ULTRA FAST RAMAN HYPERSPECTRAL IMAGING USING BRAGG TUNABLE FILTERS AND A HIGH PERFORMANCE EMCCD CAMERA

F. Thouin[a], E. Gaufrès[a], N. Cottenye[a], F. Leblond [b], R. Martel[a], M. Verhaegen[d], O.Daigle[c]

[a]*Department of Chemistry, University of Montreal, Montreal QC*
[b]*Engineering Physics Department, Ecole Polytechnique Montreal, Montreal QC*
[c]*Nüvü Caméras, Montreal, QC*
[d]*Photon Etc., Montreal, QC*

Introduction

Because of its high specificity to a variety of molecular processes and its low sensitivity to the presence of water, Raman hyperspectral imaging is regarded as a very promising technique to help pathologists improve the accuracy of medical diagnostics when compared to conventional histopathological analysis. Indeed, metastatic cells of malignant melanomas were identified by acquiring Raman maps divided by k-means clustering analysis using fiber optic probes [1] (*figure 1*) and a commercially available Raman microscope mapping system [2].

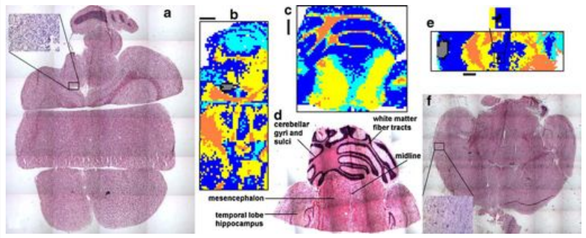

Figure 1. Pristine mice brain slices in H&E staining ((A), (D) and (F)) and corresponding Raman maps ((B), (C) and (E) respectively). Colors are assigned by cluster analysis so that tissues of high to low lipid-to-protein ratios are orange to blue, and high and medium concentrations of tumor cells are colored gray and high. Bar=1 mm. Taken from [1].

However, acquisition periods per hyperspectral data (cube) are very long - about 6 hours and they increase with spatial resolution - significantly reducing the appeal of this technique for *ex-vivo* diagnostics and rendering *in-vivo* applications impracticable.

To increase acquisition speed, we investigated the use of a high quality EMCCD combined with a Raman hyperspectral imager based on holographic Bragg tunable filters to acquire Raman maps.

The cameras

- Two cameras were used in this study: An EMN2 1024x1024 electron multiplying CCD by Nüvü Caméras and a Pixis 1024B CCD by Princeton instruments
- Cameras' quantum efficiencies, dark current, sensor and pixel size are **equivalent**.
- **Lower readout noise** with the EMCCD

	Princeton's CCD	Nüvü's EMCCD
Sensor size	1024x1024	**1024x1024**
Quantum efficiency	92% @ peak	**92% @ peak**
Dark current	0.0009 ē/pixel/s @ -70°C	**< 0.0006 ē/pixel/s @ -85°C**
Readout noise	15 ē rms @ 2MHz	**< 0.1 ē @ 20MHz**
Pixel size	13µm x 13µm	**13µm x 13µm**
Cooling	Thermoelectric	**Liquid nitrogen**

Table 1. Main specifications of the two cameras used in this study.

The imagers

- Two imagers: An **Alpha 300R** from WiTec and a **RIMA** from Photon etc.
- **WiTec Alpha 300R** (*figure 2 a)*):
  - Confocal microscope with a scanning stage
  - Uses a **transmission monochromator** and a spectroscopy CCD
  - Sample mapped by acquiring a spectrum point by point
  - Confocal excitation provided by a **30mW** 532 nm CW laser

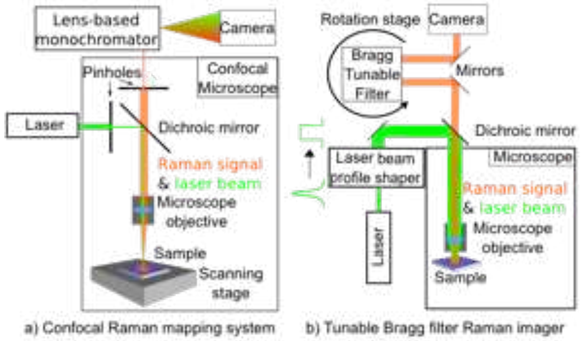

Figure 2. Schematized representation of the two Raman imagers used in this study.

- **Photon etc's RIMA** (*figure 2.b*):
  - Wide field microscope
  - Spectrum discriminated by a **Bragg tunable filter**
  - Sample is imaged by acquiring whole images at given wavelengths
  - Wide field Excitation provided by a **4W** 532 nm CW laser

The methods

- Sample: A 5 nm thick mat of **single walled carbon nanotubes** (CNT) on a Si substrate (*figure 5)*
- To compare the two imagers, we used the same:
  - spectral interval and step,
  - region of the sample
  - average power density (18 MW/m^2)
  - in-plane resolution (1 pixel=0.13µm).
  - SNR (about 26)
- To compare the two cameras, we used the same:
  - region of the sample
  - imager (RIMA)
  - integration time (4.5s)
  - excitation power (0.875 W).

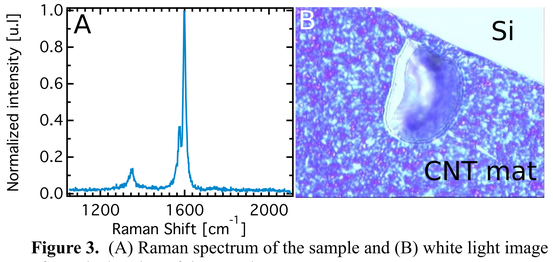

Figure 3. (A) Raman spectrum of the sample and (B) white light image of a typical region of the sample.

Experimental Results

- **RIMA** equipped with the Pixis 1024B obtained a 133µmx133µm cube in **1h13min** while the **Alpha300R** obtained a 40µmx40µm cube in **3h23min** (*figure 4 a) and b) respectively*).

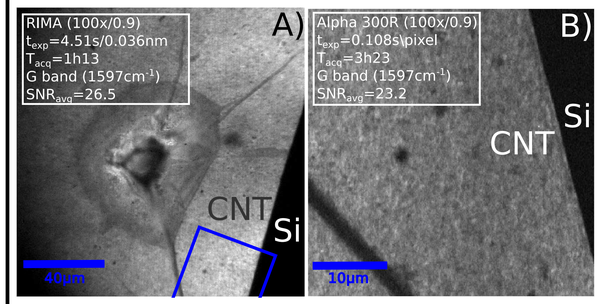

Figure 4. Grayscale images of the G band of CNT taken the hyperspectral cubes acquired by A) RIMA with a Pixis 1024B and B) by the WiTec Alpha 300R, which corresponds to the blue box of A).

- The SNR of an acquisition by RIMA with the EMCCD in analog or photon counting modes is measured and compared to the modeled SNR of the CCD

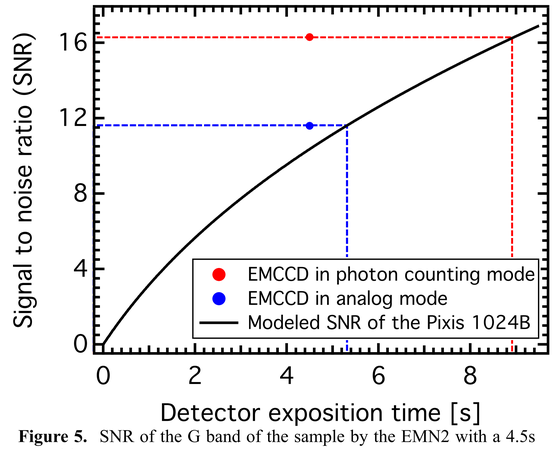

Figure 5. SNR of the G band of the sample by the EMN2 with a 4.5s exposition

- The EMCCD in analog and photon counting mode respectively achieved a **SNR of 11.59 and 16.30 in 4.5s** of acquisition.

Discussion

- Raman imaging using a tunable Bragg filter is about **30 times faster** than conventionnal point by point mapping since it acquired a map **11 times larger in 2.8 less time**.
- The EMCCD's **superior performance** is due to:
  - Negligible readout noise
  - Low clock induced charge (CIC)

$$SNR(t) = \frac{PQ_e t}{\sqrt{\left((1+\xi_{ENF})PQ_e t + I_{dark}t + CIC + \sigma^2/G^2\right)}}$$

- Acquiring the same SNR would have required 5.3 and 9 seconds with the Pixis1024B.
- This makes Nüvü Caméras' EMCCD in analog and photon counting mode respectively **1.18 and 2 times faster**.

Conclusions

- Nüvü Cameras' EMCCD was **as much as twice faster than the CCD.**
- The imager based on tunable Bragg filter was **30 times faster** than the confocal scanning microspectrometer.
- Combined together, Nüvü's EMCCD and Photon Etc's RIMA can make Raman imaging **60 times faster than standard technologies.**
- *In-vivo* and *ex-vivo* applications are now at reach, paving the way for real time tumor detection during surgery.

Future work

- Reduce the impact of background fluorescence:
  - From biological tissues (*figure 6*)
  - From the imager
- Solutions?
  - Time resolved Raman spectroscopy
  - Anti-stokes Raman spectroscopy
  - Lower excitation energies

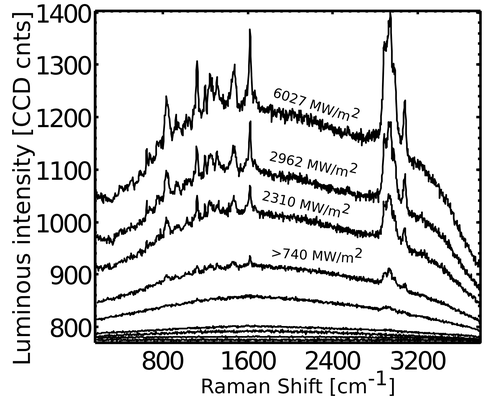

Figure 6. Raman spectrum of a slice of mouse brain fixed in formalin for various power densities. Acquired with the WiTec Alpha300R

References

[1] Krafft, C. et al., Methodology for fiber-optic Raman mapping and FTIR imaging of metastases in mouse brains, Anal Bioanal Chem 389, 1133–1142 (2007)

[2] Gajjar, K. et al., Diagnostic segregation of human brain tumours using Fourier-transform infrared and/or Raman spectroscopy coupled with discriminant analysis, Anal. Methods 5, 89-102 (2013)

Acknowledgements

We wish to thank the Montreal Neurological Institute for providing us with scientific grade mice brains.