Moving beyond single-point Raman spectroscopy: development of a hand-held Raman imaging probe for intraoperative tumor margin assessment

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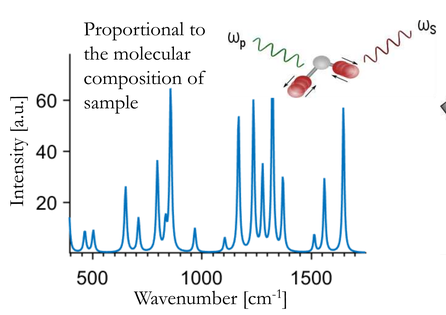
Introduction

Cancer diagnosis → Resection of the tumor by a surgeon

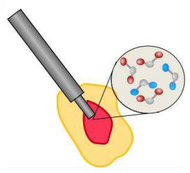
Normal / Cancer

Problem: For several oncology procedure, surgeons need to precisely identify tumor margins. Current imaging methods(for example: MRI, CT) to discriminate between cancerous and normal tissue can be limited in sensitivity and specificity.

Propose solution: **Intraoperative Raman spectroscopy**



Current Raman probe collects the inelastically scatter light for one point



Old system (point measurement)

With the old probe at least two measurements are needed to identify the tumor margin.

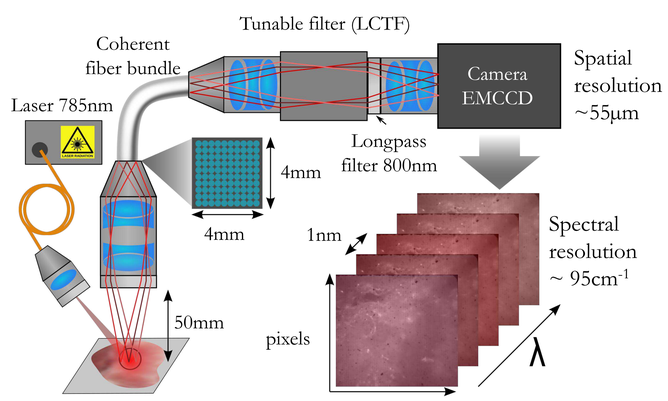
Our goal

New system (Wide-field)

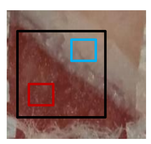
Develop a Raman imaging probe to help the surgeon clearly visualize the tumor margin using tissue molecular contrast

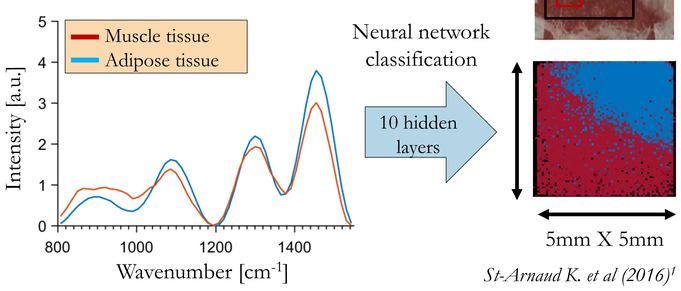
Previous work

We developed a handheld Raman imaging system using a coherent fiber bundle. At the distal end of the bundle, a relay lens insures collection of Raman signals and rejection of Rayleigh scattering. At the proximal end, a liquid crystal tunable filter (LCTF) with an EMCCD camera (Nüvü) insures detection of a Raman spectrum for each camera pixel. Hyperspectral Raman images are collected using following spectral scanning.



We were able to reconstruct images of porcine tissue base on the molecular contrast using Neural Network classification.





St-Arnaud K. et al (2016)[1]

Methods

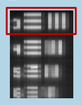
Wide-field imaging system

Instead of using spectral scanning our new system spatially scans a line across the sample to reconstitute a Raman image. Each line is projected at the entrance slit of the spectrometer. Detection is done using a Andor Newton 920BR-DD CCD camera. Illumination and detection are combined using a dichroic notch filter centered at 785nm.

Spectral resolution: 13 cm^{-1}

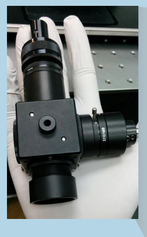
Spectral range: [755-1800]cm^{-1}

Spatial resolution: 200µm



1951 USAF target

System can resolve feature from group 2 element 3.



Signal Processing

For each pixel:

$ID(x_i,y_i,\lambda) = Raw(x_i,y_i,\lambda)-D(x_i,y_i,\lambda)$

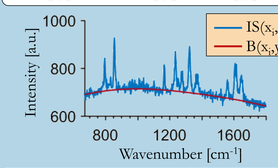
Darkcount (**D**) substraction (measurement with laser off)

$IS(x_i,y_i,\lambda) = ID(x_i,y_i,\lambda)/Tsys(x_i,y_i,\lambda)$

Evaluation of the system response (**Tsys**) using a NIST Raman standard for 785nm (SRM 2241).

Spectral and spatial normalisation

$IB(x_i,y_i,\lambda) = IS(x_i,y_i,\lambda)-B(x_i,y_i,\lambda)$



Iterative 6th polynomial fit to remove background

$I(x_i,y_i,\lambda) = F_{sgolay}[IB(x_i,y_i,\lambda)]$

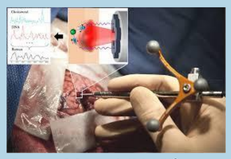
Savitzky-Golay filter (Window = 17, Order = 2)

Measurements

Hyperspectral line image acquisition

- **Repetitions:** 3 per line acquisition
- **Time:** 2s per line imaging (100 spectra)
- **Step size:** 100µm
- **Intensity:** 0.45J/cm^2 (MPE skin: 1.4J/cm^2)

Validation with Raman probe



Jermyn M. et al (2015)[2]

Validation achieved using the single point Raman probe developed for brain cancer detection. Point measurements were spatially registered with wide-field images

Gold standard: Raman probe

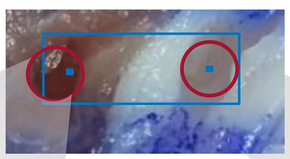
- Acquisition time: 0.2s
- Intensity: 3.58J/cm^2 (MPE skin: 1.1J/cm^2)
- Spectral resolution: ~15cm^{-1}

Results

Raman images were taken on porcine tissue at the border of muscle and adipose tissue. The spectra were compared with probe measurement using the correlation coefficient of Pearson **(r)**.

Imaging area 2.6mm X 7.1mm

- 7242 Raman spectra
- Total acquisition time: 145s
- 71 moving steps



Probe measurement Ø = 2mm

Wide-field Raman spectrum

- Average over 25 spectra

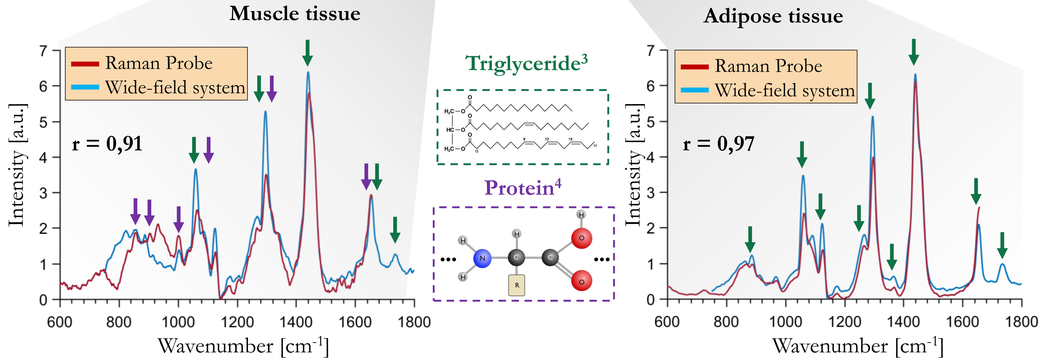
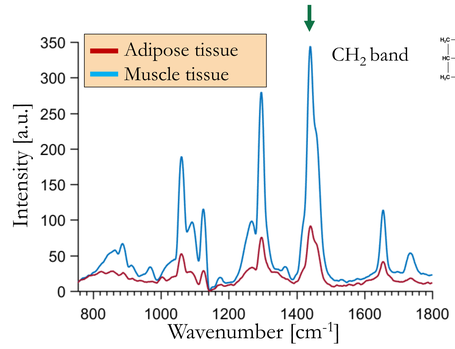
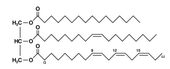


Image reconstruction

Image reconstruction is possible using Raman spectroscopy to highlight the molecular contrast between adipose and muscle tissue.

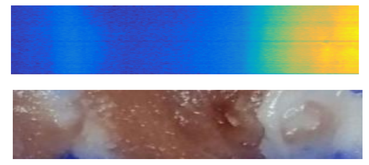


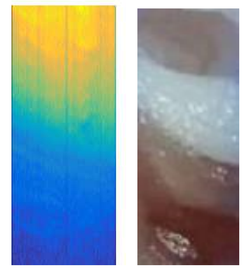


CH$_2$ peak is more intense in adipose tissue since the contribution of triglyceride is more important. The intensity of this band can be used to highlight the contrast between muscle and adipose tissue.

To the top the Raman image and below the white light image

- **Area:** 2.6mm X 14.1mm
- **# spectra:** 14 382
- **Time acquisition:** 288s



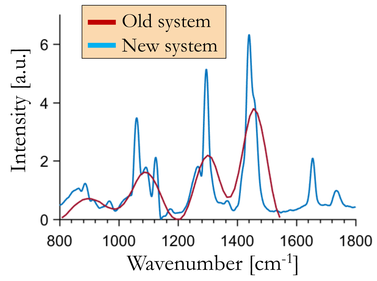


To the left the Raman image and to the right the white light image

- **Area:** 2.6mm X 7.1mm
- **# spectra:** 7242
- **Time acquisition:** 145s

Discussion

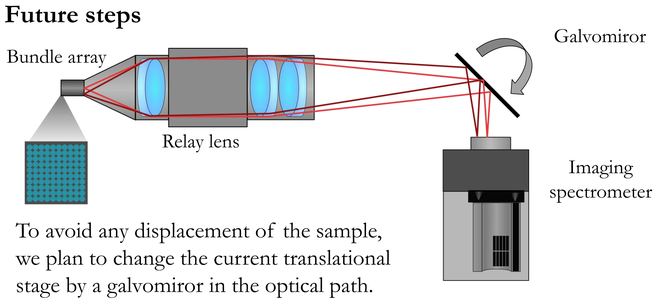
Comparaison with previous system



- **Spatial resolution** ↓
- **Spectral resolution** ↑
- **Signal-to-noise ratio** ↑
- **Field-of-view** ↓
- **Time acquisition** ↑

A few modification in the optical design could improve the spatial resolution and field-of-view.

Future steps



To avoid any displacement of the sample, we plan to change the current translational stage by a galvomiror in the optical path.

Custom made lens

We currently use off the market lens and optomechanics. Changing for custom made components could improve different aspects of the current system such as: the spatial resolution the field-of-view and the sensitivity.

References

1. St-Arnaud K. *et al*, Opt. Lett. **41**, 4692-4695 (2016)
2. Jermyn M. *et al*, Sci. Transl. Med. **7**, 274ra19, (2015)
3. J.R. Beattie *et al*, Lipids, **41**(3), 287-294, (2006)
4. J.R. Beattie *et al*, Meat Sci. 80(4), 1205-1211, (2008)