RAMANTIS: A real-time material characterisation system using multispectral Raman imaging

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ABSTRACT
A wide-field, multi-band Raman imaging setup was built that enables single-exposure snapshot acquisition of all data required to produce a map of the spatial distribution of select chemical components of a sample. First proof-of-principle measurements demonstrate the ability to distinguish, in one second, three common compounds of a painkiller tablet: paracetamol, aspirin, and caffeine. Extrapolations based on known limitations of the setup show that a further 100x speedup should be feasible, opening up the possibility of real-time application of the technology. The potential societal impact is enormous and spans many fields within fundamental research, healthcare, and industry.

Keywords: Raman spectroscopy; microscopy; multispectral imaging; Raman imaging.

1. INTRODUCTION

Raman spectroscopy is a ubiquitous technique for material inspection, offering accurate and non-destructive identification of the composition of samples. It is a rapidly growing billion-dollar market, spanning multiple application domains, among which biomedical, novel materials, pharmaceutics, semiconductors, environmental, and more. Raman imaging (RI) applies this spectroscopic technique to extended two-dimensional samples. Current RI systems require scanning times of minutes to days to produce a single Raman image/map. The RAMANTIS project aims to reduce the acquisition time by multiple orders of magnitude, thereby reducing its operational cost, improving the technique's ease-of-use, and widening its applicability, e.g. towards the investigation of dynamical processes and unpredictably moving subjects.

The breakthrough we aim to achieve is to acquire the data necessary to produce a Raman map completely in parallel, rather than via a sequential scan of a large number of measurements. This innovation is enabled by a high-speed snapshot multispectral imaging system, in combination with global illumination of the sample. The system can simultaneously acquire the intensities of selected Raman bands from 3 million different points (i.e. the number of spatial pixels). In current state-of-the-art commercial Raman imaging microscopes, each individual spatial point has to be scanned sequentially, disqualifying it for use in many applications. For example, while developing catalysts that convert biomass, waste and CO_2 into usable fuels and chemicals, it is important to understand the reaction dynamics across the full catalyst surface as various components are introduced. Raman spectroscopy could provide valuable insight, but currently no solution exists to perform a spatially resolved measurement in real time. A second potential application is the use of RI for in vivo tissue characterisation, leading the path towards image-guided surgery systems that indicate tumour margins in real-time. This could potentially revolutionize the way tumour are resected, and receives widespread attention in both academia and the medical devices industry. These are just two of many ground-breaking innovations that may be unlocked when this core technology matures.

For this project, a wide-field, multi-band Raman imaging setup was designed and constructed, wherein results can be compared with traditional Raman measurements at a single location. The original scope (characterizing graphene) was expanded to include the identification of Active Pharmaceutical Ingredients (API), based on the findings of a student team from the CERN IdeaSquare Summer School and our own market research. Due to COVID-19, the lab facilities were unavailable during a large fraction of the experimental campaign. As a result, this paper presents only the first proof-of-principle measurements with basic data analysis routines applied. It does not yet report on the real-time capabilities.

2. STATE OF THE ART

We benchmark our technology against the state-of-the-art (SOTA) in commercially available Raman Imaging

microscopes, which is set by ThermoFisher Scientific, WITec, Horiba, Renishaw, and Bruker. All leading systems have a similar mode of operation: they perform raster scans over samples in order to deliver spatially resolved data. The fastest commercial system can technically measure up to 1800 Raman spectra per second (at 20 mW laser power). This is an upper limit, as the actual acquisition time for a single Raman spectrum depends strongly on the scattering strength, laser damage threshold of the material, selected collection optics, and more.

Even if the theoretical maximum speed can be achieved, collecting a 3 Megapixel Raman map takes 30 minutes. In practice, however, acquiring such high-resolution images usually takes many hours. For this reason, Raman images are usually collected in stages, starting with a coarse raster scan over a large field of view, followed by higher-resolution scans of small regions-of-interest. These microscopes are thus impractical and time-consuming to use, pose the risk of missing important information outside of the ROIs or at length scales smaller than the chosen step size, and are not suitable for studying the dynamics of processes at time scales shorter than the total acquisition time (i.e. hours).

Some manufacturers are starting to develop strategies for automatically pre-selecting the areas to scan at higher spatial resolution, while retaining a lower average resolution. While more practical to the user, this does not alleviate the other, more substantial disadvantages listed above. It is also not possible to increase the laser power on the sample to increase the signal strength and thus reduce the acquisition time, because all power is focused onto a spot the size of a pixel (which is small for higher resolution). The resulting extremely high laser power density is typically already limited by the material limits. Effectively, the current generation of Raman imaging microscopes already operate close to the physical limits of what is possible with this mode of acquisition.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The central breakthrough of the project is that by employing a new type of multispectral camera setup, all spatial information, as well as all required spectral information is captured simultaneously, i.e. in a single exposure without any form of scanning (whether in a spatial or spectral dimension). If the available laser power were unlimited, one would be able to operate at the same power density as in the point-scanning case, i.e. just below the material's damage threshold, and perform the full measurement in approximately 10^{-3} s (vis-à-vis 10^{3} s). However, with a 1 mm diameter field-of-view (FOV), this would require excessive laser power (~10^{4}W). With suitable commercially available laser sources delivering ~10^{1} W, we still expect to be able to achieve *3 orders of magnitude speedup in the acquisition* of high-resolution maps of the spatial distribution of known chemical components. A variation of the technology would achieve a 100x improvement in the acquisition speed of full Raman spectra instead of selected bands, enabling the rapid characterization of *any* material. It is expected that RAMANTIS technology will be able to compete with the current state-of-the-art in terms of other system properties as well, as is summarized in Tab. 1.

Tab. 1. Comparison table of key characteristics of commercially available point-scanning Raman imaging microscopes and the potential of RAMANTIS technology. We note that the values given in the "point-scan" column indicate the variation in best values within the set of commercial microscopes. For example, where the "spectral resolution" reads 0.1-5 cm^{-1}, it means that the best performing system (on this front) achieves 0.1 cm^{-1}, while the worst performer achieves 5 cm^{-1}.

Property	Point-scan	RAMANTIS
Acquisition time (3MP map)	0.5 – 10h	1s
Laser power [W]	0.02 – 0.1	10
Spectral range [cm^{-1}]	5 – 30000	100-3500
Spectral resolution [cm^{-1}]	0.1 – 5	<10
Field of view [mm]	0.2-100	1
Spatial resolution [um]	0.3	<1

Since Raman spectroscopy is such a widely applied technique for non-destructive identification of materials, whose adoption is in part held back by the slow acquisition speed, the enormous achievable speedup could revolutionize many fields of research. In addition, the speedup may make RI compatible with real-time quality and process control in certain manufacturing processes. We expand upon the enabled applications in section 5.3 below.

4. PROJECT RESULTS

Experimental setup

For this project, we built a multi-spectral Raman imaging setup with 4 cameras. Fig. 1 shows a basic schematic of the setup. Light from the laser source is slightly defocused by a lens system (DFL), travels via the dichroic mirror towards the objective, where an FOV filling spot is formed (because of the previous defocusing) on the sample. The Raman signal is collected by the objective, and travels through the dichroic mirror and edge-filter to remove Rayleigh scattered light. The beam splitter cubes (BS) split the signal equally into four channels, and through the filters (F1-F4) the signal light reaches four camera systems (C1-C4), where four Raman images are detected simultaneously at different wavenumber ranges.

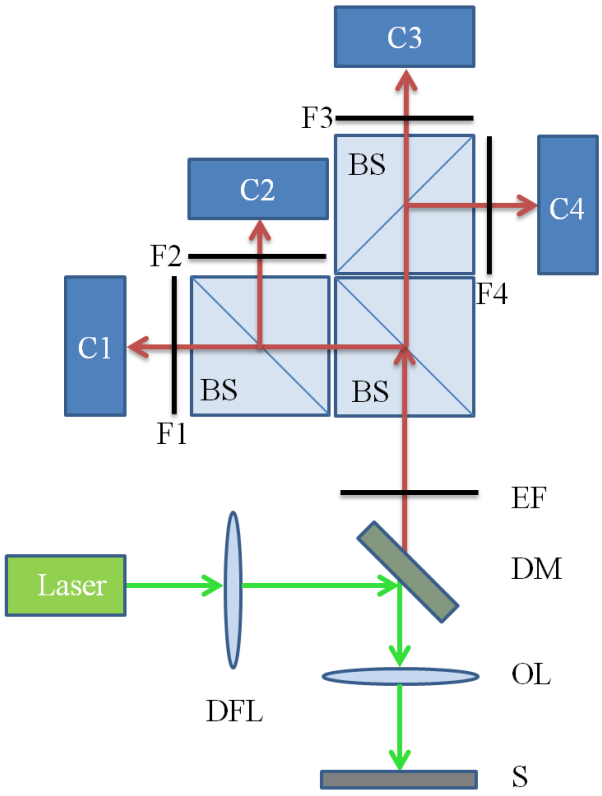

Fig. 1, schematic illustration of the setup. **DFL**: defocusing lens; **DM**: dichroic mirror; **OL**: objective lens; **S**: sample; **EF**: edge filter; **BS**: 50:50 beam-splitter cube **F1**-**F4**: filters; **C1**-**C4**: camera-lens assemblies.

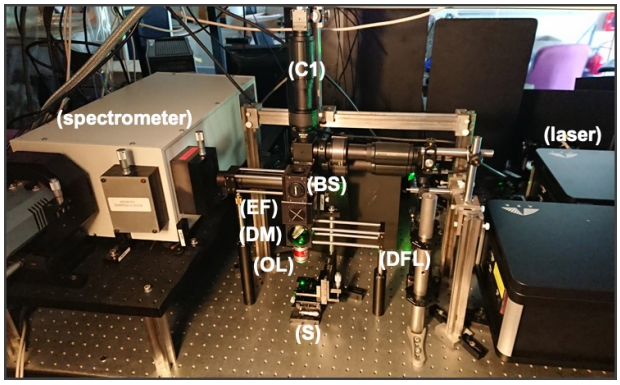

Fig. 2. Picture of the RAMANTIS setup constructed at the VU. Annotations are the same as in Fig. 1. The spectrometer branch can be activated by placing a removable mirror in the optical path.

Methods

The goal of these proof-of-principle measurements is to distinguish different API compounds in real-time. We measured samples containing aspirin (Merck, Germany), paracetamol (Sigma-Aldrich, Germany) and caffeine (Sigma-Aldrich, Germany). These compounds have significant spectral overlap, making filter selection critical. Off-the-shelf options typically do not qualify. Reference spectra of the pure compounds were taken using the spectrometer branch of the setup to support the specification of semi-custom filters. The chosen wavenumber ranges are 555±17 cm^{-1} for a specific caffeine peak, 1170±16 cm^{-1} for a specific paracetamol peak, 1962±142 cm^{-1} and 2680±130 cm^{-1} for measuring the (fluorescence) background signal.

The setup's spatial resolution is 2.19 µm or better, as determined by measuring a positive USAF 1951 target (Thorlabs GmbH, Germany). With the used 5x, NA=0.12 objective (Leica) the FOV is about 1.2×0.9 mm. For this experiment, we deliberately chose a challenging compound mixture with overlapping Raman spectra, and the filters do not separate aspirin, paracetamol and caffeine directly. First, calibration measurements were performed on the pure compounds to establish a conversion matrix from the measured data to compound identification. This conversion was performed using the partial least squares method, following the methods described in Zada et al. 2018 [1].

Results and discussion

A sample containing aspirin, paracetamol and caffeine (all white powders) was mixed and placed on a metal microscope slide. Fig. 3A shows a white-light image of the sample, in the same exact field-of-view as the actual measurement. The laser power was 10 W at 532 nm wavelength, and the measurement duration was 1 second. Fig. 3B shows the same sample, but with colour-coding per compound based on the calibrated matrix conversion of the detected Raman intensities. Aspirin is coloured red, caffeine green and paracetamol blue. The aspirin is well identified, and the caffeine and paracetamol are mostly correctly identified. Currently, we separated 3 compounds based on 2 narrow, specific filters and 2 wide, aspecific filters. To improve the separation, one wide filter could be replaced with another narrow, specific filter.

The current acquisition time of 1 second can still be significantly reduced by i) improving the light collection, and ii) enhancing the optical efficiency, i.e. reduce transmission losses between the sample and detectors. For a 1 mm FOV, a ~10x magnification suits typical camera sensor sizes well. At this magnification, NA=0.9 objectives have been made, which would collect 15x more light than the currently used objective. In addition, higher-quality optical filters (with ~2x better transmission), and a more optimized camera setup (with ~4x lower losses), would enable a total improvement in sensitivity of about two orders of magnitude. Consequently, the acquisition time could be reduced by the same factor.

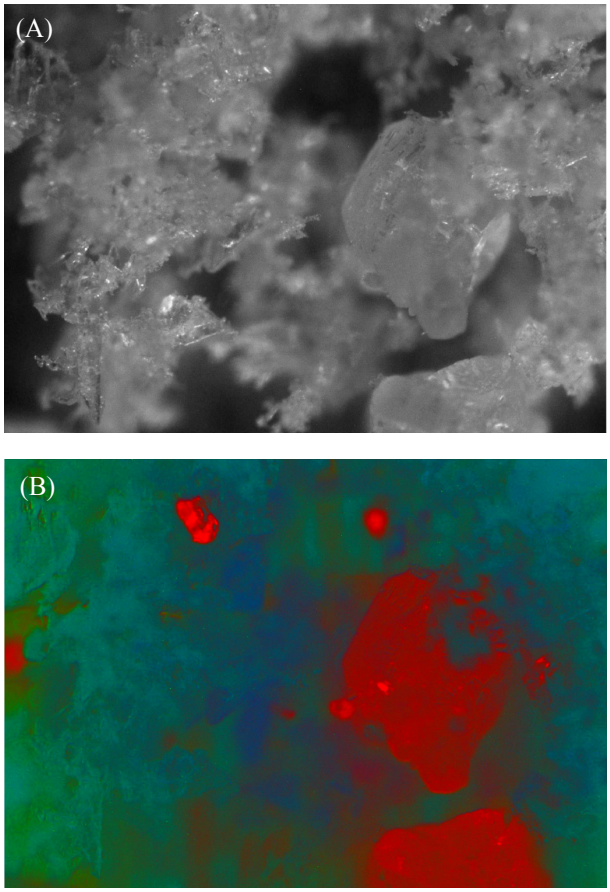

Fig. 3, (A) white-light image and (B) multispectral Raman image of the aspirin, caffeine, and paracetamol mixed sample, colour-coded per compound, reconstructed from data of a 1-second-long measurement. Red: Aspirin; Green: Caffeine; Blue: Paracetamol. FOV: 1.2×0.9 mm

5. FUTURE PROJECT VISION

The long-term goal of this project is to develop the technology into a general-purpose, high-end, high-speed, turn-key Raman imaging microscope. This instrument would compete in the marketplace with the devices produced by the companies listed in section 2. The development of such a product will require substantial funding, and involves significant commercial risks.

5.1. Technology Scaling

Given the results described above, the RAMANTIS technology is currently estimated to be at TRL 3. In order to advance to TRL 4, a setup would need to be constructed that includes a detector unit with a purpose-built RAMANTIS, i.e. one optimized for better sensitivity and more spectral channels than the current proof-of-principle setup. Furthermore, the RAMANTIS data acquisition and processing system would need to be tightly integrated in the setup, in particular in terms of software. A more integrated setup would also facilitate its relocation for the assessment of its performance and utility in multiple environments and application areas, thereby enabling the advancement to TRL 5. At this stage, a launching customer would have to be signed, for whom a more advanced prototype would be developed. This will require a revised and refined optical and mechanical design, more automation (i.e. a central electronic control unit), yet tighter integration, user-friendly alignment and calibration solutions, and a more complete software package that includes a first GUI. Completing these milestones would lead to a TRL level of 7. Further development towards a market-ready product would then be taken on by Chromodynamics.

5.2. Project Synergies and Outreach

In order to achieve the objectives for phase 2, our consortium would need to be strengthened with members bringing specific hard- and software expertise. The technology relies on high-end optical coatings to be applied to many optical components. An industrial partner is needed to develop custom coatings with all required properties. Second, close collaboration with a laser manufacturer would be desired in order to tightly integrate a custom-built laser system into the setup and explore strategies to bring down cost. In terms of software, we currently foresee transitioning to Machine Learning techniques and GPU processing to ensure the highest possible data processing speed. We would therefore likely add either an academic or an industrial partner with a proven track record in deep learning for vision processing. We would aim to demonstrate approximately three applications (see below), which would all require particular expertise from the end user. Here, it is to be determined whether the end user (e.g. from one of the Phase 1 projects) would be added to the consortium, or e.g. as a launching customer.
Assuming sufficient protection for the IP is put in place, it would be essential to generate publicity to attract commercial interest and the attention of potential investors to successfully transition into the post-ATTRACT phase. The three use cases described below would serve this purpose. If successful, it should be possible to arrange media coverage. In addition, these use cases would be presented at conferences and trade shows.

5.3. Technology application and demonstration cases

As indicated above, a Raman imaging microscope can be used for dozens of applications. The RAMANTIS technology can be used in two different operational modes: real-time identification of *selected* components, or high-speed characterization of *any* material, each with its own application areas. We have identified some of the most promising applications and summarized them in Tab. 1. The three exciting Phase 2 projects have potential societal benefits in terms of health, environment, and enabling new technological innovations.

Tab. 1. Planned use cases during and after a potential ATTRACT Phase 2 project. The use cases are divided into the categories "Process control" and "Research" in the columns, and further categorized (using *italic* or **bold**) by the foreseen operational mode of the RAMANTIS technology, namely *high-speed characterization of any material* (with 100x speedup over SOTA) & **real-time identification of selected components** (with 1000x speedup over SOTA).

	Process control	Research
Short-term ATTRACT phase 2 & initial commercialization	• **In-line monitoring of pharmaceutical product manufacturing** • *Thin film/2D material characterisation for semiconductor manufacturing*	• **High-speed characterisation of the dynamics of surface catalysis for the conversion of biomass, waste and CO2 into usable fuels and chemicals**
Long-term Post-ATTRACT	• *High-speed tissue characterisation for ex vivo medical diagnosis* • *Fast completion of crime scene forensics* • *Label-free cell cytometry with broadened component identification* • **Image-guided surgery for tumour resection** • *Rapid detection of drug counterfeiting*	• **Dynamics of uptake of drugs into cells & tissues** • ***Battery development, e.g. characterization of electrodes during charge/discharge cycles*** • *Solar cell research & development*

One of our current project partners (LaserLaB Amsterdam) is already a member of the European Research Infrastructure LASERLAB-EUROPE and once the Raman imaging setup will be fully completed and tested it will be made available for Transnational Access to the European laser research community, thereby facilitating the development of yet more applications. In summary, applications of this ground-breaking technology are numerous and wide-ranging, and could lead to new scientific discoveries, higher-quality manufacturing, and contribute to a more sustainable, healthier & safer society.

5.4. Technology commercialization

From the end-point of an ATTRACT Phase 2 project (TRL 7), significant further funds would likely need to be raised in order to develop a commercial product that is robust, easy to use, cost-competitive, manufacturable, and maintainable. This is foreseen to be performed by consortium member Chromodynamics. This company was founded early 2019 to develop and market real-time chemical imaging systems. The company has grown to 3 FTE, is profitable, and is currently validating the business plan in preparation of its next funding round. It is in very close contact with a large investment firm.

5.5. Envisioned risks

While there are technical risks involved in the execution of the technology roadmap, we foresee the greatest risk to be commercial. First, Raman imaging microscopy is an existing market with the competition in the form of large well-established companies. Breaking into this market as a new player is challenging. Second, certain key technologies, in particular high-power lasers, will need to come down in price to build a price-competitive product. Third, the envisioned product is a complex instrument with a long development roadmap. Even upon success during ATTRACT Phase 2, significant further investments and development time will be needed to reach TRL 9. Chromodynamics will need to have achieved commercial success with earlier products for the associated risks to be acceptable.

5.6. Liaison with Student Teams and Socio-Economic Study

As mentioned, we have already liaised with a student team during Phase 1. The project's lead technologist was the contact point for the students in case they had questions. We evaluated their findings at the Chromodynamics office and took their input at heart. This was a valuable addition to the project and we would welcome further such input during a potential Phase 2 project. Furthermore, at the Vrije Universiteit Amsterdam there is a successful BSc and MSc curriculum "Science, Business and Innovation" from which student or student teams can be involved to investigate business opportunities.

6. ACKNOWLEDGEMENT

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222. Funding was also received from the Netherlands Space Office, grant # ALW-GO/15-19. The authors express their gratitude for the technical support provided by Hennie van der Meiden and Jordy Vernimmen of DIFFER.

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