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

Most biomedical applications based on:

- UV absorption spectroscopy
- NIR spectroscopy
- Raman spectroscopy

Source: SDI 2011
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 granulocyteties, 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

- 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

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

\[ c = \frac{(A \times e)}{b} \]

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
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 various states including disease.
# Biospectroscopy – Comparison Raman vs IR

<table>
<thead>
<tr>
<th></th>
<th>Near-IR</th>
<th>Mid-IR</th>
<th>Raman</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spectral range (cm⁻¹)</strong></td>
<td>13,300–3300</td>
<td>4000–400</td>
<td>4000–50</td>
</tr>
<tr>
<td><strong>Analysis of:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gases</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Liquids</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Solids</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Aqueous systems</td>
<td>Difficult</td>
<td>Very difficult</td>
<td>Yes</td>
</tr>
<tr>
<td>Macroscopic samples</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Microscopic samples</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Signal</strong></td>
<td>Strong</td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td><strong>Sampling</strong></td>
<td>Easy</td>
<td>Difficult</td>
<td>Easy</td>
</tr>
<tr>
<td>Through glass windows</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>In situ</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Quantitative</strong></td>
<td>Yes</td>
<td>Difficult</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Noninvasive</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Fiber optic interfacing</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Information content</strong></td>
<td>Low, Limited to O–H, N–H, and C–H vibrations</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>Reaction monitoring and modeling</strong></td>
<td>Requires chemometrics</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Biospectroscopy – Examples of NIR Spectroscopy – IVF

- In vitro fertilization application
- Embryo viability testing

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

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

Single use sample cell with RFID
5.4 cm (H) x 2.2 cm (W) x 0.8 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

Biospectroscopy – Examples of Raman Spectroscopy

Raman Spectral Pathology of Atherosclerosis

- Calcified plaque
- Lipid-rich plaque
- Normal artery

- Ca hydroxyapatite
- Proteins
- Cholesterol
- β-carotene
- Proteins
- Collagen
- Elastin
- Actin

Image credit: Image courtesy of Dr. Alan S. Zelcer, Department of Pathology, University of Toronto, Canada.
Biospectroscopy – Examples of Raman Spectroscopy

Biospectroscopy – Examples of Raman Spectroscopy

Normal Breast Tissue

Malignant Breast Tumor

1Gloucestershire Royal Hospital, Gloucester, UK

100 mW excitation, 785 nm, 1 second collection
Biospectroscopy – Examples of Raman spectroscopy
Biospectroscopy – Examples of Raman spectroscopy

<table>
<thead>
<tr>
<th>Application</th>
<th>Raman spectroscopy</th>
<th>Market potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological macromolecules (nucleic acids, proteins, lipids)</td>
<td>UV resonance Raman</td>
<td>Microspectroscopy</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
<td></td>
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<tr>
<td>Organelles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microorganisms</td>
<td></td>
<td></td>
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<tr>
<td>Bacteria</td>
<td></td>
<td></td>
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<tr>
<td>Phytoplankton neurotoxins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
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<tr>
<td>DNA in chromosomes</td>
<td>Visible</td>
<td>MEDIUM/HIGH</td>
</tr>
<tr>
<td>Pigment in granulocytes &amp; lymphocytes</td>
<td></td>
<td>Bioassay reader</td>
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<tr>
<td>Immunoassay</td>
<td></td>
<td>Cytometry</td>
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<tr>
<td>Protein identification</td>
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</tr>
<tr>
<td>Tissue: Alzheimers, artery, blood (analytes, LGR, bone, brain, breast, cervix, colorectal, esophagus, GI, glucose, larynx, prostate, skin, thyroid, tumors)</td>
<td>NIR</td>
<td>HIGH</td>
</tr>
<tr>
<td>Portable instrument with fiber probe vs FT-Raman vs. immunofluorescence staining (tissues)</td>
<td></td>
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<tr>
<td>In vivo</td>
<td></td>
<td></td>
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<tr>
<td>Peripheral blood</td>
<td></td>
<td></td>
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<tr>
<td>SERS &amp; endoscopy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal/vetnarian</td>
<td></td>
<td></td>
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<tr>
<td>Human/clinical</td>
<td></td>
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</tr>
</tbody>
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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

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

![Human Antibody Detection Graph](image)
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