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Towards quantum-enhanced Raman spectroscopy for bioimaging applications

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ABSTRACT

In the QuantERA project QURAMAN (Quantum Raman) are we aiming for a combination of breakthroughs and improvements of existing components and already existing setups for building a commercial quantum Raman microscope. By combining the project partners' expertise and skills in quantum optics, nonlinear optics, Raman spectroscopy and medical device design we will develop the next-generation Raman microscope for bio-imaging with quantum-enhanced sensitivity. The background knowledge and idea behind the QuRAMAN project is described in our recent publications (Optica 7, 470-475 (2020)). Where we have demonstrated that the use of continuous wave (CW) squeezed light can improve the SNR of weak Raman signals. However, to beat the performance of state-of-the-art SRS microscopes by means of squeezed light, one must employ amplitude squeezed picosecond pulses in a strongly focusing configuration (using an objective with a numerical aperture above unity). This will enable the imaging of weak Raman features and will push the Raman technology beyond the state of the art by applying pulsed amplitude squeezed light for signal enhancement.

Keywords: Quantum sensing, Raman spectroscopy, squeezed light, bioimaging

1. INTRODUCTION

Optical quantum sensing exploits the unique quantum correlations of non-classical light to enhance the detection of physical parameters beyond classical means [1–4]. While several different quantum states of light can, in principle, be used to provide such a quantum advantage, so far, it is only the ubiquitous squeezed states of light that have been demonstrated to be beneficial in practice [5–8] due to their generation simplicity and relative brightness. Quantum enhancement can be useful for measurements of extremely weak signals, with the crowning example being the detection of gravitational waves [9-10]. One field that can greatly benefit from sub-shot-noise detection is Raman spectroscopy of bio-samples. Raman spectroscopy is an ideal contrasting method for chemically resolved microscopy with no prior preparation or fluorescent tagging of the target molecule required. However, the major challenge for Raman sensing is the relative weakness of the Raman response, which is orders of magnitude weaker than fluorescence. Stimulated Raman spectroscopy (SRS) has become a powerful tool to study the spatio-dynamics of molecular bonds with high sensitivity, resolution, and speed. SRS employs two laser beams, known as the pump and probe (Stokes) beams, to coherently excite a selected molecular vibration of the system under investigation [11]. If the vibrational frequency of the chemical bond matches the frequency difference of the pump and probe laser, the Raman interaction is stimulated and, as a result, significantly amplified by orders of magnitude. In the stimulated Raman effect, a photon is annihilated from the pump beam and, simultaneously, a Raman-shifted photon is created in the background noise of the probe beam. The sensitivity and speed of state-of-the-art stimulated Raman scattering (SRS) spectroscopy are currently limited by the shot-noise of

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the light beam probing the Raman process. In principle, the sensitivity can be arbitrarily improved by simply increasing the power of the input beams. However, in biological systems, and especially in living biological specimens, the optical intensity levels must be kept below certain thresholds to avoid damaging or changing the biological dynamics and thereby leading to erroneous results. Leaving the optical power at a constant level, the sensitivity and bandwidth of the SRS can instead be boosted by overcoming the shot-noise level using amplitude squeezed states of light [8,13]. To detect the stimulated scattering of photons from pump to probe, a modulation scheme is often employed. An intensity modulation is applied to one of the two beams and gets transferred to the other beam by SRS. The resulting modulation is detected with an intensity detector and a lock-in amplifier [12]. The precision by which the Raman signal can be measured depends on the background noise of the probe beam. This background noise is fundamentally limited by the shot noise when the probe beam is in a coherent state produced by a conventional laser, but it can be reduced significantly using amplitude squeezed states of light. State-of-the-art high-sensitivity Raman spectrometers are based on strongly focused pico- or femtosecond pulsed lasers with very high peak powers [13,14]. Pulsed SRS microscopes attain sensitivities that are orders of magnitude larger than cw SRS microscopes, due to the large peak powers, they cannot necessarily be applied when interrogating fragile light- and heat sensitive biological specimens. Therefore, to beat the performance of current state-of-the-art SRS microscopes by means of squeezed light, one must employ squeezed picosecond pulses in a strongly focusing configuration.

In the QuRAMAN project we will develop a novel quantum Raman microscope [9,13], which pushes forward the field of optical microscopy for bio-medical and bio-imaging use. For a conceptual layout see figure 1. We will exploit a new paradigm of quantum bio-optical measurements, which uses pulsed non-classical squeezed light combined with SRS. The QuRAMAN system is intended to support intraoperative decision making for cancer screening. Currently the assessment done by a trained pathologist is subjective. An additional product to objectify diagnostic decision making would be unique and beyond state-of-art. During this project, the following main features will be developed and evaluated:1.: Development of the quantum Raman microscope. 2.: Application to human tissue to support fast, objective, and reliable histopathological lung cancer screening. Where several studies have shown that the capabilities of RS can be significantly strengthened when assisted by chemometric tools and machine learning (ML) [17]. 3.: OEM light source and compact microscope will be provided as plug&play modules, for supporting bioimaging and quantum technologies in education, science, and industry.

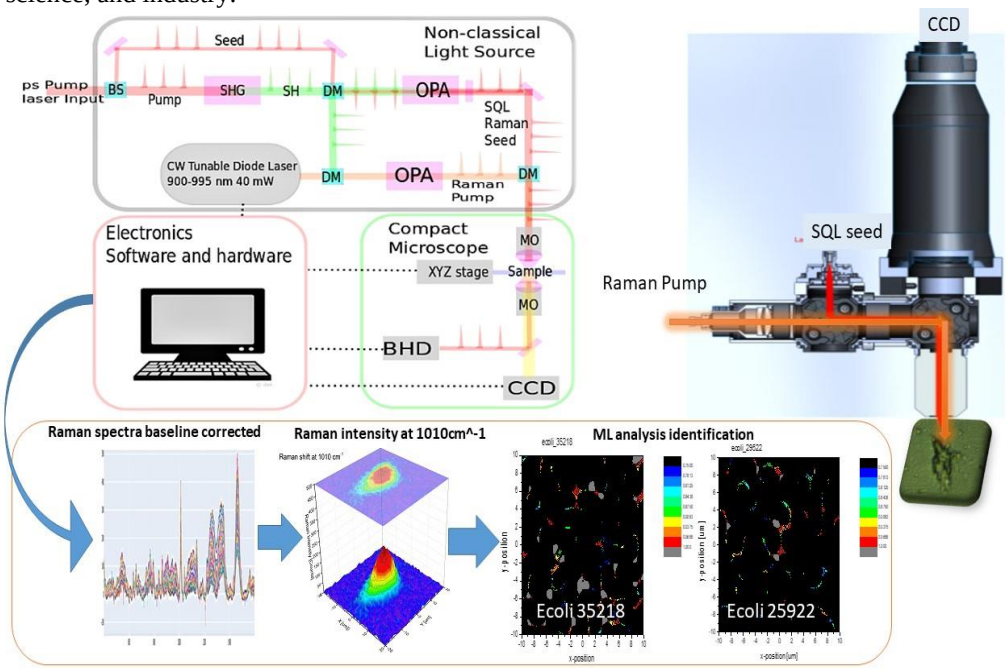

Fig.1.: Left: Block diagram of the envisioned QuRAMAN system. The two subsystems (OEM products, sub-boxes) will be controlled by third standalone box containing the electronics and software. Pump laser (the 1064 nm ps PicoTrain, is not shown). The right picture illustrates the main part of the Quantum microscope with the pump beam and imaging system shown. Finally, the acquired Raman data is analysed using neural network ML software developed at DFM [17]. See the figure in the bottom, shown in the case of two types of E. coli bacteria (The data is acquired using spontaneous Raman spectroscopy at DFM). This software can easily be adapted to the QuRaman project and the study of normal/chancer tissue.

2. USER CASE FOR THE QURAMAN MICROSCOPE

Despite recent advancements in targeted and personalized therapies, lung cancer still represents a widespread health threat and accounts for a major contribution to the mortality in industrialized nations. An early and accurate histopathological diagnosis of lung cancer and its subtype is paramount for treatment decisions and patient outcome. The diagnostic accuracy of intraoperative frozen section consulting remains imprecise in many cases and even after complete diagnostic workup on formalin fixed paraffin embedded tissue samples including histochemical and immunohistochemical methods, some tumors remain difficult to classify. Lung cancer is the most common cancer in men and the second most common in women worldwide. Histologically, lung cancer is separated into non-small cell lung cancer (NSCLC, approx. 80%) and small cell lung cancer (SCLC, approx. 20%). A fast diagnostic workup for the distinction of both is important, as SCLC are highly proliferative tumors and need to be treated by chemotherapy rapidly. The main NSCLC subtypes are adenocarcinoma (ADC) and squamous cell carcinoma (SqCC), which together account for ~70% of all lung cancers. Differentiation between these two subtypes is very important because some genetic alterations are mainly restricted to non-SqCC, and some therapies, such as treatment with bevacizumab, are contraindicated in SqCC. In poorly differentiated tumors, the distinction between ADC and SqCC is often not possible on morphology alone but requires adjunct immunohistochemical (IHC) stainings with up to four markers. To save tissue for subsequent molecular analyses, current guidelines recommend the use of only two markers to confirm the histological subtype. However, a less extensive workup will result in a decreased diagnostic precision and may lead to a delayed diagnosis. Thus, methods supporting a rapid and accurate diagnostic workup are highly warranted. The aim of this study is to develop a prototype capable to classify the most common lung cancer subtypes based on spectra acquired by spectroscopy. There is some evidence in the literature that this might be a promising approach. However, most studies suffer from lack of pre- and post-analytical histopathological quality control including the documentation of the tumor cell content and a very small sample size.

3. CONCEPT AND METHODOLOGY

The non-classical light source: The pump source used has picosecond pulses at the fundamental wavelength 1064 nm together with a CW laser tunable at 900-1000 nm. Frequency-doubled picosecond pulses will then pump two traveling-wave optical parametric amplifiers (OPAs), one of them generating the Raman pump for the SRS. This way, the system will be made synchronization-free and simpler than typical Raman setups [15]. The other OPA, seeded by the fundamental pulses, will produce amplitude-squeezed light at 1064 nm, further used as the Raman probe. Both OPAs will be based on periodically poled lithium niobate (PPLN) second-order nonlinear crystals, providing very high parametric gain. The OPA producing the tunable Raman pump will have a fan-out structure, allowing for fast wavelength scanning between 900 nm and 1000 nm and thus covering the Raman fingerprint region from 600-1700 cm^{-1}. Recent studies with near-infrared Raman spectroscopy work on lung cancer suggest that the ratio of Raman intensities at 1445 to 1655 cm^{-1} could be used to identify malignant bronchial tissue [16]. The values of squeezing achieved in the pulsed regime, 7.8 dB for twin-beam squeezing and more than 4 dB for quadrature squeezing in a multimode traveling-wave OPA, give grounds for targeting more than 6 dB squeezing in this project. To improve the squeezing, we will seed the OPA with transform-limited pulses, therefore eliminating the multimode structure.

The Raman microscope: A compact Raman microscope is to be developed for compatibility with picosecond pulsed pump and stokes lasers to achieve SRS mapping capabilities. Based on the needs of QuRaman microscope will be designed as a reflectance and transmittance light microscopy, where the optics does not impact the quality and losses of the SRS signal beam. This is achieved using low-loss high-quality optics and microscope objectives (>x60) with high optical transmittance in the NIR region. The designed QuRAMAN system will have delivery ports for picosecond pump and stokes beams and detection in transmittance mode by balanced detectors also to developed in the project. The QuRAMAN microscope will be equipped with raster scanning system (spatial resolution of sub-micrometers) for automated lateral and axial sample positioning. The image processing will be achieved using a standard CMOS sensor in transmission and reflection modes.

4. BACKGROUND KNOWLEDGE FOR QURAMAN

We have so far demonstrated in a proof-of-principle experiment an enhancement of the sensitivity of continuous-wave stimulated Raman spectroscopy by reducing the quantum noise of the probing light below the shot-noise limit by means of amplitude squeezed states of light [8]. Probing polymer samples with Raman shifts around 2950cm^{-1} with squeezed states, we demonstrate a quantum enhancement of the stimulated Raman signal-to-noise ratio (SNR) of 3.60 dB relative to the shot-noise limited SNR. Our proof-of-concept demonstration of quantum-enhanced continuous-wave Raman spectroscopy paves the way for more elaborate demonstrations using state-of-the-art stimulated Raman scattering microscopes, and thus constitutes the very first step towards a new generation of Raman microscopes, where weak Raman transitions can be imaged without the use of markers or an increase in the total optical power [8].

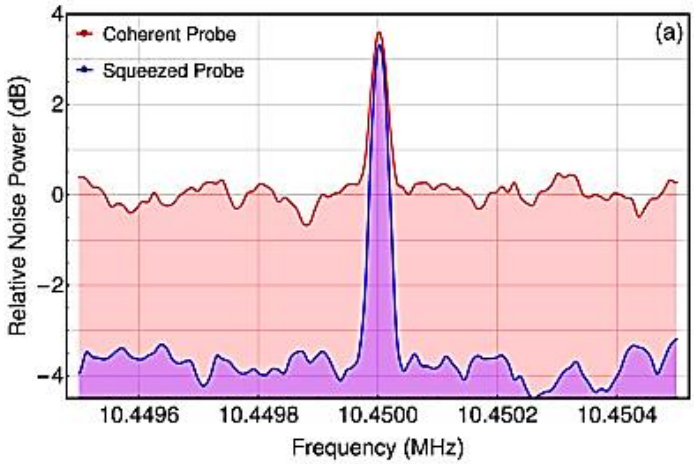

Fig. 2. Demonstration of quantum enhanced SRS spectroscopy using probe powers of 1.3 mW and pump powers of (a) 24 mW. The red SRS traces correspond to the realizations where the probe beams are in a coherent state while the blue traces correspond to the beams being in a squeezed state with −3.60dB noise suppression below the shot-noise. In both cases, the signals are normalized to the shot-noise level [8].

5. DISCUSSION AND EXPECTED OUTCOME

The QuRAMAN device will push the field of microscopy for bio-medical and bio-imaging use. We will exploit a new paradigm of quantum measurements, using pulsed non-classical light combined with SRS for early and accurate histopathological diagnosis of lung cancer. An early and accurate histopathological diagnosis of lung cancer and its subtype is paramount for treatment decisions and patient outcome. Thus, the target of the QuRAMAN project is to take known quantum effects (squeezed light) and translate them into technological applications and develop a quantum Raman microscope. This will enhance the interdisciplinarity in crossing traditional boundaries between quantum optics and Raman spectroscopy for bioimaging and will offer novel medical diagnostic tools for cancer screening. The direct impact of the QuRAMAN project will be as an additional system to support intraoperative decision making for cancer screening. Currently the assessment done by a trained pathologist is subjective. An additional product to objectify diagnostic decision making would be unique. In addition, a shortened time to the result would be very desirable and advantageous for both hospital and patient.

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