Implementation of a novel low-noise InGaAs detector enabling rapid near-infrared multichannel Raman spectroscopy of pigmented biological samples

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Pigmented tissues are inaccessible to Raman spectroscopy using visible laser light because of the high level of laser-induced tissue fluorescence. The fluorescence contribution to the acquired Raman signal can be reduced by using an excitation wavelength in the near infrared range around 1000 nm. This will shift the Raman spectrum above 1100 nm, which is the principal upper detection limit for silicon-based CCD detectors. For wavelengths above 1100 nm indium gallium arsenide detectors can be used. However, InGaAs detectors have not yet demonstrated satisfactory noise level characteristics for demanding Raman applications. We have tested and implemented for the first time a novel sensitive InGaAs imaging camera with extremely low readout noise for multichannel Raman spectroscopy in the short-wave infrared (SWIR) region. The effective readout noise of two electrons is comparable to that of high quality CCDs and two orders of magnitude lower than that of other commercially available InGaAs detector arrays. With an in-house built Raman system we demonstrate detection of shot-noise limited high quality Raman spectra of pigmented samples in the high wavenumber region, whereas a more traditional excitation laser wavelength (671 nm) could not generate a useful Raman signal because of high fluorescence. Our Raman instrument makes it possible to substantially decrease fluorescence background and to obtain high quality Raman spectra from pigmented biological samples in integration times well below 20 s. Copyright © 2015 John Wiley & Sons, Ltd.

Introduction

Raman spectroscopy is widely used to characterize biological tissues and to detect the molecular changes associated with pathological processes, e.g. distinguishing malignant from non-malignant tissue. However, the application of Raman spectroscopy in the analysis of highly pigmented biological samples presents a problem. When using laser excitation wavelength up to about 850 nm intense laser-induced tissue fluorescence often makes it difficult or impossible to obtain high quality Raman spectra.

Several strategies to reduce the interference from fluorescence have been developed, such as time gated detection,1,2 photobleaching,3,4 a confocal signal detection scheme,5 surface enhanced Raman spectroscopy (SERS),6 and resonance Raman (RR) scattering. However, these solutions have insufficiently solved the problem for (in vivo) Raman spectroscopy of pigmented tissues because they are either not sufficiently effective, lead to complex measurement setups, and/or have not shown to be applicable in vivo.

Even though confocal Raman instruments can significantly reduce fluorescence background from out-of-focus regions, this reduction is insufficient for highly fluorescent samples. Also digital background subtraction techniques7–9 are not an appropriate solution to the fluorescence problem because they may be able to subtract the background but not the shot noise that is generated by the fluorescence. Some studies have used 785-nm excitation to obtain spectra of several types of pigmented skin lesions in the so-called fingerprint region (500–1800 cm⁻¹); baseline removal algorithms have to be employed to remove the very strong fluorescence background. This inevitably results in spectral artifacts, in particular when the Raman signal is weak compared to the fluorescence background. Moreover, background subtraction cannot remove the shot noise that is added by the background. Nevertheless,
using a large measurement volume (200-µm core diameter single fiber that illuminates a 3.5-mm diameter skin area), Lui et al. obtained useable Raman signals from excised pigmented tissues.[13]

The most established approach to reduce sample fluorescence in Raman spectroscopy is the use of excitation wavelengths outside the visible range: either in the near infrared (NIR) region, far above 700 nm,[14] or in the ultraviolet (UV), below 250–300 nm.[15] For application on biological tissues in vivo UV laser excitation is not desirable, as it may cause cell and DNA damage.[16] In addition, the penetration depth of UV light in tissue is in the order of a few microns.[16,17] Several studies have suggested that using an excitation wavelength with a photon energy far above 700 nm,[14] or in the ultraviolet (UV), below 250–300 nm.[15]

Fourier-transform (FT) Raman has been successfully used to obtain spectra of pigmented skin lesions.[20,21] This proves that the problem of tissue fluorescence can be overcome by using a longer laser excitation wavelength (1064 nm in the case of FT-Raman). However, FT-Raman spectroscopy is a multiplexing single-channel technique for which signal integration times are typically several orders of magnitude longer than for multi-channel Raman spectroscopy. This is not compatible with in vivo medical applications. Patil et al. have reported dispersive Raman spectroscopy of tissues with strong auto-fluorescence using 1064-nm excitation in combination with an indium-gallium-arsenide (InGaAs) detector array.[14]

Laser-induced tissue fluorescence was significantly reduced as compared to laser in the visible wavelength range, but at the cost of much lower signal-to-noise ratio (SNR) because of the high detector noise of the traditional InGaAs detector technology.

The ideal solution would be to use a higher wavelength laser excitation to reduce fluorescence in combination with low noise multi-channel Raman spectroscopy to enable short signal collection times.

Further reduction in interference from tissue fluorescence is seen in the high wavenumber (HWVN) spectral region (ca. 2500–4000 cm⁻¹), which is the part of the Raman spectrum with the largest Stokes shift from the laser line and thereby in most cases also away from the spectral region with the highest tissue fluorescence intensity.

Although the HWVN spectral region is not as rich in spectral features as the fingerprint region, it has been shown to provide clinical diagnostic information just like the more commonly used fingerprint region (400–1800 cm⁻¹), enabling the distinction of malignant and healthy tissue. Ample evidence supports the presence of sufficient spectral features required for demanding biomedical applications, such as a diagnostic tool for tissue malignancies.[1,2,22,23] In addition, in this spectral range the interfering Raman signal from fused silica in optical elements such as lenses and optical fibers is virtually absent, enabling very simple fiber optic probe construction.[24]

Until now the detection of Raman signals in the SWIR region (>1100 nm) was constrained by limitations of the detector technology. For visible to NIR excitation the state-of-the-art detector is the cooled charge coupled device (CCD) detector, which combines very low readout noise and very low dark current with high quantum efficiency. However, light with wavelength above 1100 nm cannot be detected because of the band gap of silicon. An alternative in this spectral range are InGaAs detectors, which enable detection at wavelengths well above 1100 nm.

During the past years there has been an increasing demand for low-noise dispersive spectroscopy solutions in the SWIR wavelength range. Dispersive spectroscopy allows multichannel detection. This, in contrast to for instance FT-Raman, enables simultaneous detection of Raman signal over a range of wavelengths, reducing the total integration time. Several companies have recently moved towards the SWIR range and have introduced InGaAs-based Raman devices. However, as mentioned above, InGaAs detectors show a very high inherent readout noise in comparison with CCD detectors. Also the dark current is orders of magnitude higher than in CCDs, even when cooled to 77 K with liquid nitrogen. As a result of the relatively poor noise characteristics of InGaAs detectors the SNR of the Raman signal is limited by the detector noise of the InGaAs detector and not by shot noise of the Raman signal, which is typical for CCD-based Raman spectroscopy. This is not ideal for demanding Raman applications such as Raman spectroscopy of biological tissues.

A new type of deep-cooled InGaAs detector for SWIR imaging applications has recently been introduced by Xenics (Leuven, Belgium). This detector exhibits extremely low noise characteristics, approaching those of high-end CCD detectors, combined with a high quantum efficiency (>90%) up to 1570 nm.

We have developed a Raman instrument based on this novel low-noise InGaAs imaging camera. We show how it can be used for Raman spectroscopy and provide examples of its performance in obtaining high quality HWVN-Raman spectra with low fluorescence background of samples that are difficult to obtain using CCD-based instruments. In this paper we test the feasibility of this detector for Raman spectroscopy and demonstrate shot-noise limited multichannel Raman spectroscopy of biological samples in the SWIR region.

Materials and methods

Instrumentation

SWIR multichannel Raman instrument

A SWIR multichannel Raman instrument was constructed in-house (Fig. 1). The excitation light source was a single-mode continuous wave diode laser with a wavelength of 976 nm and an output power of 150 mW (Model R-type, Innovative Photonic Solutions, Monmouth Junction, NJ, USA).

The collimated light from the diode laser was expanded to a beam of 7.8 mm in diameter using two achromatic lenses (f = 9 mm, Ø6 mm and f = 100 mm, Ø25 mm, Edmund Optics Barrington, NJ, USA) and focused in the sample to a Gaussian spot with a diameter of ~6 µm using an achromatic lens (f = 35 mm, NA 0.36, Edmund Optics Barrington, NJ, USA). The backscattered Raman signal is collected by the same lens and focused onto the entrance slit (25 µm) of the spectrometer using an identical achromatic lens (f = 35 mm, NA 0.36). The achromatic lenses used in this setup have an anti-reflection coating (<0.5% reflection in the SWIR region of 900–1700 nm). Two long pass edge filters (OD > 6.0, cutoff at 1064 nm, Model Raman Edge Filter, Edmund Optics, Barrington, NJ, USA) were used for laser light suppression in the signal detection path in front of the spectrometer. The f/6 spectrometer has an entrance slit with a width of 25 µm and a 5 cm⁻¹ spectral resolution and was customized to cover the spectral range between 1300 and 1550 nm (BaySpec Inc., San Jose, CA, USA).

The detector for the Raman signal is a Cougar-640 InGaAs imaging camera (Xenics, Leuven, Belgium) with an InGaAs focal plane array sensor with 512 × 640 pixels, cooled with liquid nitrogen to 77 K. The Cougar-640 camera offers two distinct readout modes. In the classical Integrate-Then-Read mode, the
photo-electrons are collected for a fixed exposure time after which the accumulated charge is probed and the buffering capacitors are reset. This scheme results in a relatively high capacitor-reset noise. The second scheme uses the Read-While-Integrate (RWI) mode. In this scheme the accumulating photo-electrons are probed non-destructively during the integration period without resetting the buffering capacitors. In combination with a noise level that is already low for a single readout, the non-destructive sampling option can be used to virtually eliminate the effective readout noise of the detector. This will be explained in more detail in the section Data pre-processing.

The captured spectral range of 1340–1540 nm corresponds approximately to a Raman spectrum from 2780 to 3750 cm⁻¹. The Raman spectrum is imaged on 512 pixels. The direction of the pixel readout process of the InGaAs-chip is perpendicular to the spectral direction, which avoids possible cross-talk effects between adjacent pixels.

A custom-made sample cartridge was used to hold an excised skin sample between two fused silica windows (Fig. S1A, Supporting information). For Raman measurements the cartridge was inserted inside an anodized aluminum cartridge holder that was mounted perpendicularly to the laser beam (Fig. S1B, Supporting information). The skin sample was placed in the laser beam and the focus position optimized on the Raman signal.

State-of-the-art commercial Raman device

We used a commercial spectroscopic Raman device (Model 3510SCA Skin Analyzer, RiverD International B.V., The Netherlands), equipped with a 671-nm laser to record Raman spectra in the high wavenumber region, to compare the high wavenumber Raman spectra obtained with a CCD-based Raman multichannel system to the spectra obtained with the new SWIR-Raman setup. The CCD detector was an air-cooled back-illuminated CCD with 1024 × 128 pixels (Andor iDus type DU401A-BR-DD, Andor Technology Ltd., U.K.). The spectral range of this instrument is 2500 to 4000 cm⁻¹, with a spectral resolution better than 5 cm⁻¹.

Data pre-processing

The Cougar-640 InGaAs detector was originally designed for SWIR imaging. In order to use it for Raman spectroscopy software algorithms were developed and implemented to read and pre-process the raw data that are generated by the camera using the RWI scheme.

Non-destructive readout detection

The RWI mode has been described for low light level imaging in astronomy[25–28] and is also known in the literature as the up-the-ramp readout scheme. During the course of a single integration the electrons generated in each pixel are buffered on a capacitor with a fixed pixel clock frequency. The voltage across the capacitor is then probed without resetting the capacitors, at regular time intervals, that can be chosen by the user. The total integration time is thus divided into N equidistant intervals. RWI operation starts with a simultaneous global reset of the buffering capacitors of the entire array, followed by multiple non-destructive readouts which trace the evolution of the accumulating charge for each pixel (Fig. 2). Because the noise in the successive non-destructive readouts is uncorrelated, the effective readout noise for the full signal integration time can be reduced by fitting a straight line through the successive readouts.

To employ this readout scheme, the accumulating signal in each pixel was translated into a slope of a line fitted through all
intermediate sampling points for that individual pixel. The counts in Analog-to-Digital converter Units (ADU) are then converted to the number of generated electrons, which equals the number of detected photons. The global conversion gain for the Cougar-640 camera is 0.168 photons per ADU. From the slope (number of detected photons per number of readouts) and the sampling rate (number of readouts per second), the signal intensity detected by each pixel was computed as the number of detected photons per second.

Using \( N \) uniformly spaced readouts, the readout noise in the total acquisition \( r_{\text{out}} \) is given by \[^{26}\]:

\[
r_{\text{out}} = \sigma_r / \sqrt{N}
\]

where \( \sigma_r \) is the standard deviation of the residuals with respect the fitted line and defined by:

\[
\sigma_r = \sqrt{\frac{\sum_{i=1}^{N} (y(i) - p(x(i)))^2}{N}}
\]

In this equation \( y(i) \) are the measured intermediate readouts, and \( p(x(i)) \) are the values of the fitted line.

Linearization of pixels response

During the characterization of the camera the response showed progressive non-linear behavior after the accumulated signal exceeded a certain threshold. We identified a cutoff threshold for the linear behavior. An algorithm was implemented to correct for this non-linear response of the pixels above the threshold value. A first-order polynomial was fitted to the linear range during the first part of the integration period (Fig. 3B). The ratio between the fitted line (in blue, Fig. 2) and the intermediate pixel readouts (red dots, Fig. 2) was stored in a lookup table together with the corresponding pixel readout values. The lookup table was created from the averaged result of 10 ratios. Thus for each pixel readout the lookup table provided a correction factor by linear interpolation. The response for each pixel was then linearized by multiplying the readout by the corresponding correction factor from the lookup table.

For each pixel a first-order polynomial was fitted to the linearized data to determine the slope (see Fig. 3B) which represents the number of photons detected per second (Fig. 3C).

Finally the slopes of the pixels in a region of interest of 12 pixels perpendicular to the spectral direction were co-added (Fig. 3D) to result in a single detected intensity per super pixel for each spectral point.

**Figure 3.** Schematic overview of the RWI processing algorithm. A) Only 12 rows of pixels are used to record the spectrum. The readout direction is indicated in the figure. B) For each pixel, the response as a function of time is linearized and the slope is determined; C) the slopes for different pixels are then co-added in the readout direction as indicated in A; D) the final Raman spectrum is obtained.
Calibration of Raman spectra

Raman spectra were corrected for wavelength-dependent detection efficiency of the setup, using a NIST calibrated glass (NIST, Gaithersburg, Maryland, USA). Because no standard reference material (SRM) was available for 976-nm excitation wavelength, we used an SRM for 830-nm excitation (SRM 2246, NIST, USA) and compared the spectra acquired with an 830-nm laser to the spectrum acquired with the default 976-nm laser. The relative difference was smaller than 6%. We decided that this difference would be sufficient to use the SRM 2246 with an excitation wavelength of 976 nm to correct for the wavelength-dependent detection efficiency of the setup with satisfactory accuracy. The theoretical curve for 830-nm excitation and the curve obtained with the 976-nm laser were divided to determine a correction curve.

At the start of each measurement session a spectrum of the SRM2246 was measured (with 976-nm excitation) to determine the wavelength-dependent response of the instrument. All recorded Raman spectra in that session were corrected for this response.

The relative wavenumber calibration was determined using a calibration neon-argon lamp. The exact wavelength of the laser was determined using cyclohexane as a Raman standard calibration neon-argon lamp. The exact wavelength of the laser line position was computed from the Raman bands at 2852.9, 2923.8 and 2938.3 cm⁻¹. The Raman background that originates from the optics was subtracted from all spectra.

Sample preparation

Ex vivo human skin. Pigmented human skin lesions suspected of melanoma were excised in the dermatology outpatient clinic of the Leiden University Medical Center (LUMC) as part of the standard patient care. Skin samples were obtained from one female and from one male subject. This study was approved by the medical-ethical committee at LUMC (C13.06). Informed consent was obtained from the patients.

The freshly excised skin samples were rinsed with NaCl 0.9% solution, gently flattened between two fused silica windows and measured within a maximum of 30 min after surgery with the in-house built SWIR Raman instrument. After measuring, the skin samples were put in a formaldehyde aqueous solution (4%) and sent to the pathology department for the routine diagnostic procedure. During this procedure it was also verified that no visual damage had occurred because of the measurements.

Human hair. A dark brown hair was clipped from one Caucasian female subject and measured immediately after collection. No hair treatment such as dyeing, waving, or other styling methods were used. Spectra were collected on the first third of the total length of the hair, close to the hair root.

Red wine. Dark red wine (grape type Aragonez, 13% alcohol) was obtained from a local store and measured in a fused silica cuvette of 1 x 1 cm diameter. The cuvette was placed in the sample holder and positioned in the laser focus.

Results

Readout noise and dark current

Table 1 shows the detector readout noise for a single readout and the dark current with the detector operating in a room at 23 °C.

Table 1. Experimentally determined noise characteristics of InGaAs Cougar-640, Xenics®

<table>
<thead>
<tr>
<th>Noise source</th>
<th>Electrons ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Readout noise (e⁻ per single readout)</td>
<td>22.7 ± 5.9</td>
</tr>
<tr>
<td>Dark current (e⁻/s/pixel) at room temperature</td>
<td>69.4 ± 4.5</td>
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The readout noise (per single readout) was determined according to Eqn (2), described in Materials and methods.

Non-linearity in the detector response

The detector response was measured for different pixels until the saturation level of 3.5 x 10⁶ detected photons was reached, using the Raman signal of cyclohexane (Fig. 4A). Figure 4B shows the detector response of pixels exposed to different light intensities. The accumulated charge, expressed in the number of detected photons, is shown as a function of time. With the time-axis normalized on the time to reach detector saturation the response of pixels exposed to different light intensities overlap (Fig. 4C). This demonstrates that the non-linearity in the pixel response is a function of the total number of detected photons only and independent of the light intensity or rate at which photons are detected.

Figure S2 (Supporting information) shows two HWVN-Raman spectra obtained ex vivo of a skin sample and illustrates the effect of non-destructive readout. Both spectra were obtained using a signal collection time of 10 s. The upper spectrum (spectrum a, Fig. S2, Supporting information) was obtained using non-destructive readout at a sampling rate of 10 readouts/s (100 intermediate readouts in 10 s), and the bottom spectrum (spectrum b, Fig. S2, Supporting information) was obtained with a sampling rate of 0.2 readouts/s (2 intermediate readouts in 10 s).

The readout noise given in Table 1 decreases significantly when more sampling readouts were performed during the integration time, in accordance with Eqn (1). Table 2 presents the readout noise (e⁻ /pixel) and the signal intensity in generated electrons of the spectra shown in Fig. S2 (Supporting information), which were measured with a different number of readouts. The signal intensity is the maximum intensity in the CH band at 2938.3 cm⁻¹. The readout noise was determined according to Eqn (1) (Materials and methods).

Figure 5A shows how the spectral noise develops with increasing signal collection time. All spectra are acquired at the same non-destructive readout rate of 10 readouts/s. The HWVN-Raman spectra were obtained ex vivo of a pigmented skin lesion using different exposure times of 0.5 s, 1 s, 2 s, 4 s, 8 s, and 16 s. Figure 5B shows the measured spectral noise plotted against the calculated shot noise defined as square root of the signal. The empirical noise in signal was determined after fitting a 2nd-order polynomial to part of the OH-band (3360–3470 cm⁻¹) and then calculating the standard deviation of the residual.

To test the feasibility of acquiring Raman spectra of pigmented samples, we tested a number of samples which show high fluorescence when excited with visible light (red wine and dark brown human hair). This was done using 671-nm laser light and using 976-nm laser light (Fig. 6).

Figure 7 presents two examples of HWVN Raman spectra of highly pigmented excised human melanocytic nevi using the 976-nm SWIR Raman instrument. The spectra were acquired from eight different locations of the pigmented lesions shown in Fig. 7B and D. The spectra presented were intensity-normalized and offset along the intensity axis for clarity.
The purpose of this in-house built SWIR Raman instrument was to enable rapid acquisition of high quality Raman spectra of freshly excised pigmented human skin lesions. For detection of the Raman signals a novel SWIR InGaAs imaging camera was employed and adapted to Raman spectroscopy.

The choice of an excitation wavelength of 976 nm was guided by the requirement to collect signal in the 2780–3750 cm\(^{-1}\) spectral range with NIR excitation and by the 1570-nm sensitivity cut-off of the low-noise InGaAs detector. Using 976-nm excitation laser, the spectral range of 2780–3750 cm\(^{-1}\) corresponds to signal detection in the 1340 nm–1540 nm wavelength range. This spectral interval covers the CH-stretching, NH-stretching, and OH-stretching regions, which enables the detection of protein, lipid, NH, and OH vibration bands. We have shown earlier that this wavenumber range provides diagnostic information to differentiate malignant tissue from benign tissue.[1,2]

**Noise characteristics SWIR InGaAs camera**

The RWI readout scheme was specially designed for low light intensity applications, which also makes this camera of special interest for application in Raman spectroscopy. This readout scheme is a novelty for Raman spectroscopy and enables virtual elimination of readout noise. The detector noise characteristics were experimentally verified and approach those of high-quality CCD detectors, which is unprecedented for InGaAs detectors (Table 1). For instance, in a 10-s exposure time with a frame rate of 10 Hz, the effective readout noise is reduced to 2.27 electrons (Table 2), which is comparable to the readout noise levels found in cooled slow-scan CCD detectors used in spectroscopy.

The experimentally determined dark current value ($69.4 \pm 4.5 \, \text{e}^-/\text{s/pixel}$) is about seven times higher than specified by the manufacturer ($<10 \, \text{e}^-/\text{s/pixel}$). In this manufacturer specification the dark current is measured in a cold room with an additional cold shield mounted in the detector housing, which significantly reduces the influence of thermal radiation from the environment. The dark current in this paper is determined under standard operational conditions without an internal cold shield and therefore reflects the effective dark current including collection of normal thermal radiation from the environment. The pixel reset of the InGaAs detectors introduces small variations in the pixel-capacitor values at the start of an integration. This results in a significant pixel-noise component, which could limit low-light applications. Using the RWI readout scheme, this reset noise component can be completely canceled because the slope of the accumulated charge for each individual pixel is used, not the offset (see Figs 2 and 3).

During integration cycles with a constant light source the detector showed a strong non-linear response of accumulated pixel charge versus time. The characteristic curve resembles an S-shape and is because of a fundamental feature of the type of capacitors used in this detector (see Fig. 4). This non-linearity is essentially caused by the bias leakage in the pixel circuitry of the readout chip that decreases during the integration period, which results in decreasing sensitivity. In order to obtain a linear response of the Raman spectral intensities, a non-linearity correction was performed on the individual pixels. This was possible because we could
Figure 5. A) Ex vivo Raman spectra of pigmented human skin measured with different integration times with a non-destructive readout rate of 10 readouts/s. The spectra are offset along the intensity axis for clarity. a) 16-s integration time; b) 8-s integration time; c) 4-s integration time; d) 2-s integration time; e) 1-s integration time; f) 0.5-s integration time. B) Measured spectral noise of the spectra shown in A versus the theoretical shot noise. The dashed line indicates the level at which the empirical noise equals the theoretical noise.

Figure 6. Raman spectra of pigmented biological samples. A) HWVN-Raman spectra of red wine obtained using 976-nm laser excitation and the SWIR Raman instrument built in-house. Signal collection time: 10 s; non-destructive readout rate: 10 readouts/s; B) HWVN-Raman spectra of red wine obtained using 671-nm laser excitation and a CCD-based Raman instrument (Model 3510 SCA, RiverD International B.V., the Netherlands). Mean spectrum and spectral variance are shown (25 spectra measured with a signal collection time of 1 s each); C) HWVN-Raman spectra of a human brown hair obtained using 976-nm laser excitation, a 10-s signal collection time, and non-destructive readout rate of 10 readouts/s; D) HWVN-Raman spectra of a human brown hair obtained using 671-nm laser excitation. Mean spectrum and spectral variance are shown (25 spectra measured with a signal collection time of 1 s each). In all figures the solid line represents the mean spectrum, and the shadow area represents the spectral variance (eight repeated measurements).
verify that the non-linear response was dependent on the accumulated charge of a pixel and not on the intensity or photon flux, and did not vary between pixels. This means that a single lookup table could be used to convert the non-linear scale of accumulated charge to the linear scale of number of detected photons for a given integration time. The practical implication is that linearization of the detector response can easily be implemented on-line in the acquisition software and does not require much processing power.

Shot noise or Poisson noise is associated with the particle nature of light and can be quantified by the square root of the number of detected photons in a given time interval. For very strong signals the associated shot noise is generally the dominant noise factor but for weak signals, such as Raman signals from biological tissues, the detector noise can become a limiting factor. Ideally, the signal-to-noise ratio in a Raman spectrum is not limited by the detector noise but only by the unavoidable shot noise in the Raman signal. To test whether we could obtain quality shot-noise limited Raman spectra with the developed instrument we measured some biological samples that give low intensity Raman signals. Figure S2 (Supporting information) illustrates that readout noise is drastically reduced by using the InGaAs detector in RWI mode with multiple non-destructive readouts during acquisition. Figure 5 shows how Raman signal evolves after 0.5, 1, 2, 4, 8, and 16 s of acquisition. For shorter integration times the readout noise exceeds the signal shot noise. At high signal intensities the shot noise dominates other noise sources. The effective noise in the spectra approaches the theoretical shot noise values for exposures longer than 4 s, i.e. the noise in the spectra is determined by Poisson statistics and no longer by detector noise. The fact that the effective noise is slightly higher (about 9 photons) than the square root of the signal reflects the inaccuracy in the empirical determination of the effective noise in the spectra. It is clear from Fig. 5 that, using the non-destructive readout scheme of this InGaAs detector (RWI), we were able to obtain shot-noise limited detection of weak HWVN-Raman signals from biological tissues with excitation and detection in the NIR and SWIR region.

Figure 7. Photographs and corresponding Raman spectra of freshly excised human pigmented skin lesions using 976-nm laser excitation and the SWIR Raman instrument. Exposure time: 10 s. A and B) Melanoma in situ; C and D) benign melanocytic nevus.
We present some example spectra of naturally highly pigmented samples, of which it is extremely difficult to obtain high-quality and low-background Raman spectra with visible laser excitation. In the first example (Fig. 6A and 6B) of red wine it is evident that the spectrum acquired with the 976-nm setup shows hardly any fluorescence background and a strong Raman signal is well visible, whereas the spectrum acquired with 671-nm excitation shows a very strong fluorescence background in comparison to the superimposed Raman signal. In the second example (Fig. 6C and D), we could get a strong Raman spectrum from human brown hair, with the protein band and CH vibration features well perceptible on top a still noticeable fluorescence background. When measuring the same sample with 671 nm the background largely obscures the Raman signal, which is only marginally noticeable on top of the strong background. The first measurements also show the feasibility of the in-house built instrument to obtain high-quality Raman spectra of biological pigmented samples in just few seconds of integration time. We also presented two examples of high quality Raman spectra of pigmented human skin lesions measured in only 10 s. Now that technologicallimitation of multichannel detection above 1100 nm with extremely low noise levels is overcome, it is possible to use longer excitation wavelengths in Raman spectroscopy in order to decrease tissue fluorescence that would otherwise obscure the Raman signal. This detector enables low-fluorescence spectra from pigmented biological samples with a multichannel NIR detector.

Conclusions

In this paper, we describe a novel Raman instrument for obtaining high quality spectra from pigmented samples, using short signal integration times. The key element in this setup is the low-noise InGaAs detector. It enables near shot-noise limited signal collection and opens a wide field of demanding applications, e.g. in medical diagnosis, food quality control, or other fields in which high quality Raman spectra of pigmented samples are required.

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References


Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s web site.