Introduction

Time-resolved Raman and fluorescence lifetime spectroscopy and imaging is a key application of single photon avalanche diode (SPAD) sensors (Finlayson et al. 2023). Raman photons typically arrive before fluorescence emission photons and these can therefore be distinguished from each other using time-resolved detection. New material research becomes possible as a result, in applications including biomedical diagnostics, quantum optics, carbon materials, and battery development. Here we describe time- resolved Raman spectroscopy and imaging using the Sirona sensor.

Materials and Methods

Sirona supports in-pixel CMOS construction of time-resolved spectral histograms in each of up to 1024 spectral channels, with up to 50ps time resolution. Photon arrival time events are recorded in every pixel histogram from the initial excitation of a laser excitation pulse to a time range extending from tens of picoseconds to hundreds of nanoseconds. Simultaneous acquisition of Raman and fluorescence signals are carried out in each spectral channel (Usai et al. 2019). Motorized and beam scanning microscopy systems can then be used to produce combined Raman and fluorescence images composed of highly detailed 4D data-cubes having two spatial dimensions, one spectral dimension and one time dimension.

The Sirona sensor is a CMOS Single Photon Avalanche Diode (SPAD) line sensor with per-pixel histogramming time-to-digital converters for time-resolved multispectral imaging. Shot-noise limited sensitivity, time-correlated single photon counting (TCSPC) functionality and room temperature operation set the Sirona SPAD sensor apart from CCD device technology. Extensive triggering options are available for integration with a range of scanning systems and laser sources. A delay generator with 63 ps time resolution and 8 configurable histogram time-ranges is included.

Optical System

The time-resolved optical microscope built around the Sirona SPAD sensor is shown in Fig. 1. A pulsed laser (typically NKT Katana, wavelength 532nm, pulse duration <50 ps, repetition rate 1-40 MHz, linewidth 0.15nm) is directed at the sample using a dichroic mirror. The laser beam incidence position on the sample can be changed using either a motorised stage or with scanning mirrors, producing an XY scan at an image plane at the back aperture of the primary objective.

Raman and fluorescence signals are transmitted back through the dichroic, through an ultra-steep long-pass emission filter and coupled to a custom f/1.5 spectrometer through a 10× 0.25NA microscope objective and 50 µm diameter fiber optic patch cable that acts as the system pinhole. Light emerging from the other end of this fiber is collimated with an achromatic doublet lens and then diffracted by an 1800 lp/mm transmissive holographic grating. Finally, the photons are focused using an achromatic doublet lens and collected by the CMOS SPAD line sensor. The spectrometer spectral range is approximately 80 nm, and the spectral resolution is approximately 0.16 nm.

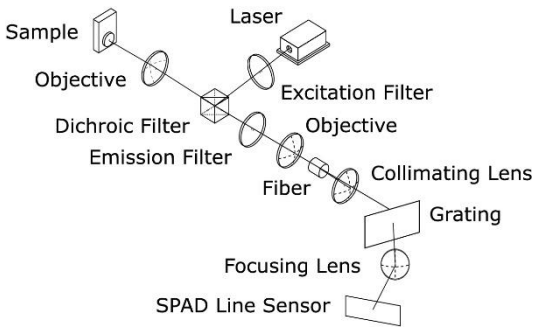

Fig. 1 Optical Raman Microscope (Finlayson et al 2021)

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Sesame Oil

In Fig. 2, we present a time-resolved spectrum of sesame oil. Raman and fluorescence signals are captured using an exposure time of 10 s. Known sesame oil Raman peaks at 1080, 1265, 1300, 1440, 1660, and 1750 cm−1 are visible, together with a broad fluorescence background with a lifetime estimated to be 2.7ns.

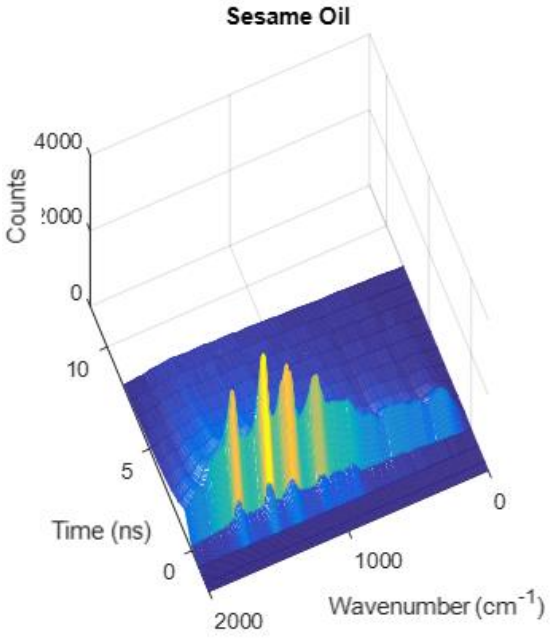

Fig. 2 Time-resolved Raman and fluorescence of sesame oil

Raman and Fluorescence Imaging

Raman imaging was investigated using mixed samples consisting of calcite and carbon single wall nanotubes (SWNT). Calcite has strong easily identifiable Raman lines, while carbon allotropes such as diamond, graphite and more unusual allotropes such as SWNT and graphene are materials of considerable interest with distinctive Raman signatures.

The SWNT sample was fabricated by high-pressure decomposition of carbon monoxide. Individual SWNT were found to have diameters in the range 0.7–1.4 nm. Raman spectra of SWNT demonstrate these typical labelled spectral features: SWNT diameter dependent radial breathing mode (RBM) in the range 150- 280 cm^{-1}, the so-called D-line at 1350 cm^{-1}, the G-line at 1585 cm^{-1} which splits into metallic (G-) and semiconducting (G+) species, and the G'-band at approx. 2700 cm^{-1}.

The SWNT sample was overlaid onto a calcite background sample. The samples can readily be distinguished in these images through their distinctive time-separated Raman spectral features. In addition, highly fluorescent regions in the images can also be identified at later times, arising from defects.

The resulting time- resolved 64x64 pixel motorized scanner images are shown in Fig 3. A 6 frame time-sequence highlighting the SWNT region at a Raman wavenumber of 1585 cm^{-1} (the G+ line position) is shown in the top row. Each frame is separated by an interval of 100ps. A 6 frame time-resolved sequence highlighting the calcite region at a Raman wavenumber of 1086 cm^{-1} is shown in the bottom row. Both sequences highlight the growth of the Raman signals in the 0-200ps time range. It is important to note that both rows of images are subsets of a *single* time-resolved datacube. The calcite and SWNT regions are strongly differentiated through their distinctive Raman signatures, lighting up or essentially invisible at quite distinct wavenumbers.

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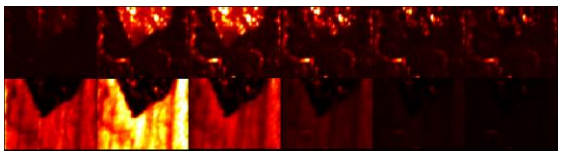

Fig. 3 Time-resolved Raman and fluorescence images of calcite and carbon nanotubes. The frames in each sequence are obtained from successive 100ps histogram bins at two different wavenumbers. Top row: carbon single wall nanotube G^+ image region at a wavenumber of 1585cm^{-1}. Bottom row: calcite image region at 1086cm^{-1}. Calcite, carbon nanotube and longer lived fluorescence regions are clearly distinguished (Finlayson et al 2023)

Conclusion

The capabilities of Sirona SPAD-based time-resolved Raman and fluorescence detection were highlighted in this note using oil, carbon and calcite samples. In-pixel photon timestamp histogramming in each spectral channel ensures that both Raman and fluorescence photon signatures are acquired simultaneously.

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References

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