Applications of IR and Raman methods from molecules to microorganisms

Revision

- Vibrational Spectroscopy – generalities
- IR Methods
  - FTIR, NIR
  - ATR-IR/AFM
- Raman methods
  - Raman, FT-Raman, Resonance Raman, SERS, Raman-AFM
  - Non-linear Raman methods
  - CARS
  - Hyper-Raman
- Raman and FTIR biomedical applications

Raman/FTIR on hair

Photomicrograph of longitudinal section of human hair representing 1 day’s growth. IR spectrum is from a 5.5 um spot representing 22.5 min in the life of drug user.

Forensic applications

White light and Raman image of crossing inks. The Raman image shows that two different inks were used to form the figure and reveals their deposition order.

Forensic applications

Trace amount of cocaine particles in a fingerprint. Fingerprint residue (red) and cocaine particles (white) were collected at high spatial resolution.
Microfluidic (lab-on-a-chip) device for SERS detection.
a Schematic illustration of a microdroplet channel for SERS detection.
b SERS spectra of paraquat at different concentrations.
c Plot of SERS intensity at 1651 cm⁻¹ versus the paraquat concentration.

In situ SERS detection of pesticide thiram using (Fe₃O₄@NRs).
a Thiram solution was sprayed on apple peel.
b Fe₃O₄@NR suspension was spread on apple peel.
c Fe₃O₄@NR microspheres were transferred from contaminated apple peel to glass slide with the aid of the external magnetic field.
d The spectrum of thiram was collected by portable Raman spectrometer.

Cell imaging by FTIR

IR bands of DNA

(A) Vibration bands in the IR spectrum of mouse kidney DNA with assignment to relevant substructures.

(B) IR spectra for pancreatic DNA extracted from cancer and normal tissue, respectively. The lowest trace is the spectrum of acetate contaminated DNA from healthy tissue, resulting from the preparation.
Cellular imaging using Raman spectroscopy

Figure 2. Unprocessed Raman spectrum of live MCF7 breast cancer cells. 300 second acquisition time, 75 mW laser illumination, approximately 100 mW illumination power.


Figure 3. Fluorescence of formalin-fixed paraffin-embedded MCF7 cell line in vitro (left). Raman images after segmentation by cluster analysis (middle). Raman spectra (right) representing the nucleus (trace 1; red cluster), the cytoplasm (trace 2; cyan cluster) and lipid vesicles (trace 3; green cluster). © The Royal Society of Chemistry.

Raman imaging of a single cell of MCF7

Fatty acids

Proteins

4-Mercaptobenzoic acid


Basal cell carcinoma (BCC)

Confocal Raman profiles of live tissue with an interval of 50–400 μm. © Kneipp.

Cancer cell targeting and SERS readout

Figure 10. Cancer cell targeting and Raman spectroscopic detection by using antibody-conjugated SERS nanoparticles. Preparation of targeted SERS nanoparticles by using a mixture of 18KPEO (keto–polyethylene glycol) and a hetero-functional PEG (SH-PEG-COOH). Conjugation of an TOSeantibody epithelial growth factor receptor (EGF) fragment occurs at the exposed terminal of the hetero-functional PEG. © 2010 Nature Publishing Group.
Hand-held Spectroscopic Device for In Vivo and Intraoperative Tumor Detection: Contrast Enhancement, Detection Sensitivity, and Tissue Penetration

Raman spectrum of esophageal tissue measured using 830 nm excitation. Characteristic biochemical peaks have been labelled. Variations in peak height and position can be detected in tissue spectra and have been shown to indicate biochemical progression towards malignancy.

Advances in the clinical application of Raman spectroscopy for cancer diagnostics

Thalassemias comprise a group of genetic disorders of hemoglobin synthesis involving mutations that reduce or abolish α- or β-globin hemoglobin chain synthesis. The hallmark of β-thalassemia is an excess of α-chains due to quantitative defects in the β-globin chain; unbound α-chains denature and precipitate, shortening the lives of red blood cells.

Diabetes is an attractive target to assess the validity and accuracy of spectroscopy-based diagnostic testing. Serum glucose levels are known to change with the onset of diabetes, and serum cholesterol and triglycerides can also be affected by concurrent metabolic disorders.

Class averages of dried serum mid-IR spectra for type 1 diabetic patients and 42 controls, along with the difference between them. Most significantly, that approach proved capable of correctly categorizing the diabetic samples for which none of the three conventional serum assays (glucose, cholesterol, and triglycerides) were outside normal limits.
Resonance Raman spectroscopic evaluation of skin carotenoids as a biomarker of carotenoid status for human studies

Clinical use of portable RRS scanner with fiber optical module for heel skin carotenoid measurements in infants

Carotenoids show a high Raman cross-section due to the resonance Raman effect

Macular pigment is comprised of Zeaxanthin and Lutein, which are found in the center of the macula (fovea) at a natural 2:1 ratio. MPOD (Macular Pigment Optical Density) is important for three specific reasons:

1. Low macular pigment is a key risk factor for Age-related Macular Degeneration (AMD), the leading cause of significant vision loss over age 55
2. Macular pigment absorbs harmful blue light, protecting the photo-receptors from damage
3. Macular pigment improves visual performance

Correlation of RRS signals obtained for the C=C double bond vibration at 1525 cm⁻¹ with the carotenoid content of six monkey retinas as determined by HPLC. A linear fit to the data results in a correlation coefficient of 0.68.

RRS MP measurements of 33 normal eyes for a young group of subjects ranging in age from 21 to 29 years. Note the large 10-fold variation of RRS levels between individuals. Since the ocular transmission properties in this age group can be assumed to vary similar, the variations are assigned to differing MP levels. Subjects with extremely low carotenoid levels may be at higher risk of developing macular degeneration later in life.
Skin carotenoid resonance Raman detector, showing argon laser, spectrograph, light delivery/collection module, and excitation laser spot on the palm of the hand of a subject. A typical measurement involves the placement of the palm of the hand against the window of the module and exposing the palm for about 1 min at laser intensities of 10 mW in a 2-mm-diam spot.

a) Correlation of skin resonance Raman intensity measured in the inner palm of the hand with serum carotenoids determined by HPLC, obtained for a group of 104 healthy male and female adults.

b) Histogram of skin carotenoid resonance Raman response measured in the palm of hands for 1375 subjects, showing wide distribution of skin carotenoid levels in a large population.

Comparison between Raman spectra arising from freshly human whole blood collected using 785 nm excitation laser line (red line) and 514 nm excitation laser line (green line). The spectra show several molecular fingerprints: the green line (514 nm) represent the -carotene molecule while in the red line (785 nm) are clearly distinguishable the presence of oxy-haem peaks.

Comparison between Raman (red line) and SERS (green line) spectra arising from freshly human whole blood collected using the same laser excitation line (514 nm). Both spectra show several fingerprints due to the different use of Raman effect. The Raman spectrum shows the beta-carotene fingerprint, while the SERS one provides haemoglobin identification with information on the oxygenation state.

Clinical study

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Gastrointestinal cancers, including colon and esophageal cancer, are some of the most prevalent diseases worldwide. Early detection is key to patient survival, and could be aided by wide-field molecular imaging technologies. However, accurate detection is hampered by the variability in molecular expression patterns exhibited between patients and within patients over time. Therefore, we are developing in vivo endoscopic imaging devices that utilize a single laser illumination source to image surface-enhanced Raman scattering (SERS) nanoparticles that are capable of being highly multiplexed to target a large number of biomarkers.

A spectral-detection system with a contact probe for quantifying the relative concentrations of multiplexed SERS NPs topicaly applied on fresh intact tissues (ex vivo and in vivo). Y. Wang, et al., Technology 2, 1-15 (2014).