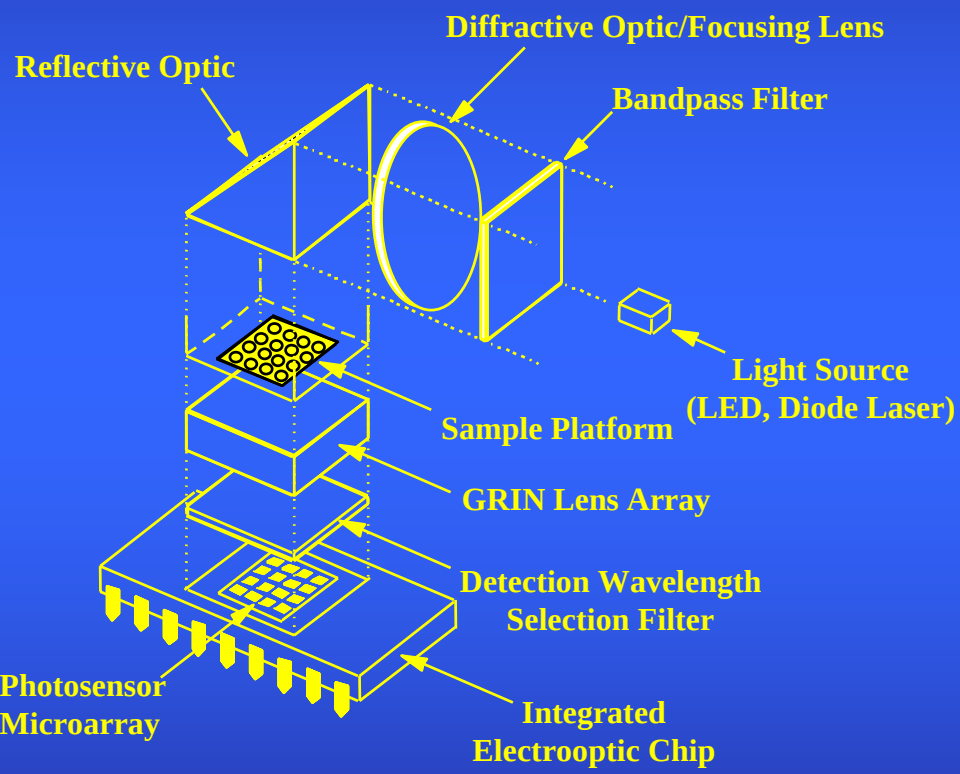
- 2-D array of independently operating photodiodes
- On-board signal amplification and data treatment
- Complementary metal oxide silicon (CMOS)-based microelectronics integrated onto a single platform
- Coupled to compact sampling system

ADVANTAGES

- Compact design
- Multiple assays possible on single platform
- Increased throughput
- Microscale sampling capability
- Low power consumption
- Cost effectiveness

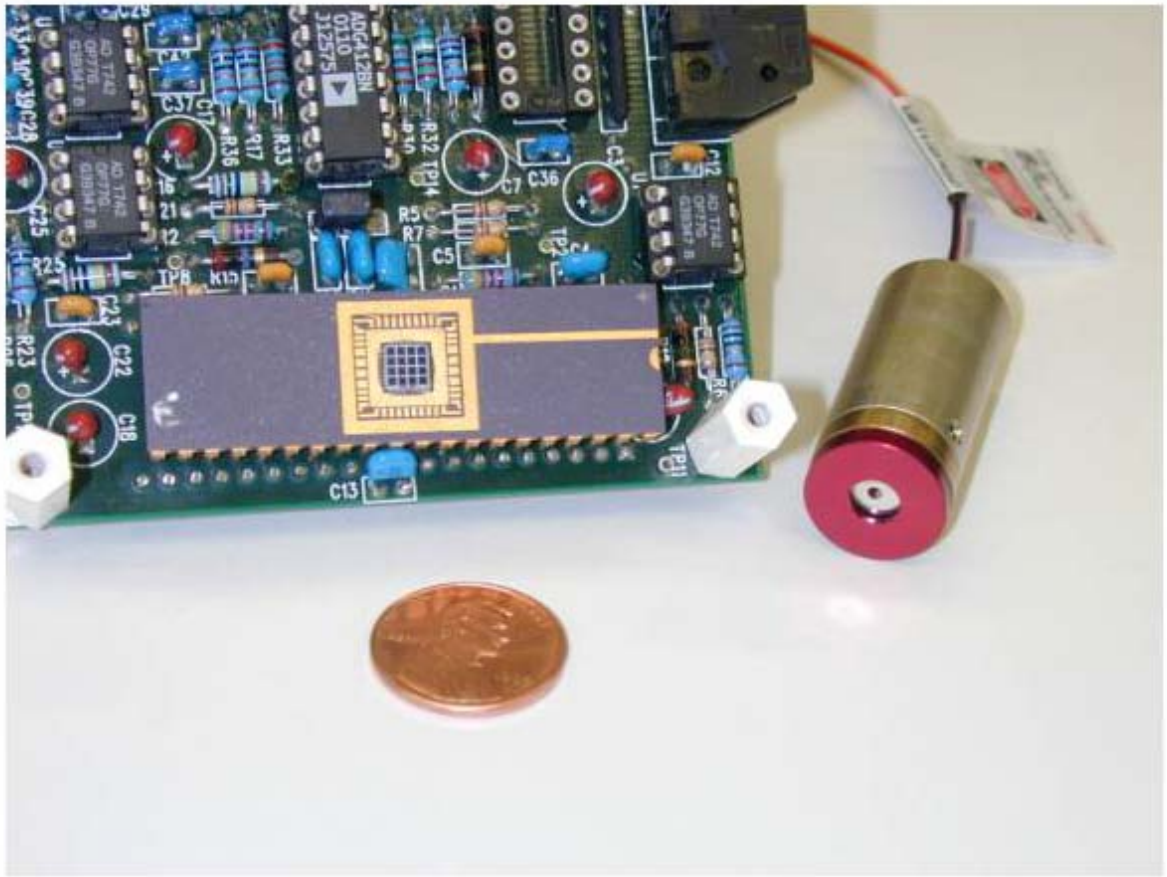
Demonstration of multifunctional capability of the integrated circuit (IC) biochip





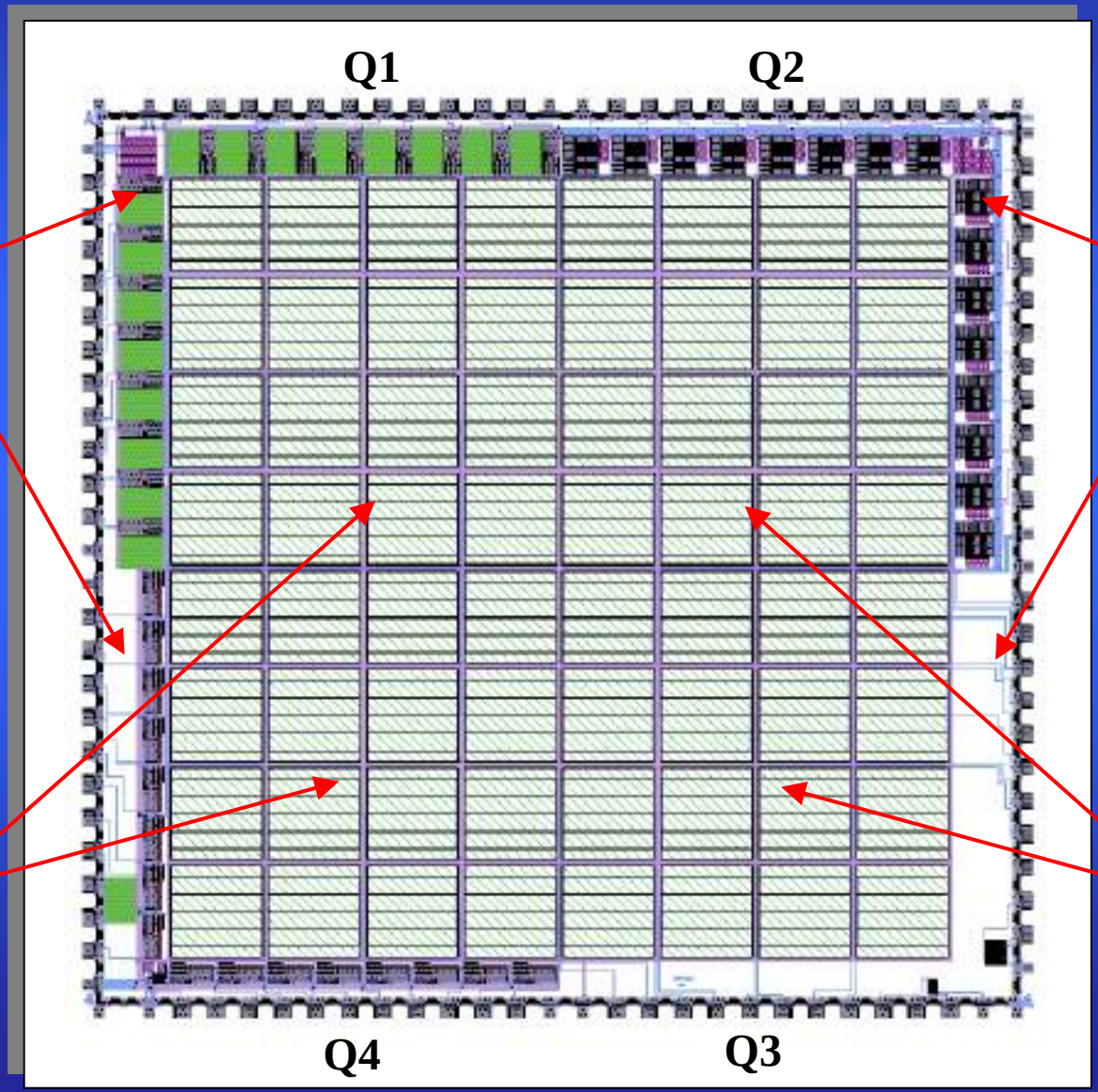
Bioreceptors: antibody, DNA, enzymes, tissues, and organelles etc.

Biochip and Miniature Diode Laser



- Biochip mounted on printed circuit board shown with miniature diode laser used for excitation
- Excitation wavelength of diode laser is 635 nm
- Diode laser power is 5 mW and uses a 5 V, 60 mA power supply

Schematic Diagram of 8 x 8 Biochip With 4 Different Designs of IC Signal Processors



Signal Processor Regions

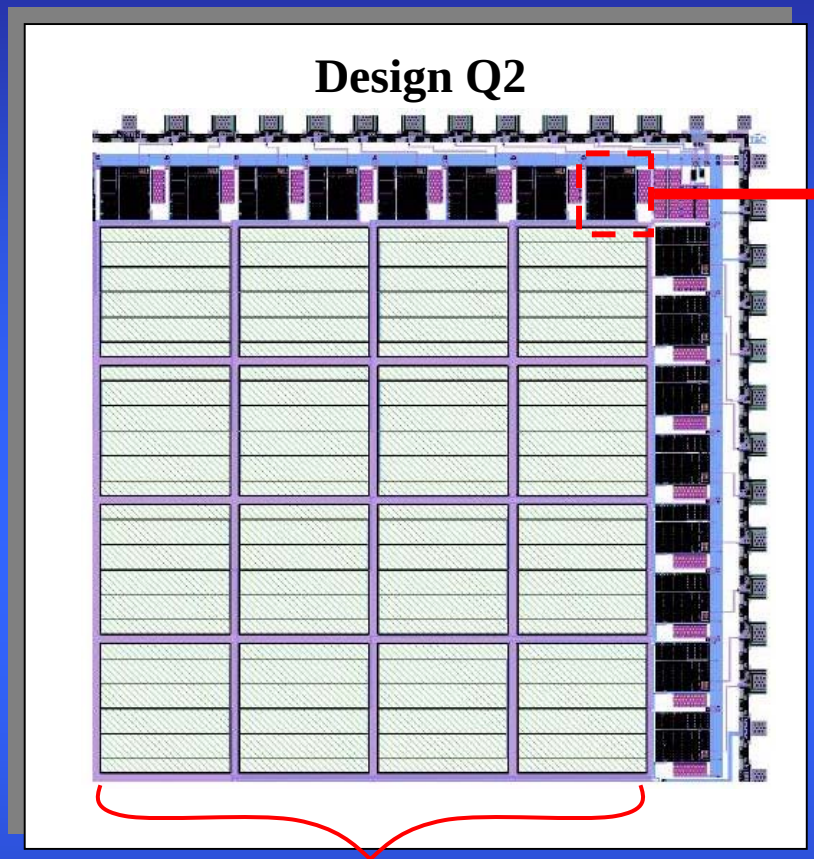
Signal Processor Regions

Individual photodiodes 900 μm^2

CMOS Photodiode Regions

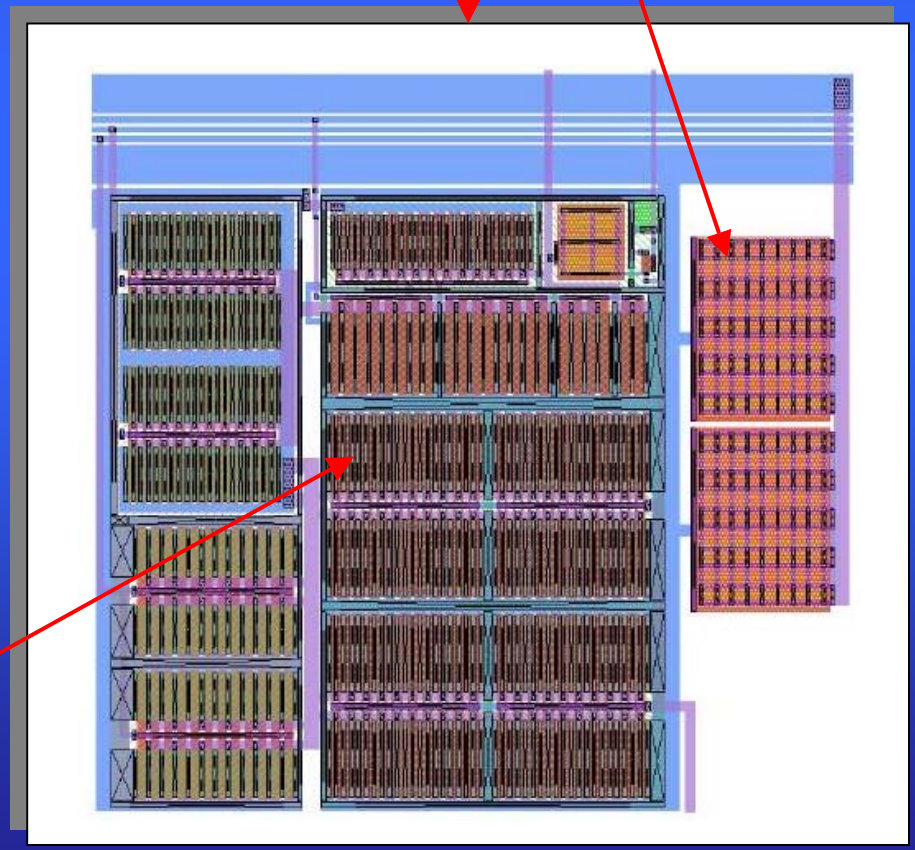
CMOS Photodiode Regions

Schematic Diagram of Design Q2 of the 8 x 8 Biochip



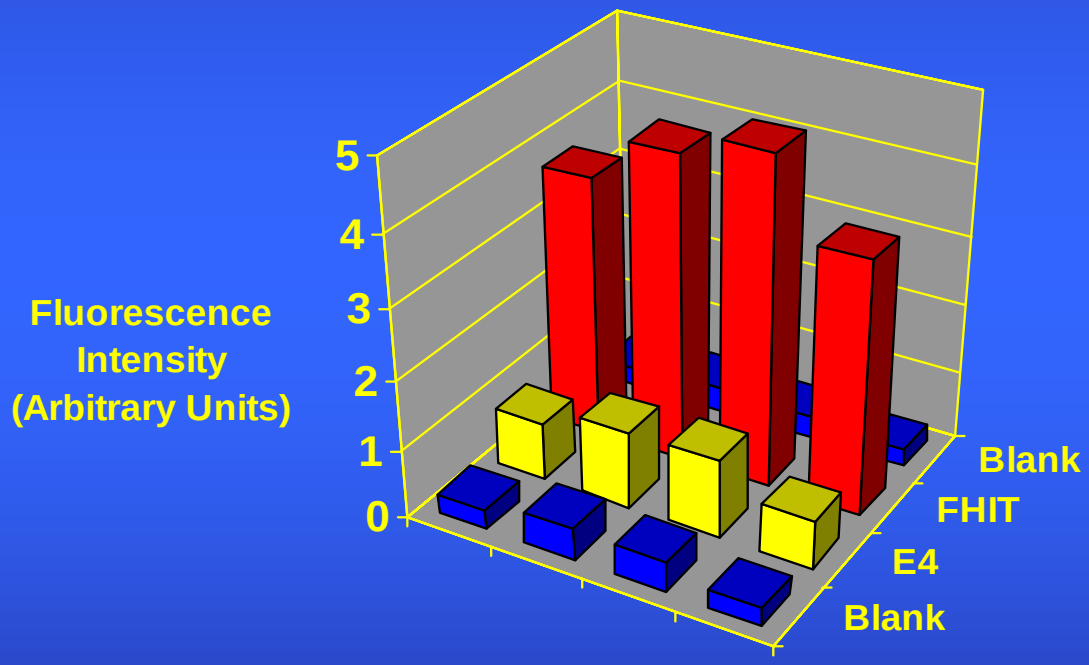
IC Processor

Integrating Capacitor



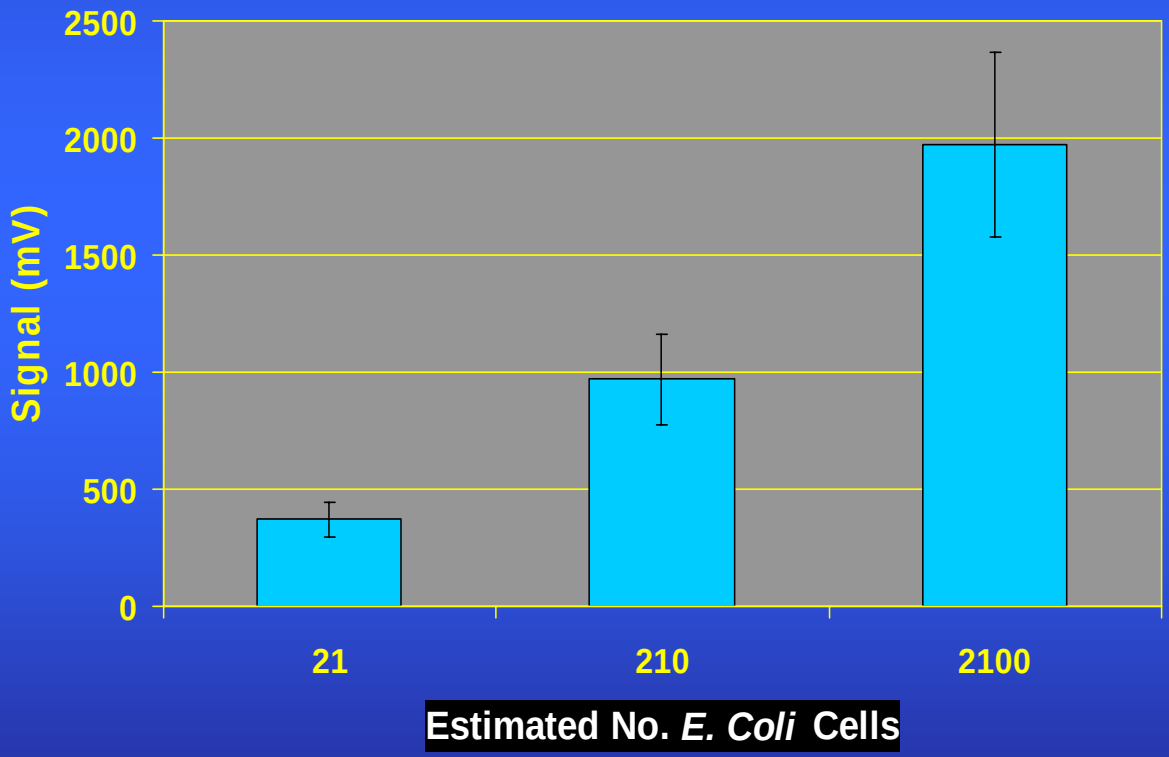
Operational Amplifier

Modified Ink-Jet Printer Technology for Rapid Formatting of Sample Platforms



- The parallel deposition process for multiple capture probes enables very rapid production of complex sample platforms.
- This figure illustrates Biochip signals observed for a DNA-based assay for the FHIT gene, performed on a sample platform which was preformatted using the modified ink-jet printer. The printer was used to produce 20 nl (0.8-1.0 mm dia.) dots of both FHIT and E4 (control) capture probes.

Sensitive on-chip detection of *E. coli* using the biochip and biofluidic system

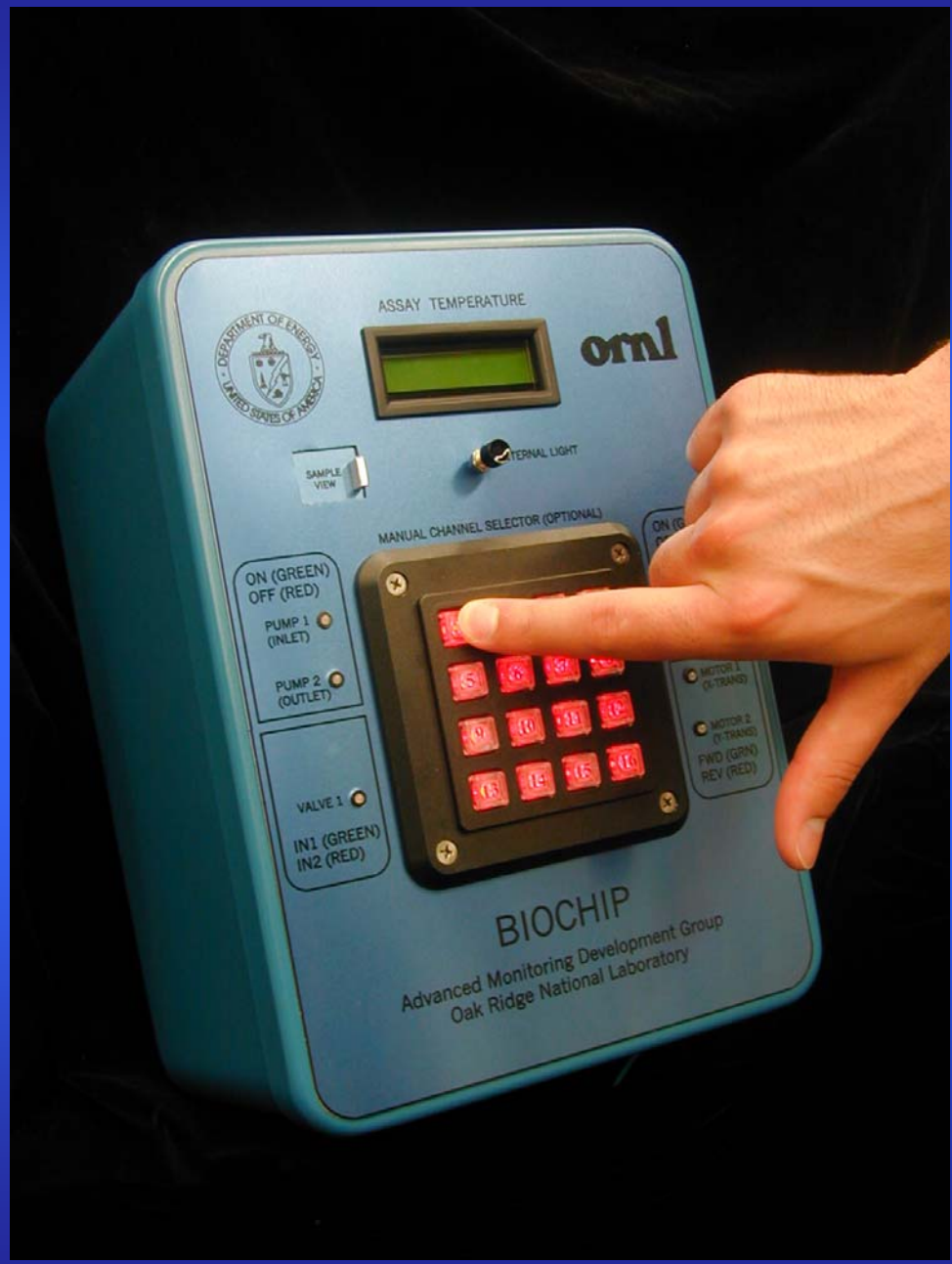


- An on-chip assay for *E. coli* has been performed using an antibody probe and 30-min incubation.
- A proportional response to the number of *E. coli* organisms deposited on a preformatted sampling platform has been observed.
- As few as 21 *E. coli* organisms have been detected through on-chip monitoring of a sandwich assay.

Portable Biochip Device

- Currently licensed to *HealthSpex, Inc.*, a local company in Knoxville, TN.

- Expected to appear in hospital/doctor's office in a couple of years

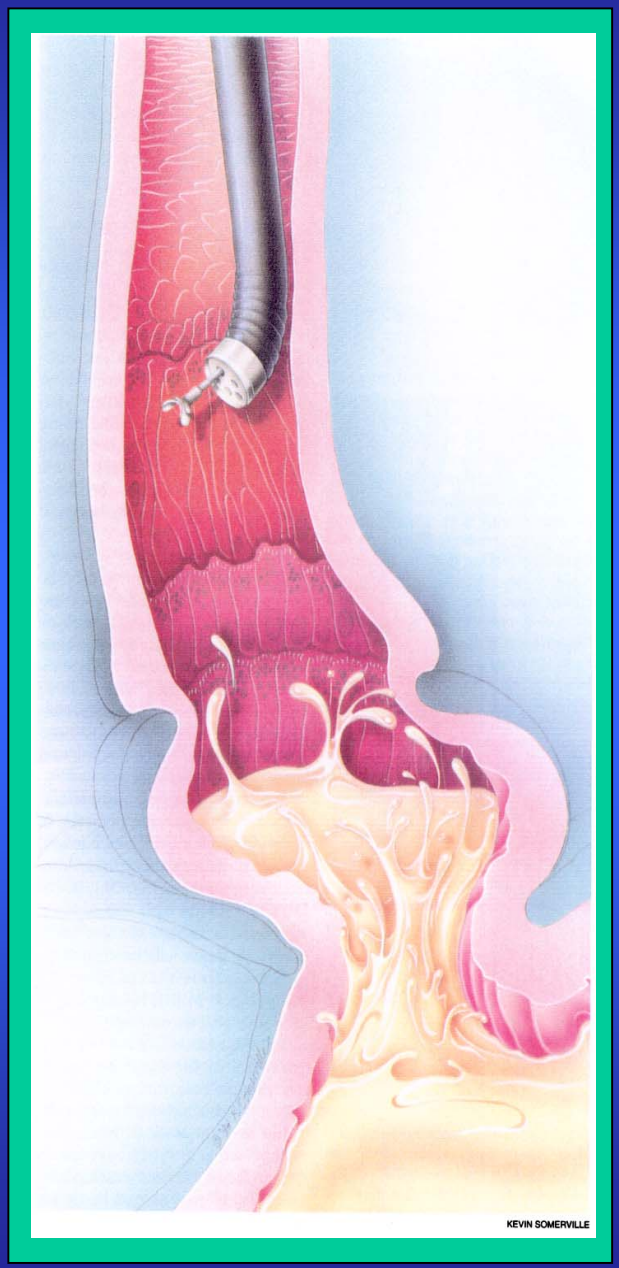


Laser-Induced Fluorescence (LIF) for Cancer Diagnosis

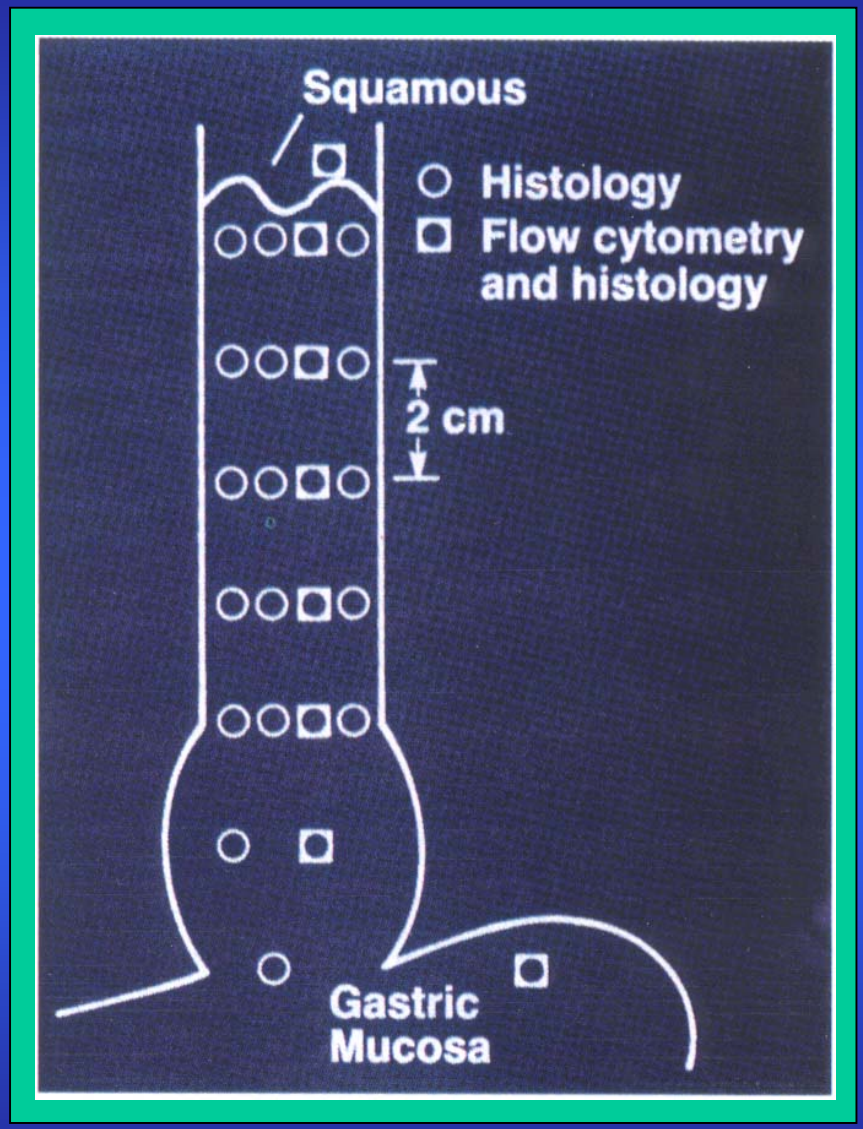
OPTICAL BIOPSY

BACKGROUND

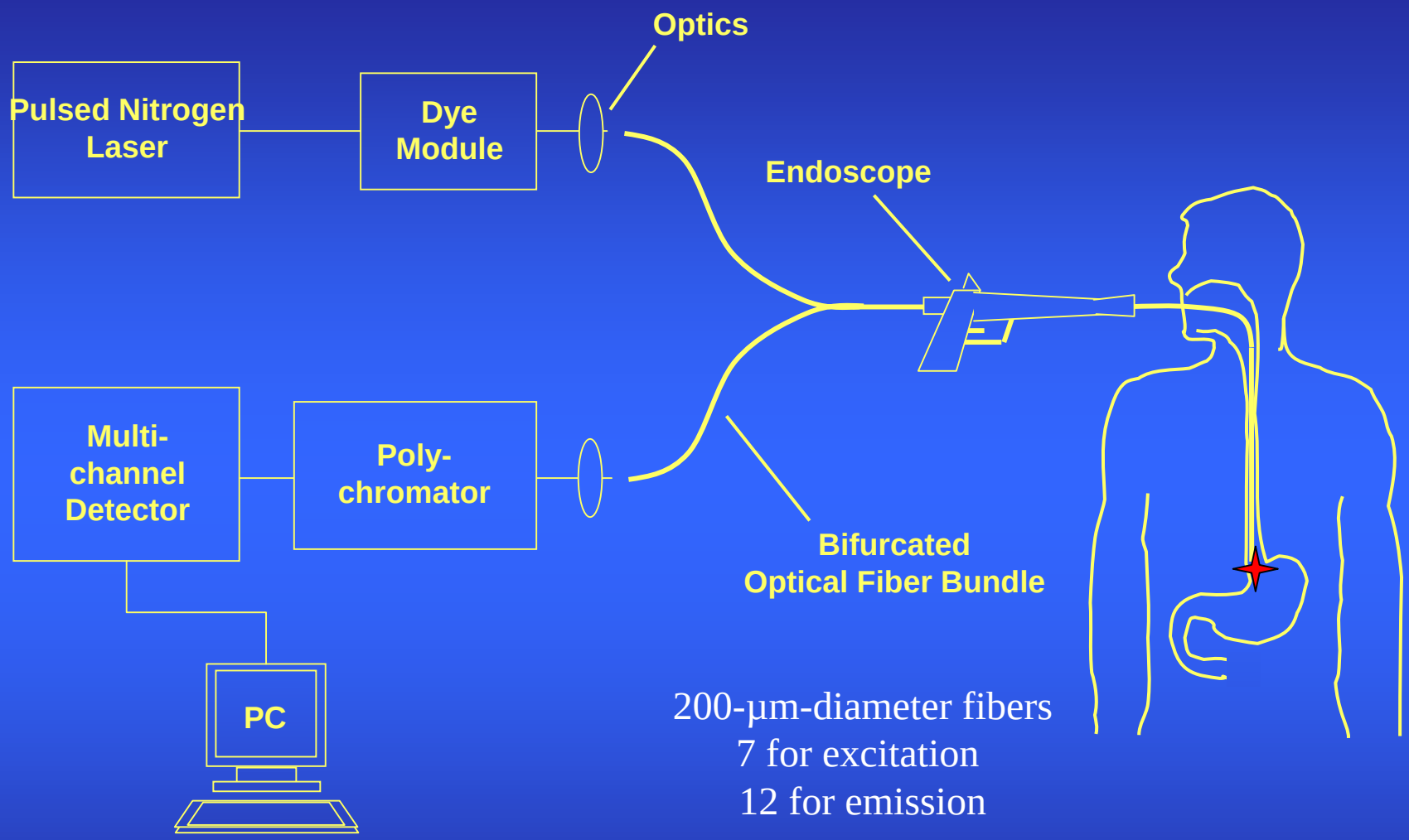
- Esophageal cancer is one of the most prevalent types of cancer
- In general, endoscopy is used to detect malignancies in the gastro-intestinal (GI) tract
- Once a suspected tumor is founded, it is removed or biopsies are taken for determination of histopathology
- The laboratory results are generally not available for several days



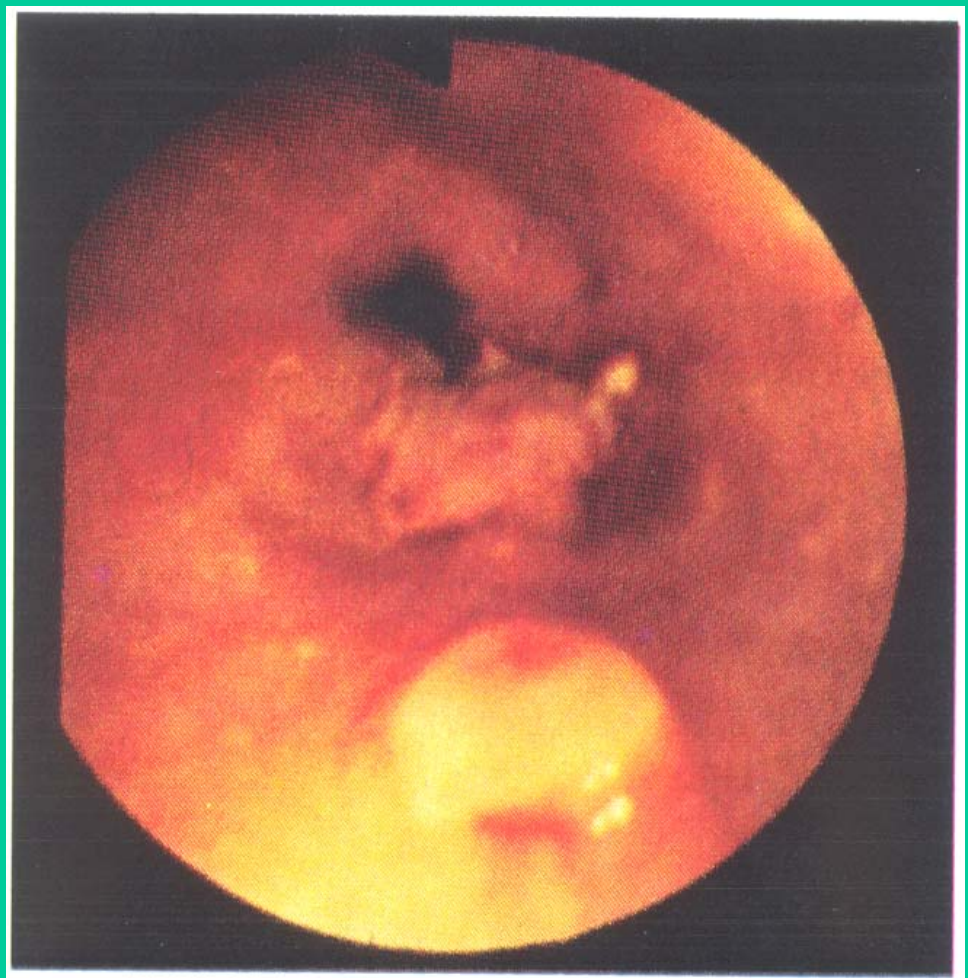
Reflux of gastric acid, pepsin, and other components of gastric juice into the esophagus, typically through a hernia, causes normal squamous epithelium (*top*) to be replaced by Barrett's metaplastic epithelium (*middle*). Endoscopic biopsy is necessary for the diagnosis of Barrett's esophagus.



Systematic endoscopic protocol, involving biopsies for pathology and for flow cytometry, permits mapping of the Barrett's esophageal segment in patients undergoing surveillance.

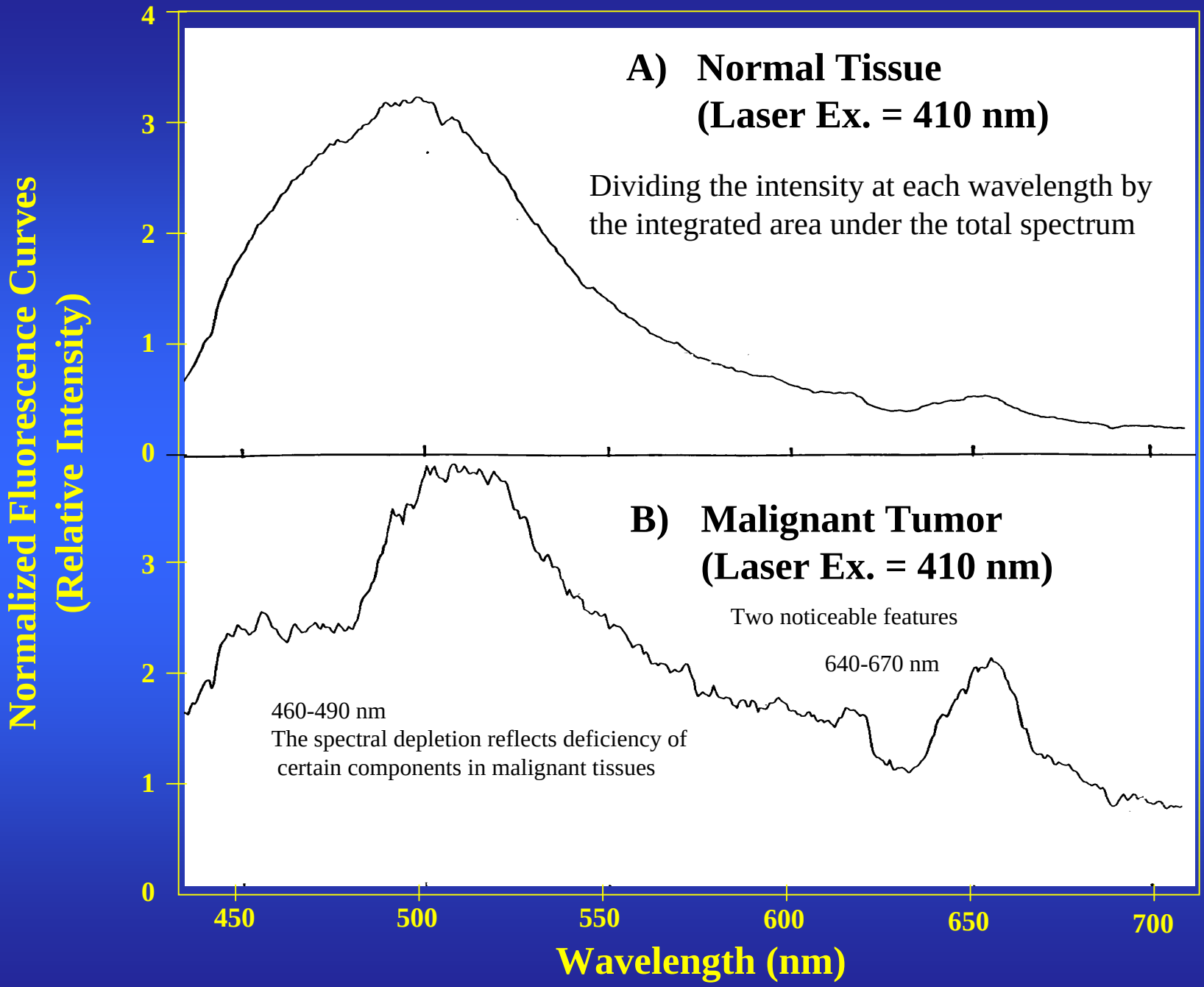


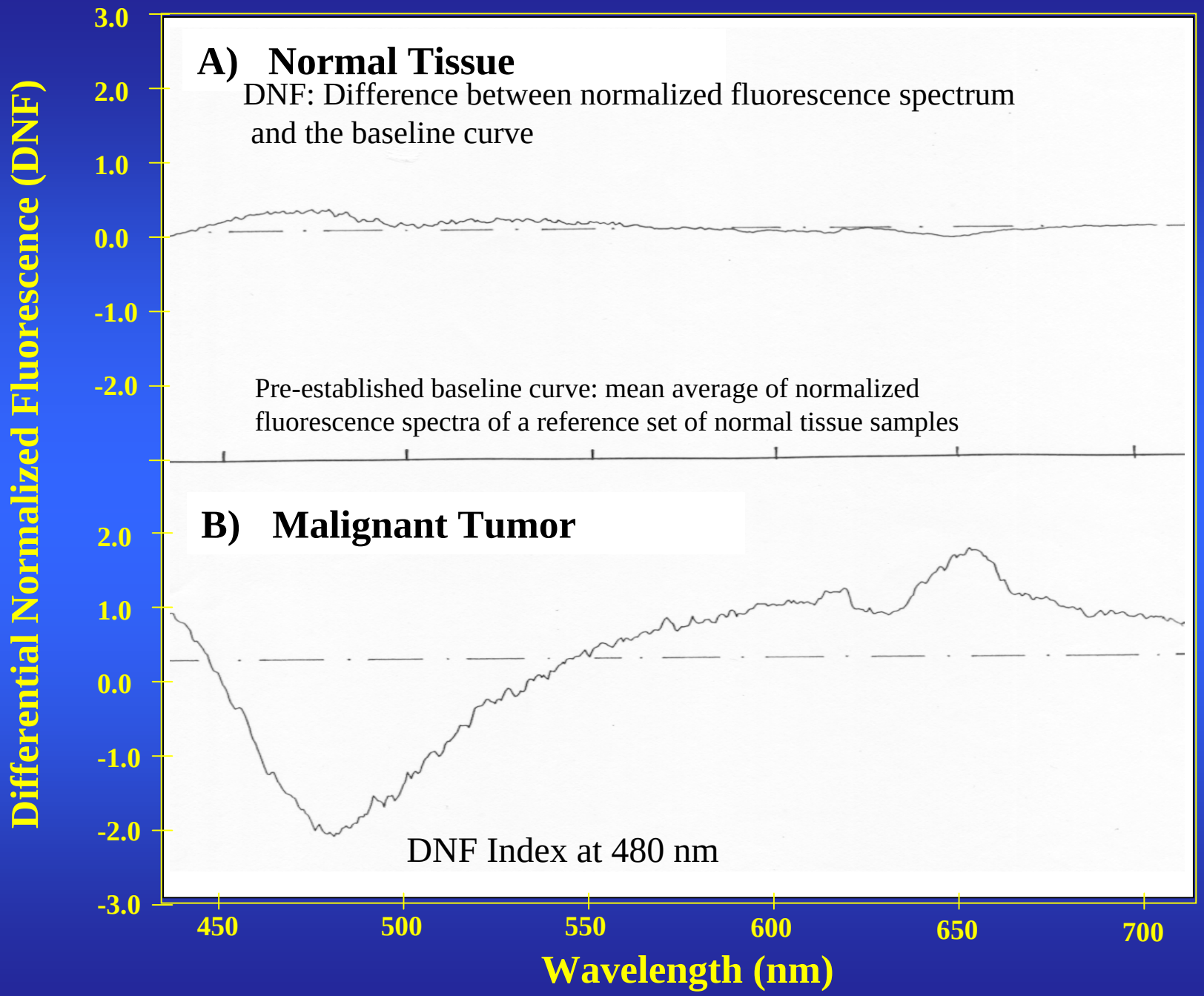
The measurement takes 0.6 s for each tissue site

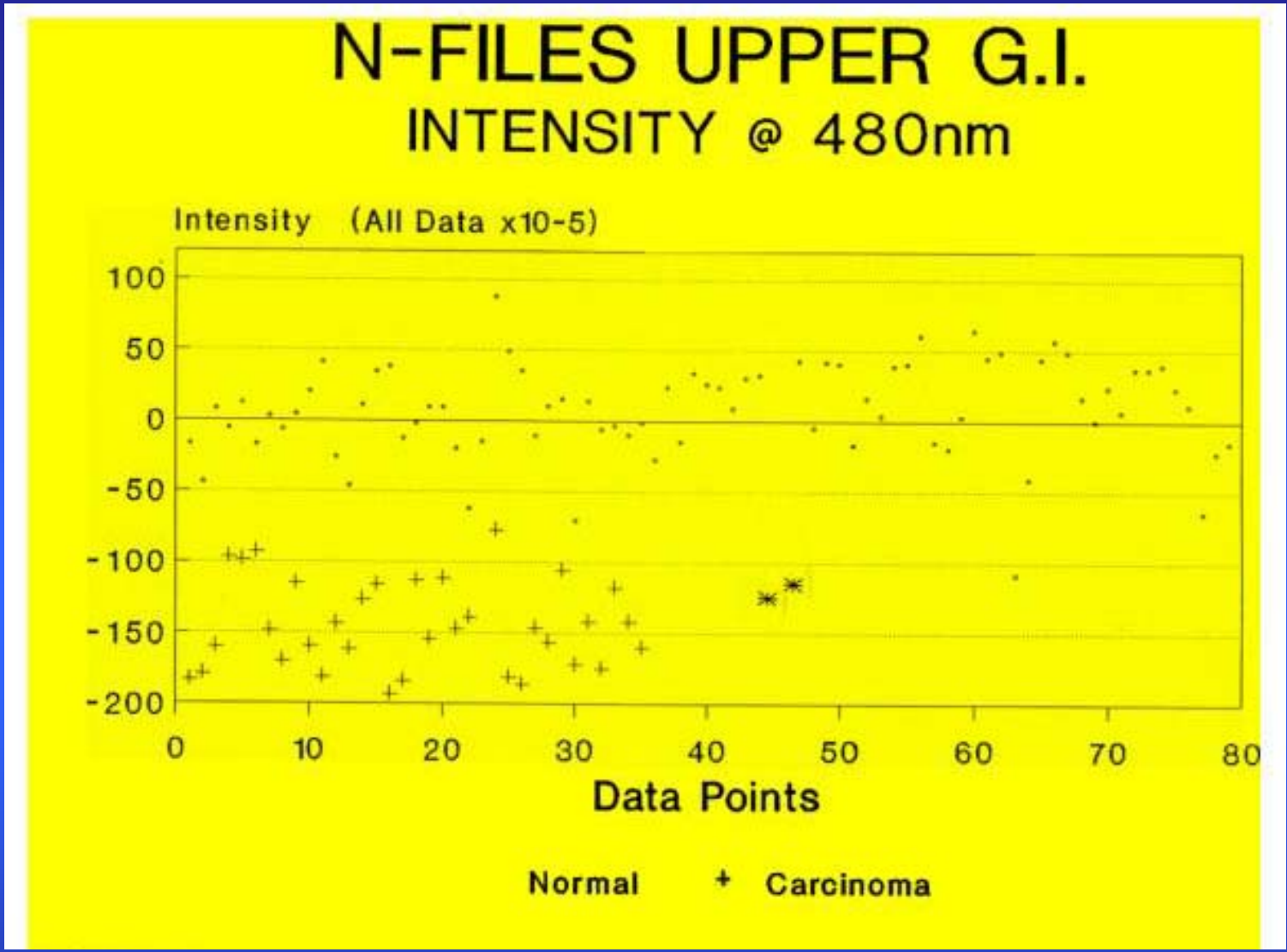


FROM SILVERSTEIN AND TYTGAT, WITH PERMISSION.

Barrett's esophageal adenocarcinoma with intramural metastasis is visible endoscopically from a proximal position


Normalized Fluorescence Curves
(Relative Intensity)
A) Normal Tissue
(Laser Ex. = 410 nm)
Dividing the intensity at each wavelength by
the integrated area under the total spectrum
B) Malignant Tumor
(Laser Ex. = 410 nm)
Two noticeable features
640-670 nm
460-490 nm
The spectral depletion reflects deficiency of
certain components in malignant tissues
0
1
2
3
4
450
500
550
600
650
700
Wavelength (nm)


A) Normal Tissue
DNF: Difference between normalized fluorescence spectrum and the baseline curve
Pre-established baseline curve: mean average of normalized fluorescence spectra of a reference set of normal tissue samples
B) Malignant Tumor
DNF Index at 480 nm
Differential Normalized Fluorescence (DNF)
3.0
2.0
1.0
0.0
-1.0
-2.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
450
500
550
600
650
700
Wavelength (nm)



Excellent agreement (98%) with histopathology in classification of normal tissue and malignant tumors of GI cancer in clinical studies involving over 100 patients

***Computer-assisted tomography (CAT) scan reveals lung cancer spreading into areas underlining the esophageal mucosa**

Conclusion

- The SERS technology has great promise for use in pathogen diagnosis, as well as in environmental monitoring.
- The RAMiTS device integrates the optical module, computer and touch screen interface into a compact, user-friendly, free-standing Raman detection unit.
- By specifically developing optical nanosensors for intracellular measurement applications and using methods analogous to those currently used in cellular and molecular biology, the transfer of technology between scientific disciplines is enhanced.

Conclusion (cont'd)

- An IC Biochip device has been developed which is very compact and cost effective, with potential for high throughput and multifunctional capability

- Optical biopsy based on LIF will hopefully preclude the need for unnecessary tissue biopsy and thus contribute immensely to early Barrett's esophageal adenocarcinoma detection critical for the timely treatment.

  "*After years of intensive effort in basic research, spectroscopy is now poised to emerge as a clinically useful tool (Gastroenterology Editor comments)*"

Advanced Biomedical Science and Technology Group

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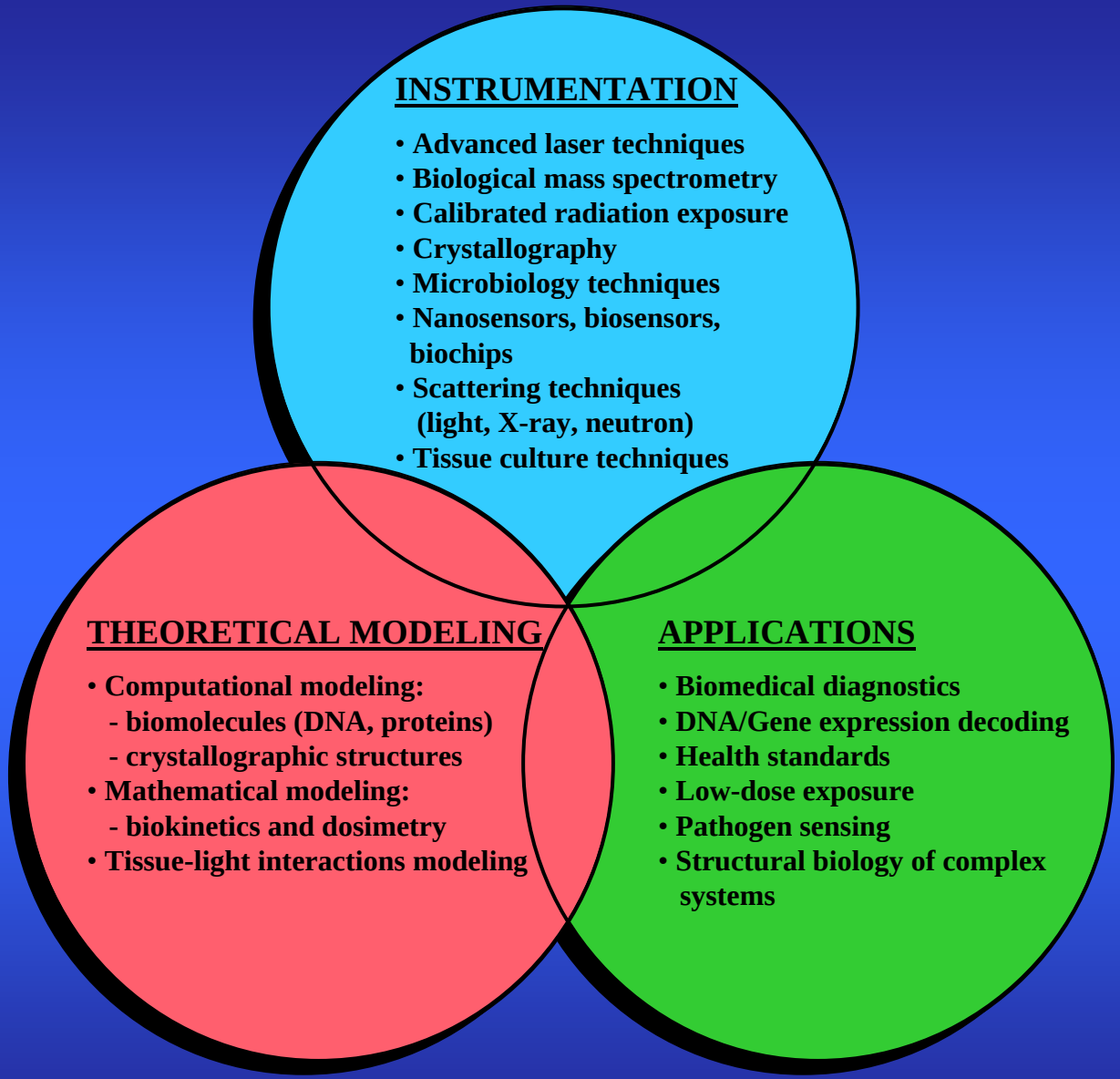
a Corporate Fellow
1 Postdoctoral Associates
2 Graduate Student
3 Part-Time
4 On loan part-time from ESTD
5 On Loan from I&C (50%)
6 Visiting Scientists/Guest Assignments


INSTRUMENTATION
• Advanced laser techniques
• Biological mass spectrometry
• Calibrated radiation exposure
• Crystallography
• Microbiology techniques
• Nanosensors, biosensors, biochips
• Scattering techniques (light, X-ray, neutron)
• Tissue culture techniques
THEORETICAL MODELING
• Computational modeling:
- biomolecules (DNA, proteins)
- crystallographic structures
• Mathematical modeling:
- biokinetics and dosimetry
• Tissue-light interactions modeling
APPLICATIONS
• Biomedical diagnostics
• DNA/Gene expression decoding
• Health standards
• Low-dose exposure
• Pathogen sensing
• Structural biology of complex systems

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Questions ?