Application for the Biophotonics 2019 Summer School

Improving Acquisition Speed via Line Scan Raman Microscopy for Biological Molecular Imaging in Combination with Line-Field Optical Coherence Tomography

Daniela Bovenkamp, M. Sc.[1]
Supervisor: Ass. Prof. Dr. Angelika Unterhuber[1]

[1]Medical University of Vienna, Center for Medical Physics and Biomedical Engineering, Waehringer Guertel 18-20, A-1090 Vienna, Austria

Research summary

Raman spectroscopy (RS) has found wide-spread applications in the biological and medical field. As it is a label-free technique no staining or tags interfere with the native environment. Upon excitation of vibrational levels of present molecules with a narrow band laser, chemical characteristics of tissue are gathered. Advantages of this technique comprise the ubiquitous applicability to a large variety of biological materials, including bacteria up to biopsies for disease analysis, and the vast information gained about the molecule distribution of the sample of interest. But, as only one in a million photons are Raman scattered, RS requires integrations times in the range of hundreds of milliseconds up to several seconds per point measurement. This disadvantage demands solutions to overcome the intrinsically slow process. Line Scan Raman Microscopy[1–3] (LSRM) allows for simultaneous acquisition of Raman spectra over a given line by creating a laser line over the sample instead of single points. Hence, raster scanning in x and y direction of the sample becomes obsolete as a sweep in one direction is sufficient to cover the area of interest. Thereby, the acquisition time is improved by a factor 6. For the sake of completeness, wide-field Raman imaging may be mentioned, as this technique also accelerates acquisition times and increases the spatial resolution but is only applicable to small samples. LSRM exceeds wide-field Raman imaging in terms of acquisition time at the cost of spatial resolution[4].

Currently, the point mapping scan of 30x30 points by our Raman spectroscopy system takes approximately 30 mins. In case of performing RS on biological samples, as we demonstrated pointwise RS measurements on bladder cancer in Bovenkamp et al.[5], acquisition times of 30mins exhibit issues in terms of the biopsy drying out and, thereby, altering its surface which potentially influences and distorts the scan. Hence, the implementation of LSRM is of high interest to perform faster RS. In figure 1, a test experiment with point mapping Raman microspectroscopy on salami is shown.

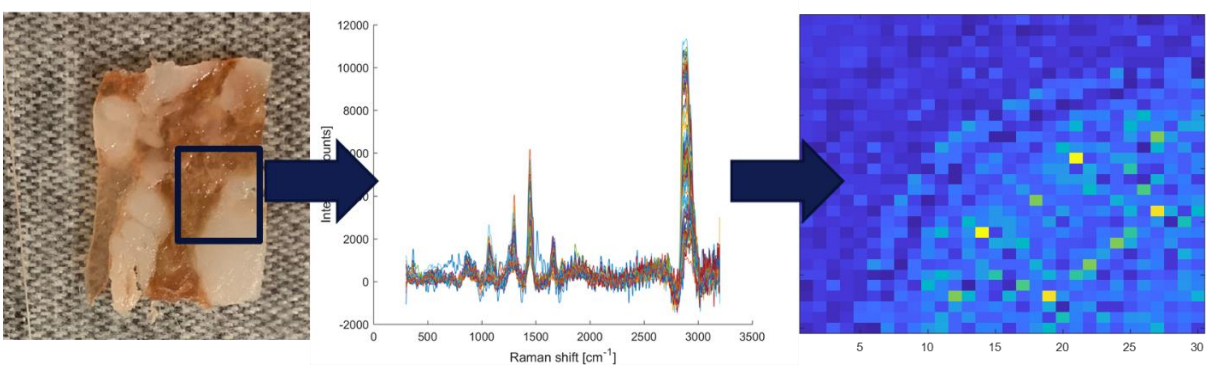

Figure 1 Point mapping Raman microspectroscopy on salami. Acquisition parameters comprised 1s integration time with 1s dwell time of the stage, 100mW on the sample, 30x30 points yielding a total acquisition time of 30 mins. Left: Image of salami with rectangular area of interest; Middle: Corrected Raman spectra; Right: Reconstructed Raman map at 2850cm^{-1}

Point mapping Raman microspectroscopy versus Line Scan Raman microscopy

As demonstrated by Schlücker et al.[4], the differences in point mapping Raman microspectroscopy and LSRM in terms of acquisition speed, applied laser power and signal-to-noise-ratio (SNR) are significant. Table 1 presents values for point mapping RS which have been measured in our current RS system, while estimations are given for LSRM. The acquisition time for point mapping measurements is composed of the number of points acquired (30x30=900), the integration time on the CCD chip and the delay for the trigger to move the stage. In our test measurements, we performed point mapping experiments with 1s integration time and 1s delay allowing the stage to move and stop yielding an overall acquisition time of 30 mins. In case of LSRM the scan of one dimension is eliminated, thereby accelerating the acquisition. Assuming a lower laser power density as for point mapping due to limited laser output power, increasing the integration time by a factor 10 is required, as demonstrated by Schlücker et al.[4], the acquisition time decreases to 5.5 mins (= 30 steps * (10s integration time + 1s stage delay)).

SNR and Raman signal are highly dependent on Raman cross sections of the material, laser power levels, integration time and entrance slit size of the spectrometer. As Raman cross sections of biological materials are comparably small, long integration times and high laser power densities are required. With increasing laser power, the signal and SNR improve, but, especially in inhomogeneous biological samples, higher laser power exhibits the potential issue of irreversible damage to the sample. Therefore, test measurements need to be performed to experimentally determine the optimal parameters.

The spatial resolution is defined the spot size of the laser beam. Currently, the microscope objective in our system provides a spot size of 10 µm. As we are interested in biological tissue as cancerous biopsies, mouse ear tissue in the wound healing process or the characterization of drugs, we aim for a larger spot size to collect an ensemble of the investigated sample. In case of biopsies containing potentially cancerous cells which exhibit sizes in the range of 10-20 µm, we aim for the bigger picture whether the biopsy is healthy or malignant rather than investigating single cells. To cover large areas, a slightly larger spot size (up to 15µm) might be favourable. Therefore, as the spot size is a characteristic of the microscope objective, different microscope objectives will be implemented depending on the required resolution.

	point mapping (measured)	*line scan (estimated)*
power density	0.31 MW/cm²	0.003 MW/cm²
spectral resolution	2 cm^{-1}	2 cm^{-1}
spatial resolution	10 µm	10-15 µm
SNR	14	62
acquisition time	30 mins	330 s = 5.5 mins

Table 1 Comparison of point mapping Raman spectroscopy versus line scanning Raman microscopy

The overall advantage of LSRM compared to point mapping RS comprises the faster acquisition at the cost of laser power per unit area.

Concept of Line Scan Raman Microscopy

Outperforming point mapping RS[6], LSRM has been applied to cells for chemical imaging by Qi et al.[7]. As these groups have shown LSRM improves acquisition times by a factor of 100 and, thereby, demonstrate the potential of LSRM. Hence, the goal is to apply this RS variant to the accelerated RS mapping on biological samples.

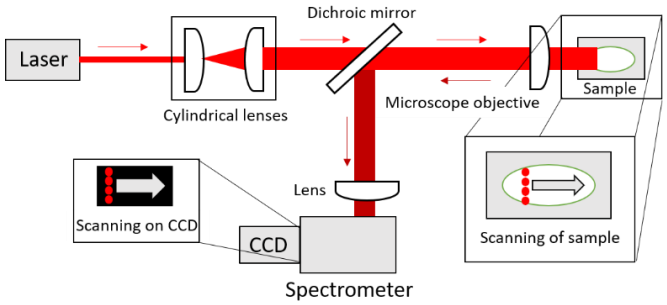

Figure 2 Illustration of the Line Scan Raman microscope. A laser at 785nm is shaped into a light sheet with cylindrical lenses followed by focusing the laser line via a microscope objective onto the sample. A stage scans the sample as it is moved. The microscope objective collects the Raman signal from the sample, followed by separation of the Raman signal from the Laser light with the dichroic mirror. Focusing into the spectrometer is achieved with an additional lens. As the sample is scanned by moving the stage, Raman spectra are collected per line.

The RS system comprises a narrow band diode laser at 785nm (VBG-Stabilized Single Laser Source, LS-1-78-1-FA), cylindrical lenses to achieve the light sheet and a microscope objective to focus onto the sample. The maximum output power of the laser is 600mW, for most measurements we apply 20-100 mW on the sample corresponding to a power density of 0.31 MW/cm². The specifications of the cylindrical lenses depend on effective focal lengths to determine the required height of the light sheet. For the detection of the Raman signal we implement a spectrometer (Shamrock 303i, Andor Technology) and a detector (Newton 920, Andor Technology). The spectrometer is equipped with a grating with 830 grooves/mm and a blaze at 900nm. The resolution at 880nm is 2cm^{-1}. Important specifications of the detector include the CCD chip size of 1024x256 pixels with a pixel size of 26µm yielding an active area of 26.7x6.7mm. The sample is placed on and moved by a stage of Zaber (X-LSM050A). Thereby, the stage facilitates the line scanning of the sample. The microstep size (default resolution) of the stage is 0.048 µm and yields an accuracy of 20 µm at a repeatability of sub 3 µm. The maximum speed of the stage is 26mm/s. As the collection of Raman scattered photons demands integration times of seconds, the detector and stage meet the speed requirements to acquire data at maximum Raman speed.

Outlook: Combination of LSRM with Line Field Optical Coherence Tomography

The strength of RS lies in providing information about the chemical distribution of molecules in biopsies but lacks morphological properties. Optical coherence tomography[8] (OCT) complements the molecular characteristics by contributing structural features. Hence, we plan on integrating a line field spectral domain OCT (LF-OCT) system featuring a Titanium:Sapphire (Ti:Sa) laser. Comparable to LSRM, the sample is probed with a laser line generated with cylindrical lenses. The systems will be combined and integrated in one system by matching the spectral requirements. A galvanometric mirror will be inserted to the existing system to scan at faster scan rates as OCT is not dependent on integration times in the range of seconds. To achieve a map on molecular as well as morphological characteristics, the line lengths and widths of the LSRM and LF-OCT are matched. Thereby, information gathered by both modalities are colocalized. Further investigations are planned on correlations between the co-registered data.

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