Measuring Lung Disease Progression using FTIR and Raman Spectroscopy

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1 Institute of Photonic Technology (Jena, Germany): Since 2008
2 University of Trieste (Italy): 2006-07
3 Dresden University of Technology (Germany): 2000-2006
Overview

- Vibrational Spectroscopy
  - Principle of Fourier-transform infrared spectroscopy
  - Principle of Raman spectroscopy
  - Imaging methodologies
- Diagnosis of lung tissues
  - Control
    - Congenital cystic adenomatoid malformation (CCAM)
    - Bronchopulmonary sequestration (BPS)
- In vivo studies using fiber optic probes
- Diagnosis of body fluids
- Diagnosis of single cells
- Diagnosis of tissue at cellular level
- Coherent anti-Stokes Raman scattering
Molecular Vibrations

Antisymmetric stretch

Symmetric stretch

Deformation

Number of vibrations in molecule with n atoms

<table>
<thead>
<tr>
<th>molecule</th>
<th>degrees of freedom</th>
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<tbody>
<tr>
<td>nonlinear</td>
<td>$3n - 6$</td>
</tr>
<tr>
<td>linear</td>
<td>$3n - 5$</td>
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</table>
Infrared Spectroscopy: Absorption of Radiation

- **Electronic ground state** \( S_0 \)
- **Electronic excited state** \( S_1 \)

Mid infrared 2.5-25 µm (4000-400 cm\(^{-1}\))

Transmission

Absorption

Wavenumber

\[ T(\lambda) = \frac{I_0}{I_0^1} \]

\[ A(\lambda) = \log \frac{I_0^1}{I_0^1} = -\log T(\lambda) \]

\[ \tilde{\nu} = \frac{1}{\lambda} \left( \frac{1}{cm} = cm^{-1} \right) \]
Raman Spectroscopy: Inelastic Scattering

- Incident light $\lambda_0$ interacts with the sample, resulting in scattered light $\lambda_0 \pm \lambda_R$.
- The scattered light is detected by the observer.

**Remarks:**
- Excitation of fluorescence is possible, but minimum fluorescence with NIR excitation due to low absorbance.
IR and Raman Spectra of Biomolecules

Spectra provide the highest information content of all spectroscopic tools. Each molecule is represented by its vibrational spectroscopic fingerprint. IR and Raman intensities depend on physical selection rules:

- Some bands not IR active
- Some bands not Raman active

Complementary to some extent
Vibrational Spectroscopic Imaging Methodologies

Confocal Raman Microscope

- Laser
- Coupler
- Single-mode fiber
- Beam splitter
- Objective
- Sample
- Motorized stage
- Notch filter
- Video camera
- Multi-mode fiber
- Grating spectrometer

Confocal Infrared Microscope

- Detector
- Single channel
- Interferometer
- Sample stage
- Schwarzschild objective
- Schwarzschild condenser
- Upper aperture
- Lower aperture
- Upper focusing mirror
- Lower focusing mirror

Mapping: Sequential registration of spectra by moving the sample
Vibrational Spectroscopic Imaging Methodologies

Imaging: Parallel registration of all spectral data within the field of view without moving the sample (128x128 arrays available)
Hyperspectral Data Cube of Spectroscopic Imaging

Data set:
- Two lateral dimensions
- One spectral dimension
- Size up to 1 Gb per data set

Chemometric analysis:
- Identification of spectral features
- Correlation with sample properties
- Univariate: one feature (band intensity, position)
- Multivariate: many features over broad range
Advantages for Biomedical Applications

Vibrational spectra
⇒ "Fingerprint" for composition and structure of biomolecules
⇒ "Fingerprint" for their assemblies: cells and tissues
⇒ Diseases connected with molecular changes and biomarkers

Vibrational spectroscopic images
⇒ Inhomogeneity accessible at cellular and subcellular level
⇒ Data collection by automatic procedures
⇒ Interpretation by computerized algorithms

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<tr>
<th>Label-free, rapid</th>
<th>IR</th>
<th>Raman</th>
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<tr>
<td>√ Radiation non-destructive</td>
<td>2.5 - 25 µm</td>
<td>0.4 - 1 µm</td>
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<tr>
<td>√ Lateral resolution</td>
<td>5-10 µm</td>
<td>1 µm</td>
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<tr>
<td>√ Penetration depth</td>
<td>µm</td>
<td>mm</td>
</tr>
<tr>
<td>√ Specificity</td>
<td>strong</td>
<td>strong</td>
</tr>
<tr>
<td>√ Sensitivity</td>
<td>limited</td>
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Congenital Disorders of Lung

**Congenital cystic adenomatoid malformation (CCAM):**
- most frequent cause of neonatal respiratory distress
- failure of normal bronchovascular formation in the pulmonary mesenchyme.
- connected to trancheobronchial tree,
- derive vascular supply from pulmonary circulation

**Bronchopulmonary sequestration (BPS):**
- benign pulmonary tissue
- lacking trancheobronchial connection
- anomalous systemic blood supply arising from thoracic or abdominal aorta

Coexistence suggests common embryologic origin

Detection by ultrasound, computed tomography, magnetic resonance imaging

Treatment of choice: segmentectomy, lobectomy within the first year of life.

Differential diagnosis important
- exclude associated anomalies
- exclude development of pathologies
  - determine treatment options
  - estimate prognosis
Raman and FTIR Image of Normal Lung Tissue Section of an Infant

FTIR: 64x64
62.5µm per spectrum
5 min per image

FTIR: 57x60
100µm per spectrum
100 spectra per hour

FTIR Spectra of Lung Tissue

Spectral contributions of proteins dominate
Scattering artefacts near tissue margins: band shift and unusual background
Raman Spectra of Lung Tissue

Spectral contributions of hemoglobin dominate

Spectra are decomposed into two components
Hemoglobin (pre-resonance enhanced)
Rest (not enhanced): proteins and lipids
Raman and FTIR Macroscopic Imaging of CCAM

Cluster Analysis: diseased tissue ⇒ blue

FTIR

Light microscopy ⇒ sponge-like appearance vs. solid tissue

Raman

Control tissue

CCAM

FTIR Spectral Signature of CCAM

Comparison of control tissue (green cluster) and CCAM (blue cluster): Less hemoglobin, more phospholipids
Raman and FTIR Microscopic Imaging CCAM

Cluster Analysis: diseased tissue ⇒ blue

FTIR
4µm per pixel

Light microscopy
⇒ morphologic
details

Raman
10µm per pixel

Control tissue
CCAM
Raman Spectral Signatures of CCAM

Comparison of control tissue and vessel (yellow cluster):
More collagen typical of smooth muscle

Comparison of control tissue and microcrystal (black cluster):
Phosphatidylcholine plus cholesterol
Raman and FTIR Macroscopic Imaging BPS

Cluster Analysis: diseased tissue ⇒ magenta

FTIR
Light microscopy ⇒ morphologic differences
Raman
Marginal tissue
BPS

Raman and FTIR Spectral Signatures of CCAM and BPS

Control tissue (1)
BPS (2)
CCAM (3)
Marginal tissue (4)

Spectral features in (3) and (4) similar: CCAM in marginal tissue

Differential diagnosis possible
Fiber Optic Probes for Raman Spectroscopy
Challenges of Fiber Optic Probe Raman Spectroscopy

![Raman spectra of unfiltered probe with and without bronchus](image)

![Difference](image)

Raman Imaging with Handheld Fiber Optic Probes

Animal model: brain metastasis in living mouse
Acquisition through cranial window (4s per spectrum)
Laser focus using InPhotonics probe (≈120 µm)
Laser intensity at 785 nm (≈200 mW)

Cluster analysis of 37x33 Raman image:
Tumor (gray)
Raman Spectroscopy with Miniaturized Fiber Optic Probes

Animal model: rabbit after high fat diet

(a) aorta bifurcation
(b) aorta thoracalis
(c) aorta arch
(d) aorta ascendens
Diagnostic Pattern Recognition by FTIR and Raman Spectroscopy

Principle:
Drop (5µl) of biofluid (blood, serum, plasma, urine…) onto substrate
FTIR or Raman spectrum
DPR model for classification or quantification

Examples:
- hepatitic fibrosis in patients with chronic hepatitis C (Scaglia et al. 2011)
- acute myocardial infarction in patients with acute chest pain (Petrich et al. 2009)
- bovine spongiform encephalopathy (Menze et al. 2007)
- quantitative serum analysis including glucose monitoring (Rohleder et al. 2004)
- rheumatoid arthritis (Staib et al. 2001)
- diabetes mellitus (Petrich et al. 2000)

Bronchoalveolar lavage?
DPR Results of Patients with Acute Chest Pain using FTIR Spectra

Clinical study of 389 patients
1429 serum samples

(a) native sample
(b) ultrafiltration cut-off 100kD
(c) ultrafiltration cut-off 10 kD

Threshold DPR score = 0.5
sensitivity 88.5%
specificity 85.1%

Comparison with clinical tests

(Petrich et al. Analyst 2009)
Identification of Circulating Tumor Cells by Raman Spectroscopy

- Detach from tumors, enter blood system, and contribute to metastatic growth
- High potential in diagnostics
- Blood sample less invasive than biopsies
- Determination of primary tumor
- Monitoring tumor spread and success of therapy
- Only few tumor cells in one million blood cells

Model:
- Leukocytes from patients’ blood
- Leukemia cell line (OCI-AML3)
- Breast cancer cell lines (MCF7, BT20)
Raman Activated Cell Sorting in Microfluidic Chips

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Sensitivity

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<td>99.5%</td>
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Dochow et al. (2011) Lab-on-Chip
Raman Spectroscopic Histopathology at Cellular Level

Tissue section of brain tumor

Raman imaging complement histopathology:
Results comparable with H&E staining
Plus
Chemical information (here: DNA and lipids)
Coherent Anti-Stokes Raman Scattering (CARS) Microscopy

Advantages:
- no fluorescence
- directed emission
- signal amplification
- rapid image
- acquisition
CARS Imaging at Video Time Frame Rates

Detection of tumor, tumor margin, tumor islets

Identification of cell nuclei in tumor

Limited molecular information due to single band imaging

T. Meyer et al. JBO (2011)
Summary

Raman and infrared spectroscopic imaging: innovative diagnostic tools
 Chemical information without labels
 Chemical fingerprint of pathologies
 in vitro: tissue sections, biopsies
 in vivo: surface exposed, endoscopic, spatial offset Raman spectroscopy

Future developments:
 Algorithms for classification
 Compact instruments
 Flexible fiber optic probes

Overview in Reviews:
 Biomedical applications of Raman and IR spectroscopy to diagnose tissue (2006)
 Disease recognition by infrared and Raman spectroscopy (2009)
 Raman and CARS spectroscopy of cells and tissues (2009)
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