Design and Construction of an X-Y-Z-motorized head to perform Deep-UV Raman measurements at microscopic level in cold environments from -30 to -5 ºC (CORaHE)

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ABSTRACT

The innovative CORaHE (**CO**ld **Ra**man **He**ad) microscopy sensor for Deep-UV Raman spectroscopy has been developed to operate under cold environments between -30 and -5ºC, performing non-destructive micro-Raman measurements of cold samples, without any limitation in size. Samples are not destroyed as it happens when using cryocells. For laboratory analysis, the microscopy sensor is placed inside a cold laboratory (-30 to -5ºC) while the Raman spectrometer and computer control of the X-Y-Z microscope stage are set outside, at room temperature. For field works in cold environments, the spectrometer must be placed in a thermal box at 15-20 ºC

Keywords: CORaHE head, Deep-UV Raman; cold laboratory; microscopic measurements.

1. INTRODUCTION

The analysis of frozen/cold samples is nowadays restricted to the use of cryocells where spectroscopic analysis can be done. However, samples are destroyed in the cutting processes to fit them into the cryocell stages. This situation could be solved with a new sensor head of vibrational spectroscopy placed in a cold laboratory where samples could be set, connecting it with the spectrometer through fibre optics that could be placed outside the cold laboratory. If the probe head is installed in a motorised controlled X-Y-Z microscope, microscopic analysis could be performed to identify inclusions trapped in the bulk of the frozen/cold samples.

Among the different vibrational spectroscopic techniques, Raman spectroscopy has the maximum capability to perform microscopic measurements, with spot sizes as small as one micron. Raman spectroscopy requires a laser power to promote the Raman dispersion effect, but the thermal impact of the laser could destroy the samples. Among the different laser excitation sources in the Ultra Violet (UV), Visible (Vis) and Near Infrared (NIR) regions, those working in the Deep-UV range require less power to produce the same Raman effect, minimising the thermal damage of samples.

To cover the absence of such a probe head for Deep-UV Raman spectroscopy in the market, the innovative CORaHE (**CO**ld **Ra**man **He**ad) microscopy sensor for Deep-UV Raman spectroscopy, has been developed to operate under cold environments, between -30 and -5 ºC. For laboratory analysis, the microscopy sensor is placed inside a cold laboratory (-30 to -5 ºC) while the Raman spectrometer and computer control of the X-Y-Z microscope stage are set outside, at room temperature. For field works in cold environments, the spectrometer must be placed in a thermal box at 15-20 ºC. This portable micrometric sensor performs non-destructive micro-Raman measurements on the original cold samples without any limitation in size of the sample and discards the need of cryostages, enabling direct microscopic measurements on the entire surface of the frozen sample. Moreover, the components of the microscopic head can work also at room temperature and hence, Deep-UV Raman microscopy measurements can be performed at ambient temperature and even at temperatures up to 80 ºC. The Raman measurements always require a previous calibration to fix the daily optimized working conditions; among the different alternative materials, diamond has been elected as the most adequate because its Raman spectrum does not suffer any variation due to temperature (between -40 and 80 ºC) nor due to laser excitation.

Moreover, Deep-UV Raman spectra have the lowest fluorescence background.

2. STATE OF THE ART

Raman spectroscopy is a vibrational spectroscopic technique used to identify inorganic and organic chemical compounds, provided they are not 100% ionic (e.g., NaCl is not sensitive to Raman). This analytical technique has been applied in most of the current areas of interest in the field of fundamental and applied studies, especially when highly sensitive samples must be analyzed [1]. The proposers of this project have measured the organic compounds [2] and inorganic gases [3] present in micro bubbles and inclusions of geological materials using Confocal micro-Raman spectroscopy, demonstrating the ability of this technique to identify compounds embedded in a completely different matrix.

Raman measurements are performed currently at room temperature. However, cold environments are getting more and more interest in several fields of research. Low-temperature Raman measurements are usually performed at room temperature with the help of cumbersome cryostages, often operated with liquid nitrogen, which generally limit the size of the sample to circa 1 cm^2 in surface area and some millimetres in thickness [4]. Since most cold samples are much bigger than available cryostage cells, such measurements are destructive, in the sense that they require a complicate selection and cutting procedure of a small piece from the original sample, which becomes ruined after the measurement, precluding reproducibility [5]. All these troubles hinder the use of Raman spectroscopy in the field, since cryostages cannot be connected to portable Raman instruments.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The new CORaHE Deep-UV Raman Head for measurements in cold environments has been designed to cover the absence of technology to perform non-destructive Raman measurements on sensitive samples having temperatures as low as -30 ºC (ice cores, frozen food, hydrated minerals, clathrates, etc.). The CORaHE sensor has been designed for laboratory and field measurements. For laboratory analysis, the sensor will be placed inside a cold laboratory (-30 and -5 ºC) while the Raman spectrometer and the computer control of the motorised X-Y-Z microscopy stage will be set outside, at room temperature. For field works in cold environments, the spectrometer will be in a thermal box at 15-20 ºC. The election of Deep-UV Raman has been conditioned from the nature of the samples; the laser focusing on samples generates always a thermal effect, and Deep-UV lasers are the most suited for low thermal effect and good Raman response of organic and inorganic compounds.

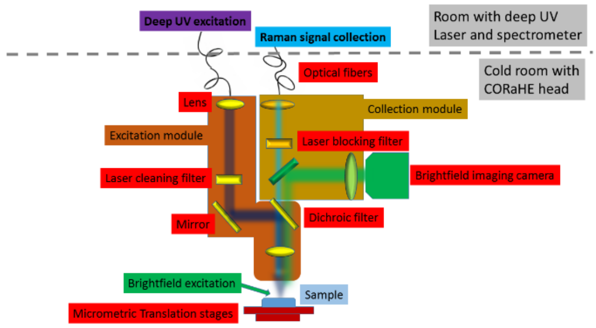

Fig. 1. The micrometric CORaHE head installed in a cold laboratory, connected through fibre optics to the Deep-UV laser source and Raman spectrometer outside of the cold laboratory.

Fig. 1 shows the conceptual design of the CORaHE sensor, mounted in a micrometric stage with a lateral resolution of one micron in X-Y-Z axis, to be installed in a cold laboratory, maintaining the spectrometer at room temperature outside the cold lab. Connections are conducted by especial fibre optics for UV light transmission and cold environmental work.

4. PROJECT RESULTS

The new CORaHE head has been implemented with components able to work from -40 to +80 ºC, using Deep-UV Raman as the identification technique for unknown compounds present in the samples. The inclusion of a micrometric stage ensures a high capacity focusing on microscopic samples embedded in complex matrixes. By simply changing the objectives, samples from 1 micron to 200 microns can be easily analysed. Details of the different parts of the CORaHE head, with the micrometric stage, can be seen in Fig. 2. Fig. 3 shows the obtained Raman spectrum of the calibrant, Diamond.

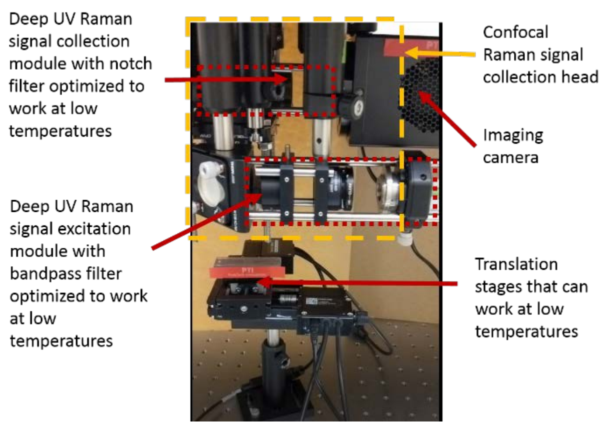

Fig. 2. Detail of the CORaHE prototype showing the critical parts that have been designed and constructed.

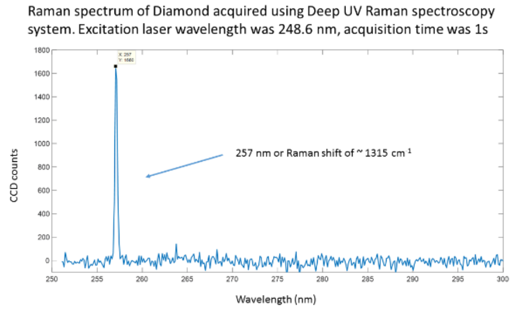

Fig. 3. Deep-UV Raman spectrum of diamond, the calibrant that must be used daily to start the work with the CORaHE head

For those not familiar with Raman spectroscopy, any Raman spectrometer must be calibrated daily to check the performance of the system. In our particular set-up, where samples from -30 to -5 ºC must be measured (even up to +40 ºC because our aim is to use the set-up not only in cold environments), the calibration sample should have the property of maintaining the same Raman response irrespective of the working temperature. This is the case of diamond.

To demonstrate the capability of the new CORaHE head coupled to a Deep-UV Raman spectrometer, Fig. 4 shows the Raman spectrum obtained for sodium gluconate. This compound shows all the expected Raman bands without any important luminescence background, a problem observed when other excitation lasers are used to promote the dispersive Raman effect.

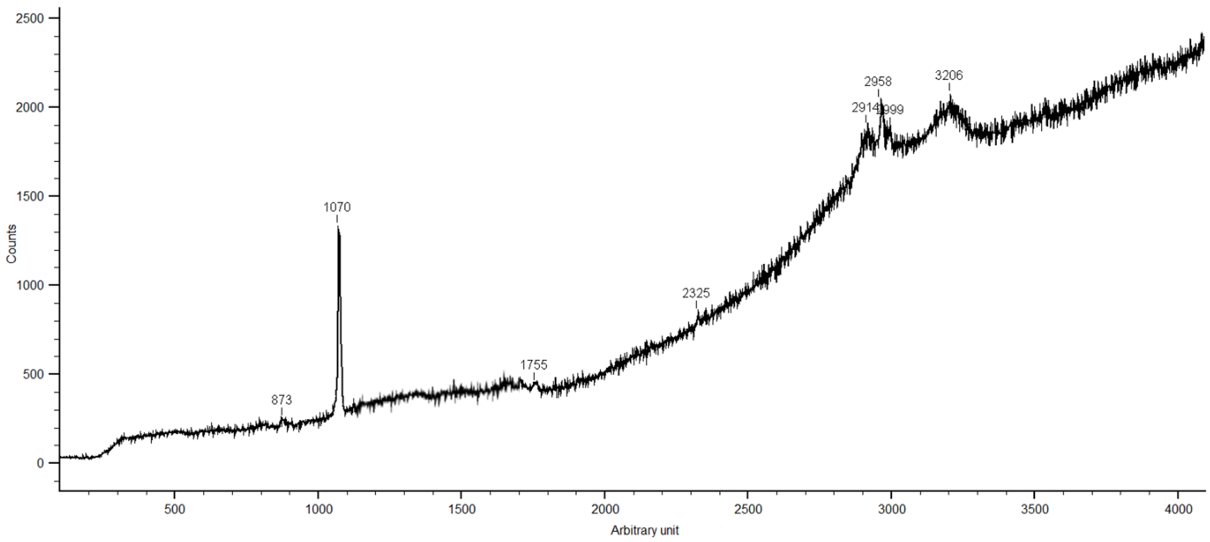

Fig. 4. Deep-UV Raman spectrum of sodium gluconate (E576 additive with a wide applicability in different agricultural, industrial and even gastronomy sectors) showing all the C-C, C=O and C-H vibrations expected for this molecule. There is not a similar spectrum published in the literature. Similar spectra were obtained for other organic compounds as well as for inorganic salts.

5. FUTURE PROJECT VISION

The results obtained in our project demonstrate that our CORaHE head for microscopic Deep-UV Raman measurements has reached a Technology Readiness Level **4.** The success obtained in the development of the CORaHE head prototype led us to consider feasible a scaled project for ATTRACT Phase 2 in order to have in the medium term a competitive product in the market, that is not similar to any other existing probe heads for Raman spectroscopy measurements. This could be achieved increasing the number and diversity of the partners of our initial consortium, strengthening the industrial and commercialization strategies around the successful prototype completed in ATTRACT Phase 1.

5.1. Technology Scaling

The main steps required for scaling our Technology Readiness Level 4 (TRL 4), achieved in ATTRACT Phase 1, to TRL 7 in the framework of ATTRACT Phase 2 are the following:

- Expand the consortium with new industrial partners
- Strengthen the scaling of the probe head, reducing its size while maintaining its performance
- Develop new software capabilities to obtain a more friendly interface between the instrument and the user

- Include databases not only to identify inorganic and organic compounds in different kind of samples (environment, agriculture, cultural heritage, biological tissues, industrial processes, research laboratories, etc.) but also to quantify its amount through the implementation of chemometric analysis of samples

- Perform laboratory and field campaign of measurements, in different environments of applicability, to demonstrate the adequacy of our approach in real scenarios

- Construct a final system prototype (CORaHE micrometric head coupled to Deep-UV Raman spectrometer) able to work in the previously defined operational environments, where differences in temperatures as hard as -30 to +40 ºC could be achieved.

5.2. Project Synergies and Outreach

To achieve TRL 7, the consortium will incorporate the next type of experts and new partners:

(a) the coordinator UPV/EHU (current Partner 1) will incorporate an expert in development of chemometric based tools for both qualitative and quantitative chemical analyses

(b) the BC3 partner (current Partner 2) will incorporate experts in sampling and measuring frozen samples in the field

(c) the PTI company (current Partner 3) will incorporate an expert in hyperspectral imaging to exploit the input for the high resolution camera included in the head

(d) a new industrial partner will be incorporated with demonstrated capabilities of constructing highly specific and sensitive spectroscopic devices, not only in the field of Raman spectroscopy but also in the field of hyperspectral imaging, and

(e) a new industrial partner with demonstrated capabilities for distributing Raman spectroscopy systems with microscopic capabilities, including dissemination actions (written documentation, web, social media, designing of tailor made solutions, etc.) to make the new device attractive for potential users.

Unfortunately we have not detected other projects developed in the framework of ATTRACT Phase 1 to which cluster as such. However, our possible industrial partners are now in the market and we have contacted them, with promising expectatives.

The new consortium will facilitate the public dissemination of our activities and results to the ATTRACT Consortium as during ATTRACT Phase 2. We will continue with our ATTRACT Phase 1 experience and the scientific outcome will be increased because we have identified research laboratories as potential end users of our final product.

5.3. Technology application and demonstration cases

To fulfil with the expected final product, including its demonstration steps in operational environments to gain TRL 7, we are envisioning an ATTRACT Phase 2 project of 3 years duration, with 1.5 million Euros funding for the described new CORaHE Consortium of 5 partners.

CORaHE will mostly detect organic molecules (also inorganic compounds can be detected) in complex matrixes and environments. Nowadays, the detection of organic compounds requires tedious analytical methods based in chromatographic analysis. In the near future, the direct detection of such organic molecules will be afforded with non-destructive analytical technologies, like the one implemented in the Deep-UV Raman spectroscopy detection through the CORaHE micro-probe head.

The technology demonstration cases, implemented in ATTRACT Phase 2, will consider the next application areas:

- Applicability in a multipurpose research laboratory processing annually more than 20 different matrices
- Direct measurements of chemospecific compounds or molecules for rapid clinical diagnosis on the surface of biological tissues
- Detection of pesticides in the skin of foods by a touch-and-go procedure
- Definition of the maturation levels of foods (tomatoes, peaches, berries) before harvesting
- Identification and quantification of micro-plastics in processed food, fresh fishes, juices, and salt/aromatics
- Organic micro-pollutants and pharmaceutical residues in treated waste-waters
- Detection of metallic micro-particles in urban atmospheres
- Identification/quantification of decay products in artworks and historical buildings
- Detection of sulphate compounds in demolition materials from old urban buildings
- Detection of dissolved nitrate in supposed bottles of high degree alcoholic beverages

The selected technology demonstration cases aim to bring **concrete** benefits in some fields in the areas of Scientific Research, Industry and Societal Challenges. In particular, our CORaHE final product will contribute to the next Societal Challenges:

- *Health, demographic change and wellbeing (CORaHE will directly measure biological tissues for diagnosis purposes)*
- *Food security, sustainable agriculture, inland water and the Bioeconomy (non-authorised chemical in food, maturity of fresh foods before harvesting, presence of micro-plastics in foods and beverages, as well as organic micro-pollutants in waters can be detected)*

- *Climate action, environment, resource efficiency and raw materials (detection of harmful compounds in urban atmospheres, diagnostics in the safeguard of cultural heritage assets, detection of non-desired compounds in secondary sources for materials reuse, etc.)*
- *Secure societies - protecting freedom and security of Europe and its citizens (direct measurements inside bottles for non-authorised chemicals, detection of hazard compounds, etc.)*

Our innovative system (Deep-UV Raman on the CORaHE micro-probe head) to measure organic and inorganic compounds, in cold and warm environments, will be implemented as portable and also as laboratory high resolution device. Thus, it could be incorporated to European Research Infrastructures due to its versatility.

The coordinator of the CORaHE project is engaged in the ERISH (European Research Infrastructure for Heritage Science) platform. There, we offer several instruments (micro-Raman spectrometers with 785, 633, 532, 325 and 266 nm excitations, High Resolution Raman imaging, High Resolution FT-IR spectrometers in Transmission, ATR and Diffuse Reflectance modes, High Resolution IR Imaging, Reflectance VisNIR spectroscopy) to the scientific community to perform measurements with high valuable samples, artworks, historic documents, archaeological objects,... CORaHE will be included in ERISH, where nobody else contribute with a Deep-UV micro-Raman system. Moreover, our system will offer the possibility to perform measurements in cold environments without the need to use the current destructive cryostages.

5.4. Technology commercialization

In the new CORaHE Consortium we will incorporate an industrial partner with its main role focused on the commercialization of the final product. The concrete steps we have envisaged for such commercialization include (a) an attractive design of the box containing the system, (b) an easy to follow software to perform measurements in an intuitive way, (c) dedicated calibration targets for defined applications, (d) technical notes based on the results of the works described in heading 5.3, (e) offer to the costumer our capability to design a tailor made solution.

In Phase 1 of the ATTRACT project we have fabricated two calibration targets, one for historical building research and other for food analyses. For the moment, we have not contacted investment stakeholders because our aim is to reach TRL 7 and the partners have agreed in incorporate their own funds if required.

5.5. Envisioned risks

The core risks of our development in a potential ATTRACT Phase 2 project are centred in the adequacy of the selected samples of the different application areas described above. Our mitigation strategy will apply the cleaning procedures of samples in field works together with the use of soft pre-concentration steps to increase the level of measurable compounds in front of the CORaHe head. These are procedures applied in standard spectroscopic measurements using portable Raman devices with different excitation laser sources.

5.6. Liaison with Student Teams and Socio-Economic Study

The CORaHE Consortium in ATTRACT Phase 1 incorporates two pre-doctoral students. One is going to present her thesis next September. The other will present his thesis by the end of May 2021. In the new CORaHE Consortium for ATTRACT Phase 2 project, we will continue with the same philosophy of incorporating pre-doctoral researchers in some of the teams, with their PhD projects hosted by the Doctoral Program (UPV/EHU) coordinated by the IP of CORaHE. Moreover, one member of the Partner 1 team is the coordinator of a Master Degree at the UPV/EHU, and he will offer Master Thesis positions to the European academic community.

Finally, the new CORaHE Consortium will contribute during Phase 2 to the expert-driven socio-economic study of the ATTRACT initiative and ecosystem. We will prepare written and video material with the results of Phase 1, and as we progress with the results of the demonstration campaigns of Phase 2, similar materials will be prepared and submitted to the ATTRACT office. These materials will include contributions of third persons that facilitate our field measurements, explaining the benefits they observe with the systematic application of CORaHE in their field of activity.

6. ACKNOWLEDGEMENT

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