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# Biomedical Applications of Molecular Spectroscopy

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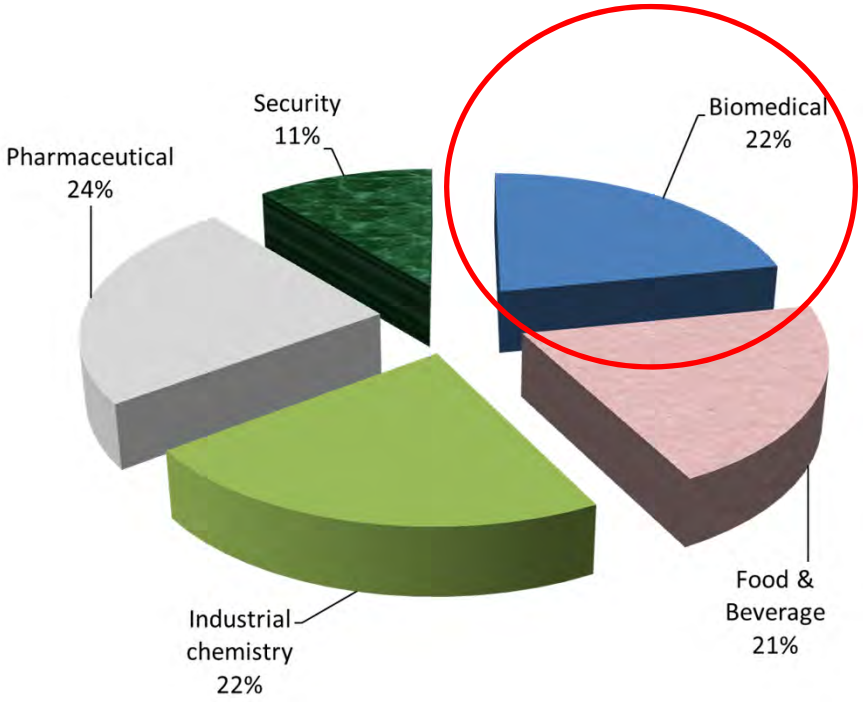
# Overview

- Molecular spectroscopy is a large and expanding discipline.
- One of the key areas that is driving this expansion is biomedical applications of molecular spectroscopy (“biospectroscopy”)
- Low cost compact modular spectrometers are enabling more applications
- Several methods are used in the analysis of DNA, proteins, viruses, bacteria, cells, tissues and these mainly use ultra-violet (UV), visible, near infrared (NIR) spectrometers together with a range of sampling techniques
- A review of biomedical applications using these different spectrometers, particularly for UV absorption, NIR transmission and reflectance, together with Raman scattering, will be discussed
- Rapid adoption of spectrometers which are now being deployed for biomedical analysis, for example: antioxidants, arteries particularly artherosclerosis, bone, cancer tumors, diabetes, fertility, infections and other examples

# Biospectroscopy – Large Area of Molecular Spectroscopy

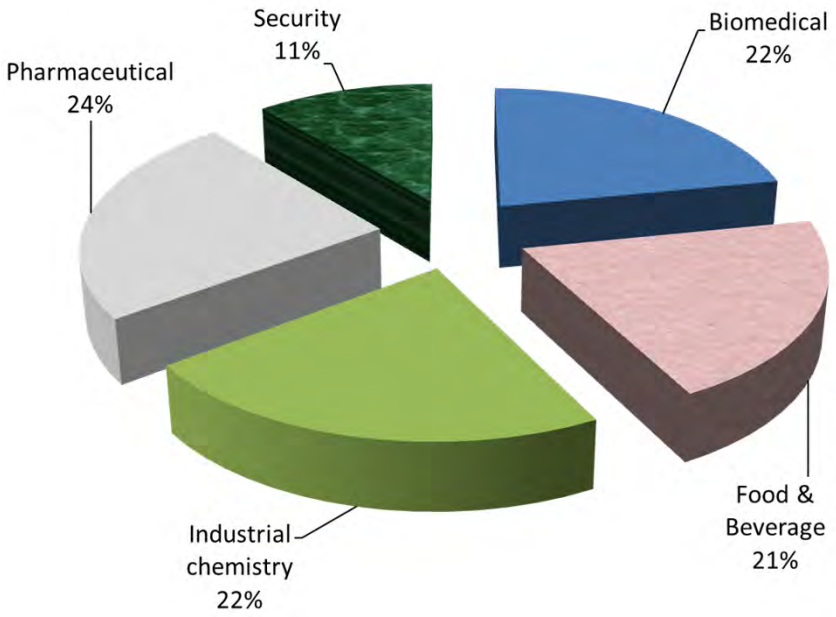



Source: SDI 2011

Most biomedical applications based on:

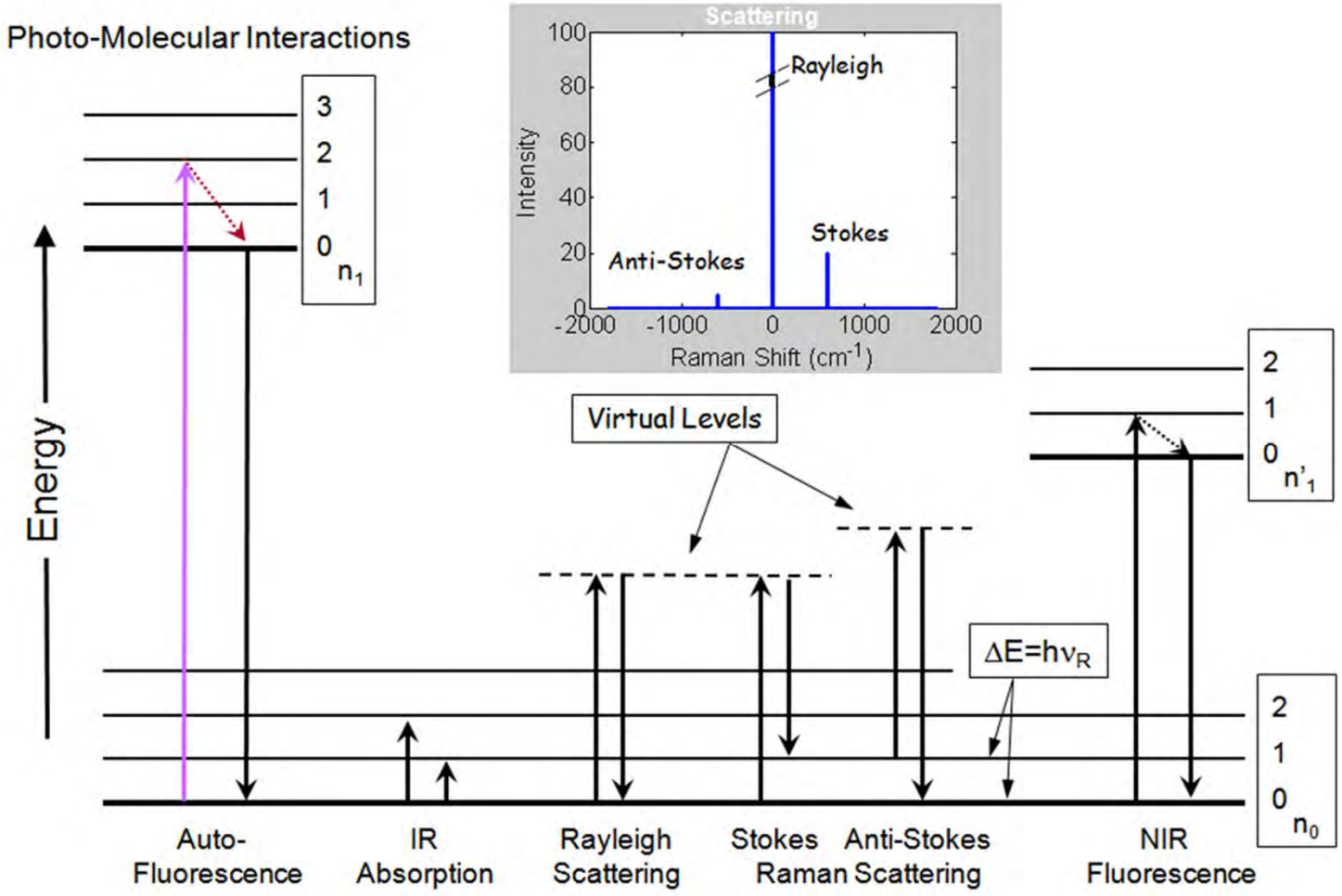
- UV absorption spectroscopy
- NIR spectroscopy
- Raman spectroscopy

# Biospectroscopy – Large Area of Molecular Spectroscopy

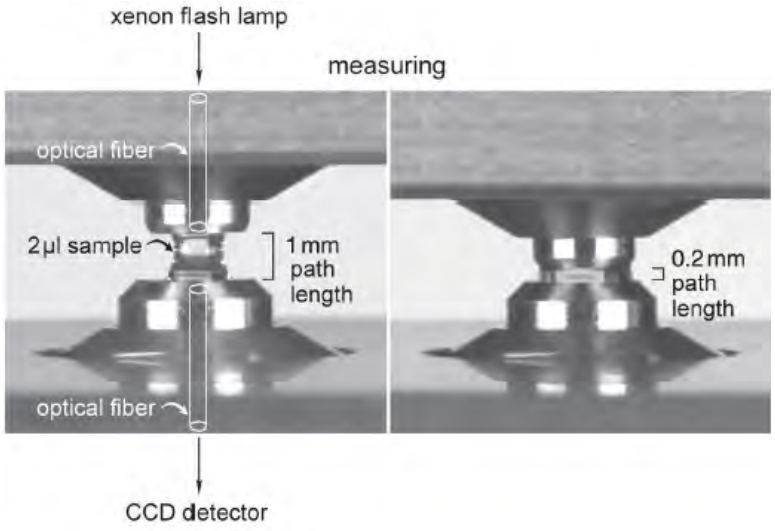



- Biological macromolecules:
  - nucleic acids, proteins, lipids
- Blood disorders:
  - anemias, leukemias, thalassemias
- Cancer diagnosis:
  - brain, breast, cervical, colon and other
- Chemical processes in live blood cells:
  - malaria, drug reactions
- DNA in chromosomes, pigment in granulcotyties, RBCs, helptocytes
- Immuno assays
- Organelles, cells, micro-organisms, bacteria, phytoplankton, neurotoxins, viruses
- Tissue analysis:
  - Alzheimer's, artery, breast, bone, cervix, embryo media, esophagus, gastro-intestinal tract, prostrate

# Biospectroscopy – Biophysics



# Biospectroscopy – Examples of UV Absorption Spectroscopy - µL




For nucleic acid quantification, the Beer-Lambert equation is:

c = (A * e)/b
Where c is the nucleic acid concentration in ng/microliter, A is the absorbance in AU, e is the wavelength-dependent extinction coefficient in ng-cm/microliter and b is the path length in cm. The generally accepted extinction coefficients for nucleic acids are:
• Double-stranded DNA: 50
• Single-stranded DNA: 33
• RNA: 40

Source: Nanodrop Technologies

- Acquire UV-visible absorption spectrum between 220 nm to 750 nm
- Measure peak ratios 260 nm/280 nm; for DNA, the peak should be at 260 nm, the 260/280 ratio should be between 1.8 and 2.0
- Concentration measurements of DNA, RNA, dyes, proteins, cell cultures
- Typically 1 pg per µL sensitivity
- Each sample is measured using two different path lengths (1mm and 0.2 mm), providing an wide dynamic range (~ 2 ng/µl - 3700 ng/µl dsDNA)
- The short path length provides 50 times higher in concentration than can be measured on classical 1 cm cuvette-based systems

# Biospectroscopy – Advantages of Vibrational Spectroscopy

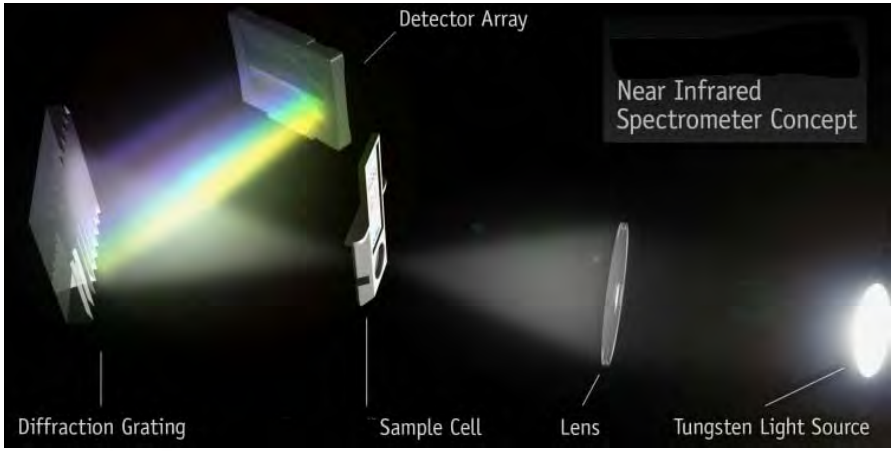
- Vibrational spectroscopy including Raman scattering and near infrared(NIR) absorption, transmission and reflectance has large and increasing potential
- Number of degrees of freedom in a complex molecule is $3N - 6$, where $N$ is the number of atoms and for a large molecule like a biopolymer with ~ O(100) atoms, there are effectively three degrees of freedom for each atom
- Spectra tend to be complex with many lines, but different types of molecules have characteristic behavior
- Different distinct spectra for tissues and pathologies
- Objective is to identify spectral characteristics that are associated with a various states including disease

# Biospectroscopy – Comparison Raman vs IR

| | Near-IR | Mid-IR | Raman |
|---|---|---|---|
| Spectral range ($cm^{-1}$) | 13,300–3300 | 4000–400 | 4000–50 |
| Analysis of: | | | |
| Gases | No | Yes | Yes |
| Liquids | Yes | Yes | Yes |
| Solids | Yes | Yes | Yes |
| Aqueous systems | Difficult | Very difficult | Yes |
| Macroscopic samples | Yes | Yes | Yes |
| Microscopic samples | No | Yes | Yes |
| Signal | Strong | Strong | Weak |
| Sampling | Easy | Difficult | Easy |
| Through glass windows | Yes | No | Yes |
| In situ | No | No | Yes |
| Quantitative | Yes | Difficult | Yes |
| Noninvasive | Yes | No | Yes |
| Fiber optic interfacing | Yes | No | Yes |
| Information content | Low. Limited to O–H, N–H, and C–H vibrations | High | High |
| Reaction monitoring and modeling | Requires chemometrics | Yes | Yes |

# Biospectroscopy – Examples of NIR Spectroscopy – IVF

- In vitro fertilization application
- Embryo viability testing




NIR-based spectrometer with touch screen
25 cm (H) x 28 cm (W) x 42 cm (D)

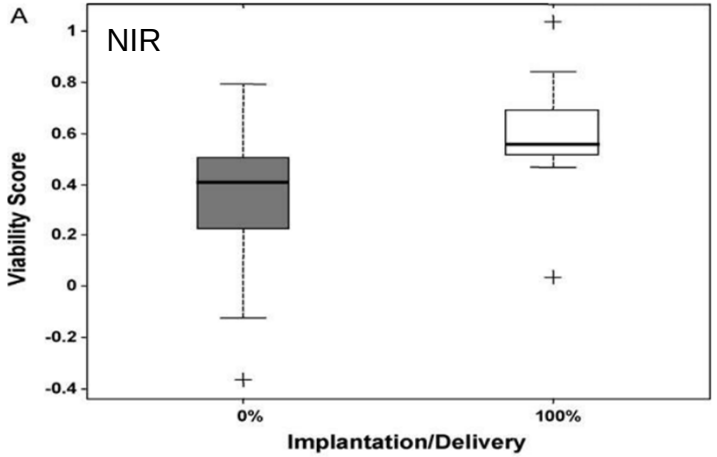
Three components:
1. Analysis instrument
2. Sample cells, dark cell & reference cells
3. Temperature stabilizer (23.8ºC)

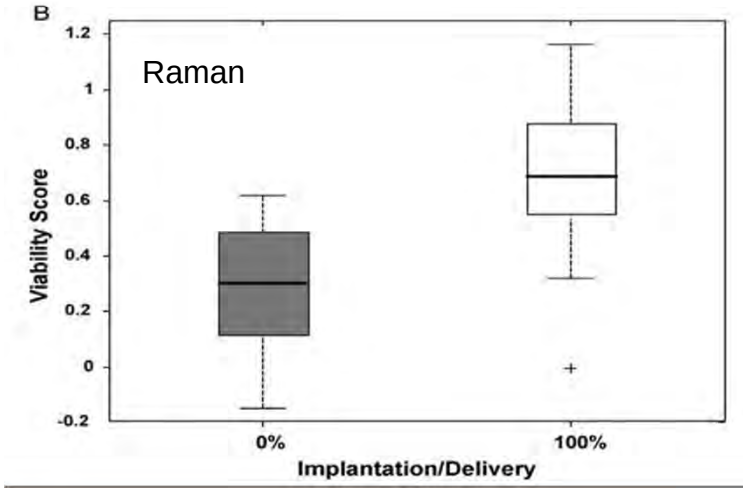
Single use sample cell with RFID
5.4 cm (H) x 2.2 cm (W) x 0.6 cm (D)

Source: Molecular Biometrics

# Biospectroscopy – Examples of NIR Spectroscopy - IVF

- Metabolomic profile of spent embryo culture (2 -3 days)
- Target specific Oxidation Stress (OS) biomarkers in embryonic culture media and measure NIR absorption
- Identify metabolomic differences in viable compared with non-viable embryos
- Non-invasive spectroscopic method for prediction of implantation potential of embryos in IVF
- Measure changes in the -CH, -NH, -CH, -SH functional groups between 900-1700 nm
- **Viability Score** indicates the SET embryo reproductive potential
- Established correlation between Viability Score and fetal cardiac activity within 12 weeks




Source: Molecular Biometrics

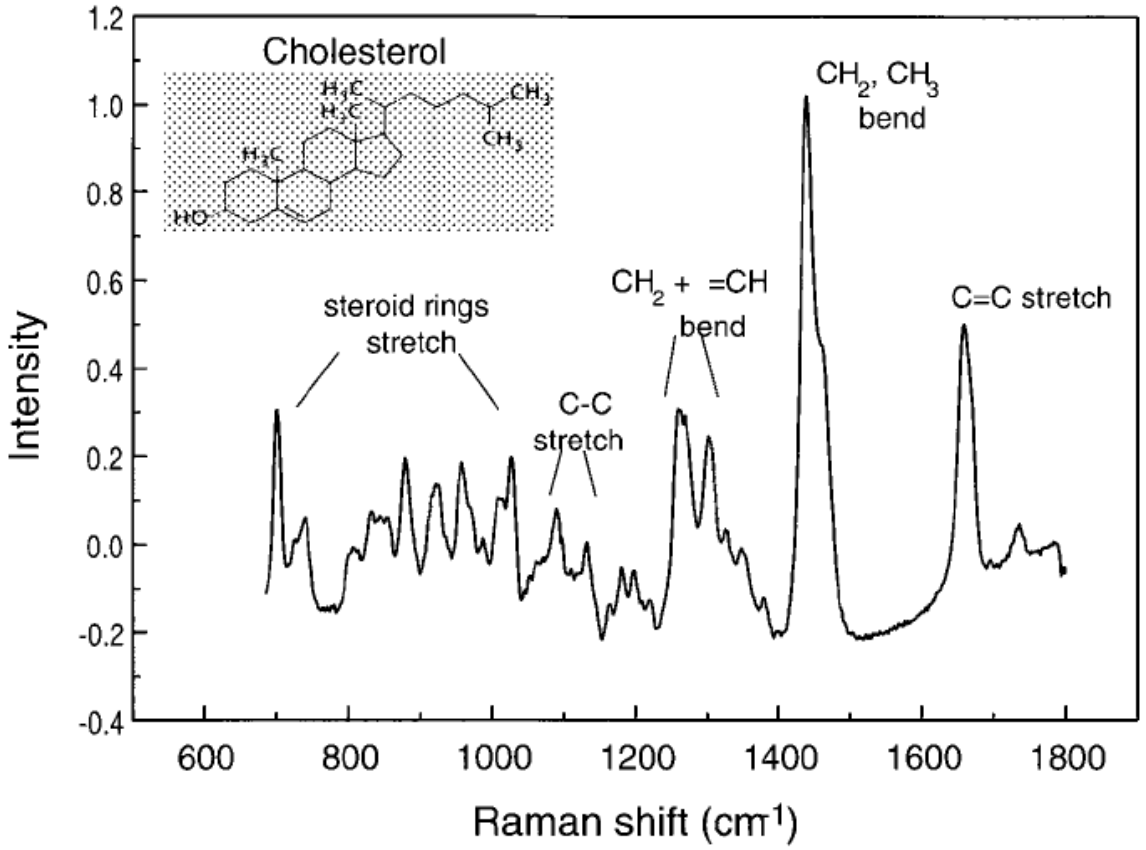
# Biospectroscopy – Advantages of Raman Spectroscopy

- Wavelength selection
- No water interference (physiological state)
- No biopsy required
- Directly measures molecules
- Small concentrations
- Chemical composition
- Morphological analysis
- Quantitative analysis from sharp spectral peaks
- *In vivo* diagnosis
- High spatial resolution
- Raman can distinguish numerous pathologies
- Raman probes can distinguish disease
- Raman can now be used in deeper tissues

# Biospectroscopy – Examples of Raman spectroscopy

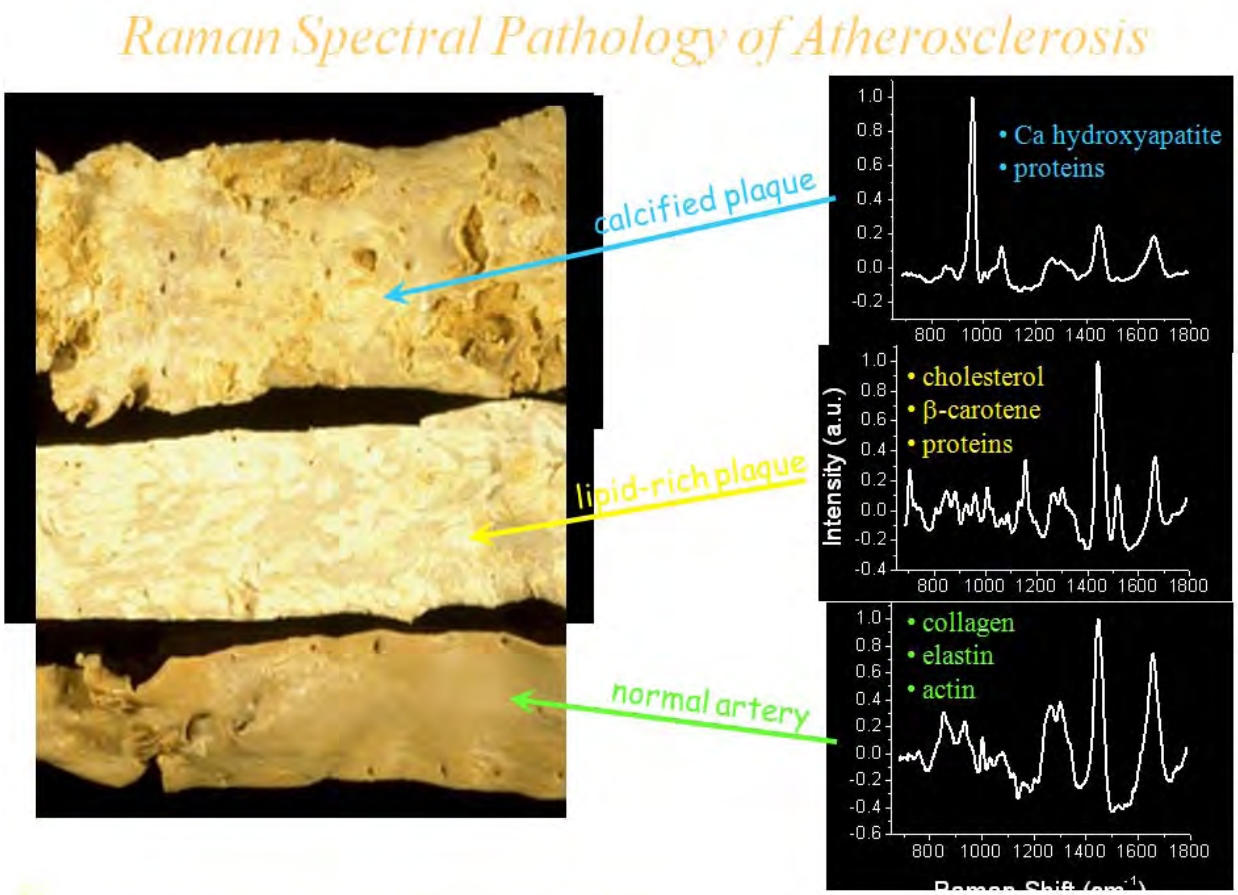
- Raman spectroscopy 'fingerprints' molecules by characterizing interactions between photons and molecular vibrations (*unique for each biomolecule*)
- Near-infrared excitation is preferred for biomedical applications
- Recent optical fiber probe developments allow accurate real-time analysis *in vivo*
- Alternative to immunofluorescence staining (*clinical diagnostics*)
- New areas of research are promising for widespread clinical applications (*cell biology, imaging, tissue engineering, pathogens, pharmacology*)

# Biospectroscopy – Examples of Raman Spectroscopy




Hanlon *et al.* "Prospects for *in vivo* Raman spectroscopy," Phys Med Biol **45**: R1 (2000)

# Biospectroscopy – Examples of Raman Spectroscopy



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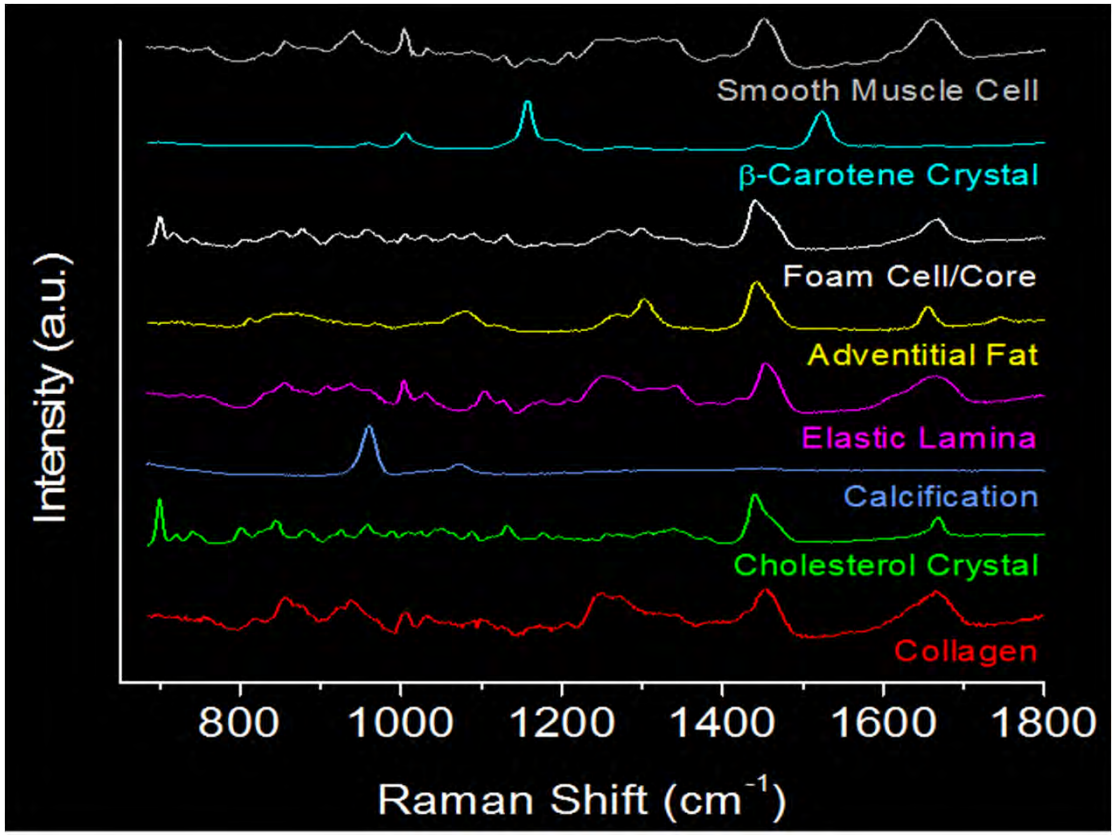
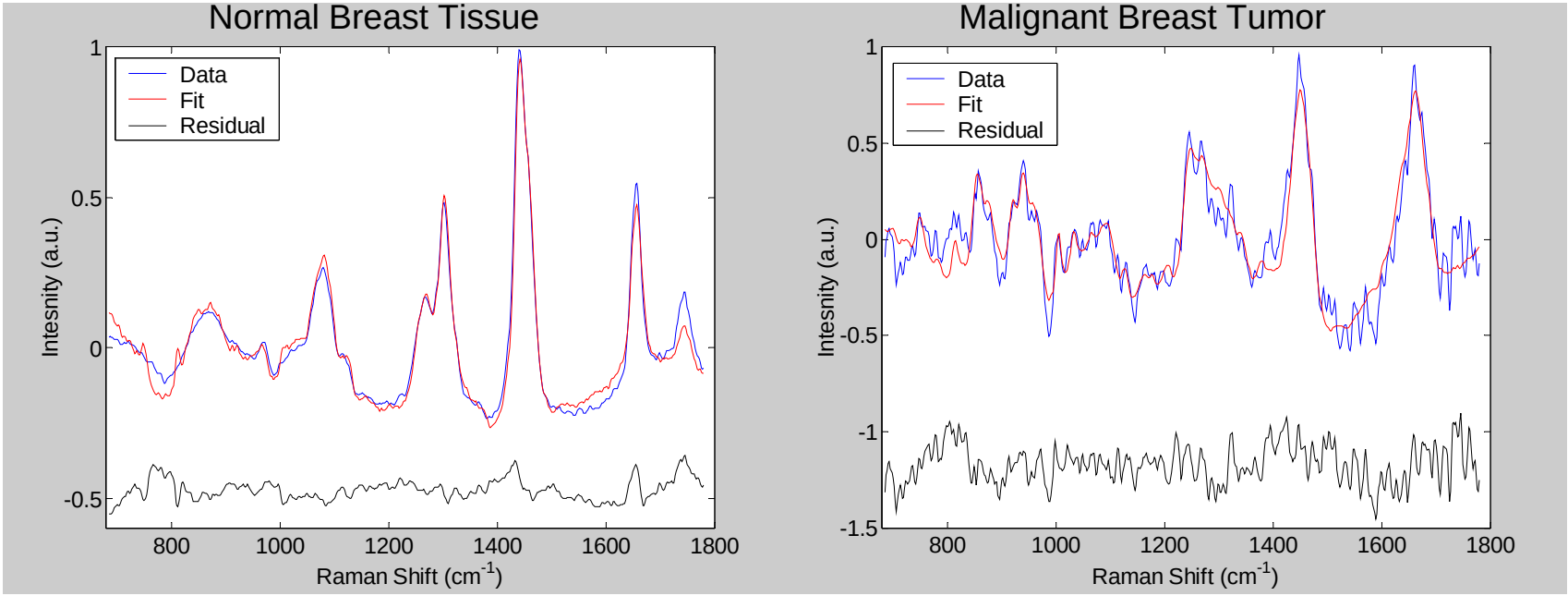



Buschman HPJ, et al. *Cardiovascular Pathology* **10**(2), 69-82 (2001)

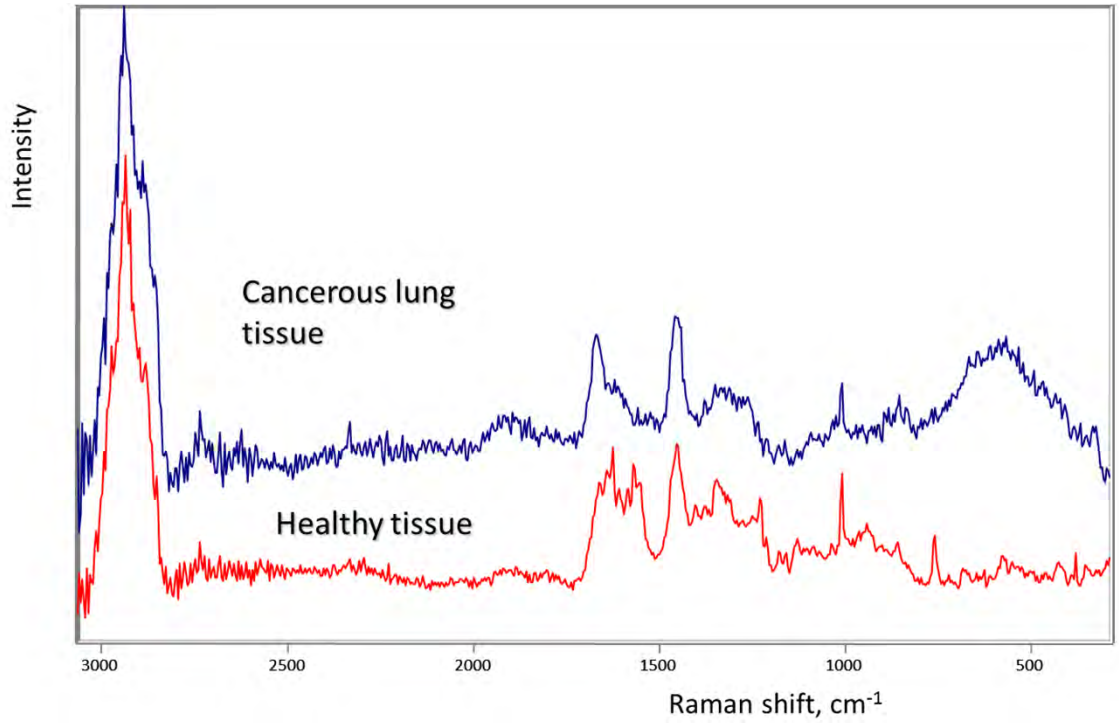
# Biospectroscopy – Examples of Raman Spectroscopy




[1]Gloucestershire Royal Hospital, Gloucester, UK

100 mW excitation, 785 nm, 1 second collection

# Biospectroscopy – Examples of Raman spectroscopy



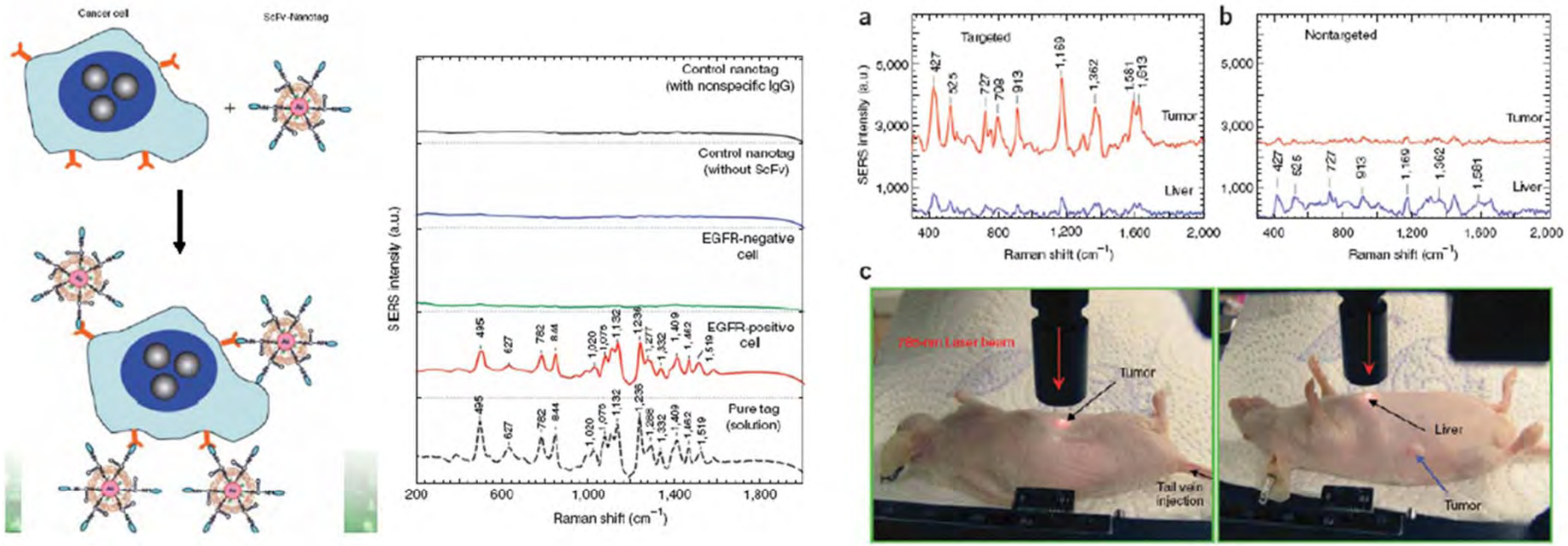

# Biospectroscopy – Examples of Raman spectroscopy

| Application | Raman spectroscopy | Market potential |
|---|---|---|
| Biological mcromolecules (nucleic acids, proteins, lipids)<br><br>Organelles<br>Cells<br>Microorganisms<br>Bacteria<br>Phytoplankton neurotoxins<br>Viruses | UV resonance Raman | Microspectroscopy |
| Cells<br>DNA in chromosomes<br>Pigment in granulocytes & lymphocytes<br><br>Major applications<br>Immunoassay<br>Protein identification | Visible | MEDIUM/HIGH<br><br>Bioassay reader<br>Cytometry |
| Tissue: Alzheimers, artery, blood (analytes, LGRL), bone, brain, breast, cervix, colorectal, esophagus, GI, glucose, larynx, prostate, skin, thyroid, tumors<br><br>Major applications:<br>Cancer diagnosis<br>Cardiology disease<br>Medical microbiology<br>Tumor detection | NIR | HIGH<br><br>Portable instrument with fiber probe vs FT-Raman vs. immunofluorescence staining (tissues)<br><br>In vivo<br>Peripheral blood<br><br>SERS & endoscopy<br><br>Animal/vetnarian<br>Human/clinical |

# Biospectroscopy – Examples of Raman spectroscopy - SERS

- In vivo tumor targeting and detection
- Biocompatible and non-toxic nano-particles (pegylated gold) for SERS
- Target tumor biomarkers, for example, epidermal growth factor receptors (EGFR) on human cancer cells and xenograft tumor models

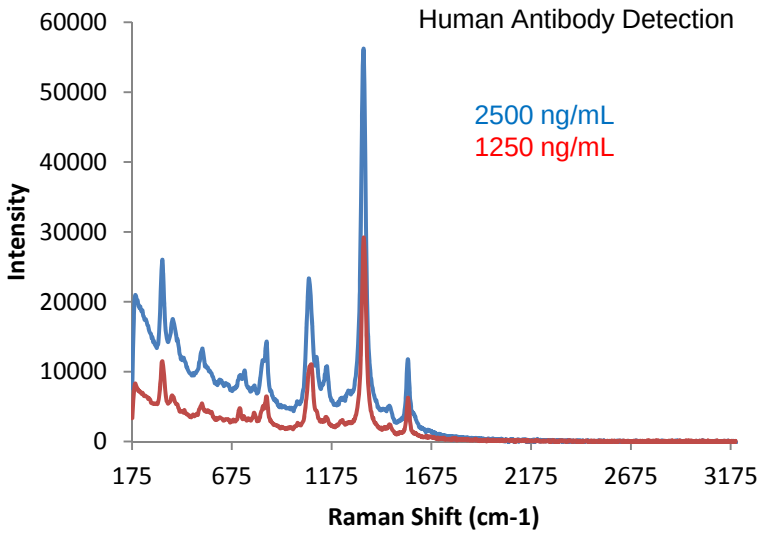
# Biospectroscopy – Examples of Raman spectroscopy - SERS




In vivo tumor targeting and spectroscopic detection and surface-enhanced Raman nanoparticle tags
*Nature Biotchnology* **26**, 83-90 (2008)

# Biospectroscopy – Promise of Enhanced Raman (SERS)

- Subtle changes within biomolecules, such as drug interactions, tissue healing, cosmetics, disease diagnosis
- Intercellular SERS localization and interaction. Identification of drug binding to cells for Drug-DNA and cellular interaction analysis
- Investigation of microorganisms in single cells; yeast cell classifications, single bacterium
- Oxygenation measurements of blood and tissue
- Molecular level cancer detection (cervical, lung, throat and
- Immunoassays using SERS and Raman readers



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