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Spectroscopy COVid-19 Analysis with mulTi-wavelength Observations (sCOVato): proof of concept

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Abstract. The sCOVato project aims to develop a rapid, specific and low-cost diagnostic methodology for the detection of viral infection in material extracted from pharyngeal swab. This project consists in two distinct methods to detect virus antigens in biological material using a spectral multi-frequency approach (microwave and visible wavelengths). Laboratory set-ups to perform preliminary tests in both spectral bands have been assembled and put in operation. These tests have been conducted on isotonic solutions with different concentrations of contaminant to assess the feasibility of the experiment.

Key words. Microwave dielectric spectroscopy – Laser Induced Fluorescence – Multi-wavelength technique

1. Introduction

In March 2020, at the National Institute for Astrophysics a call for ideas was launched by the INAF President, Prof. N. D'Amico, to contrast the COVID-19 pandemic. In response to this call, the authors of this contribution started an inter-disciplinary brain-storming on how to apply their competences and skills to this health emergency. Eventually, the so-called sCOVato project (acronym of "Spectroscopy COVid-19 Analysis with mulTi-wavelength Observations", Figure 1) was seen as an original and promising contribution to this purpose.

The proposed technique intends to detect directly the presence of the virus through a

spectrometric analysis using electromagnetic fields in two different ranges of the spectrum: microwave and visible band. The basic idea is to recognize the absence or presence of virus pathogens by measuring the spectrum of the dielectric properties of the biological sample (at radio frequency) and the fluorescence signal emitted by the aforementioned sample (visible band).

The long-term goal of the proposed project is to quickly, easily and accurately detect and diagnose a virus outbreak (as for instance COVID-19 pandemic) overcoming the current diagnostic challenges, such as the needs of laboratory environment to perform sophisticated tests, highly prepared operators and time-consuming procedure.

At the time of the INAF workshop (March 2021), the status of the project was that laboratory tests were conducted to assess the sensitivity and reliability of the measurement systems. It is worth noticing that at that time no virus have been used in the tests.

In this contribution, Section 2 briefly summarizes the most common techniques used for virus detection. Then in Section 3 the general methods adopted by the authors are detailed. Finally, in Section 4 we show two proofs of concept to perform measurements in the two frequency bands with also some preliminary results.

2. Virus detection techniques

The existence of a rapid determination of epidemic pathogens is one of the basis for the control and eradication of infections. To date, the most accurate virus detection technique is based on Real-Time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR), a time-consuming procedure that requires highly prepared operators. rRT-PCR, known as quantitative PCR (qPCR), consists in the amplification of the nucleic acid of these microorganisms. qPCR testing is a highly sensitive technique, ten copies per reaction is the sensitivity that the researcher is relying upon to avoid false-negative tests that can result in the failure of detection of virus-infected patients.

At the same time, the enormous gap between the large number of patients/contacts and the laboratory capacities to perform rRT-PCR in a timely manner is a major limitation of current public health containment strategies. Moreover, rRT-PCR has some operational limitations such as the costs of diagnostic tests, the operators expertise, and finally, the necessary logistics for the safe preparation of the samples. The pandemic onset of SARS-CoV-2 has dramatically highlighted this problem, which can be generalized to other important viral pathogens such as the dengue virus, the west nile virus or the yellow fever virus just to mention a few of the organisms that cause more victims and with higher morbidity.

For this reason, rapid, low-cost, transportable, standardized and relatively easy-to-implement diagnostic tools that can be inserted into a reproducible laboratory equipment represent one of the research priorities to stop the epidemic that causes COVID-19 pandemic or other similar ones that could emerge in the future. As alternative assay, in the last months the antigen detection tests have been widely applied thanks to their ability to detect the presence of the virus in respiratory samples within 15-30 minutes even if with lower sensitive than rRT-PCR (Huggett et al. 2021; Pavia 2021).

Ths sCOVato project aims to propose the direct detection of virus antigens by means of other alternative strategies based on microwave dielectric spectroscopy and Laser Induced Fluorescence (LIF). Some works in the literature show measurements conducted using microwaves to detect structural information of cells in biological solutions (Denzi et al. 2015; Ning et al. 2014). Although dielectric spectroscopy applications for the detection of molecular components such as glucose (Yilmaz et al. 2019) and nanometric particle such as liposomes (Merla et al. 2009) are present in the literature, specific works on the detection of viruses carried out in the same frequency range of the present project are not reported. Bridging this gap is one of the objectives of the sCOVato project.

Furthermore, we plan to use LIF technique for the detection of viral pathogens. A recent study performed on Picornaviruses (Gabbarini

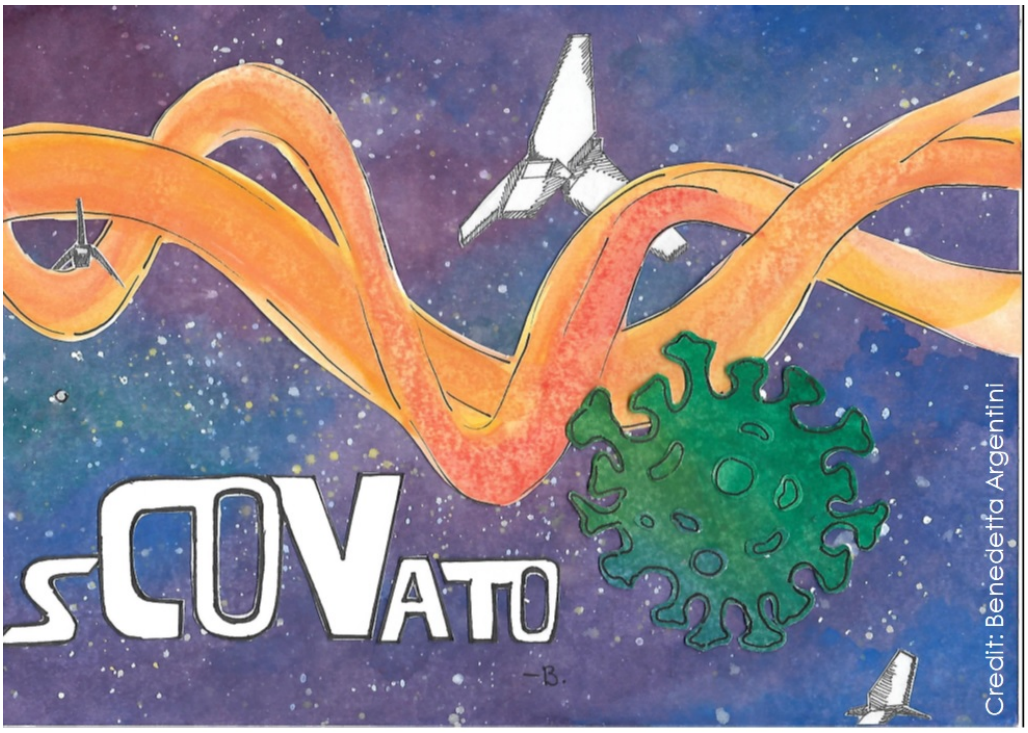

Fig. 1. Visual concept of the sCOVato project with EM signals at different wavelengths implemented to detect the virus.

et al. 2019) supports the idea proposed in this project to apply the technique to viral agents here prepared with specific fluorophores attached to antibody markers.

3. Multi-band method

Nowadays, the multi-band approach is widely adopted in modern astrophysics to reveal the complex phenomena of the Universe. Therefore, a similar synergy of observations in different frequency bands will be proposed by the authors to increase the success probability in the detection of viral infection. The interaction between the two approaches to access the advantages of each of them will be defined at a later stage after both methods have been carefully tested and their intrinsic limitations are fully understood.

Hereafter the two methods operating in microwave and visible band will be presented.

3.1. Microwave dielectric spectroscopy

The technique proposed for the microwave approach is the dielectric spectroscopy. This is a very common method to use radio frequency electromagnetic waves to measure the electrical characteristics of the material under test.

Among numerous applications, dielectric spectroscopy is used in bioelectromagnetics to probe biological materials. When the electromagnetic signals cross the biological medium, the electric properties of the biological elements interact with the field, providing an electric signature of the sample that can be extracted from the measured dielectric permittivity. The latter is a complex quantity that characterizes the interaction between the material and the electromagnetic radiation.

The use of microwave dielectric spectroscopy has resulted in several breakthroughs in biological investigations, particularly in molecular and cellular analysis. In fact, it of-

fers the advantage of being contactless, and label free, allowing for non-destructive characterization of the sample under test with high measurement speed. Moreover, possible contamination or perturbation of the biomaterial is suppressed during characterization and the detection area may be easily sized to very small volumes of biological samples (i.e., microliters and below). The sensing technique is non-invasive for cells or other biological specimen thanks to the application of low power fields to avoid any interference with the living matter; thus, cells can be kept alive during tests, thereby promoting the real-time monitoring of biological reactions.

Our idea is to use this technique to detect small variations of the dielectric properties of the sample thanks to the different dimensions of the constituents. By applying an electromagnetic wave to a biological sample we should be able to recognize the presence of a virus by using specific markers that interact with the virus.

3.2. Laser Induced Fluorescence

For the detection in the visible band, the LIF technique will be used, i.e. the use of an artificial source (laser light) that excites molecules called fluorophores (Lakowicz, 2006).

Fluorophores can have natural or synthetic origin, moreover they can occur naturally, or they can be added to other components of the sample to provide fluorescence. Each fluorophore is characterized by a specific absorption spectrum and this feature has a fundamental impact on the selection of the excitation light source. Fluorescence emission instead, typically occurs at lower energies or longer wavelengths than that of the absorption light (Stokes Shift).

Generally, fluorescence spectroscopy dataset are presented as emission spectra. Fluorescence spectra are intrinsically connected to the chemical structure of the fluorophore and the solvent in which it is dissolved, so they can show very different shapes.

Historically, the first detection of fluorescence from a quinine solution in sunlight was obtained by Sir John Frederick William Herschel in 1845. Nowadays, fluorescence spectroscopy is considered of fundamental importance in biochemistry and biophysics, extensively applied in in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, and genetic analysis (Duschek et al. 2017; Owoicho et al. 2021).

In particular, our experiment can be collocated in the immunoassay field (Cox et al. 2019) where fluorophores are used to mark the viral agent to be detected. When excited the fluorophores will emit a fluorescence signal that will attest the presence of the virus within the sample. Differently from the microwave approach, the biological sample will go through a cleaning process to remove the exceeding fluorophore. The main advantage of the LIF approach is the possibility to quantify the fluorophore in the sample and therefore the amount of virus.

4. Measurement and preliminary results

4.1. Microwave band

The measurement technique is conceived with a 2-port transmission/reflection method (i.e. given by a 2x2 scattering matrix) to characterize the material under test in a wide frequency band. A Vector Network Analyzer (VNA) instrument is first calibrated and then connected to an ad-hoc designed rectangular waveguide specimen holder containing the biological sample under test (see Figure 2). The Ka band (26-40 GHz) is considered a very interesting frequency range for the experiment, best suited to host ideal volumes of samples guaranteeing, at the same time, sufficient biological material for the test and signal attenuation levels within the measurement dynamics of the instrument. From the reflection and transmission coefficients, it is then possible to retrieve the spectra of complex dielectric permittivity and loss tangent of the material under test.

Preliminary tests have been conducted at the INAF-Arcetri Astrophysical Observatory using isotonic solutions (the same base commonly used in tests with chemical buffer

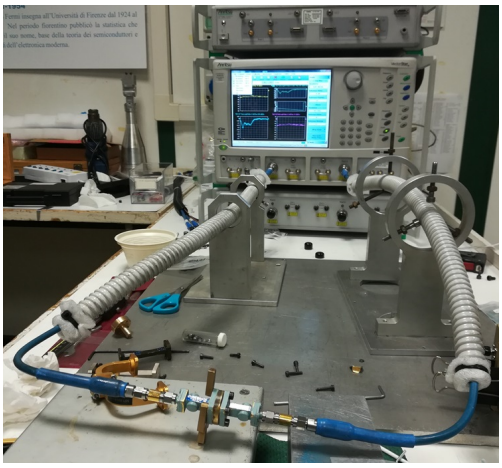

Fig. 2. Measurement setup consisting of a VNA (in the background) and input/output coaxial cables connected to the coaxial line - waveguide transitions and to the sample's holder (in the foreground).

reagents) plus the addition of sugar at different concentrations. The main purposes for this series of tests were to verify the mechanical stability and the watertight sealing of the holder. We wanted also to evaluate the losses in the isotonic-based medium and assess them with respect to the overall sensitivity of the measurement system. Another objective was to verify the repeatability in the results for a sequence of consecutive measurements; drift of the instrument as well as mechanical instabilities and inaccuracies in the procedure applied by the operator to fill and seal the holder could seriously impact the results.

Figure 3 (top panel) shows in the frequency band 26 - 40 GHz the transmission coefficient of the isotonic solution contamined by different levels of sugar (from 1% to 10%). For each level of concentration, three measurements have been conducted always repeating every single step of the procedure (inserting the sample in the holder, sealing the holder, connecting it to the VNA and running a new acquisition). The averages of the three measurements with same concentration are reported in Figure 3 (bottom panel). The different curves in the top and bottom panels of Figure 3 clearly indicate that: the loss in the medium increases with the frequency (by a factor of about 15 dB from 26 to 40 GHz); the higher the concentration of the sugar, the higher the transmission coefficient (this relation is frequency-dependent, for instance at 26 GHz there is about 2.5 dB difference between losses without sugar and with 10% sugar concentration); the same amount of sugar brings to very similar transmission coefficient values; even for the lowest transmission coefficient value (no sugar at 40 GHz) the Signal-to-Noise Ratio (SNR) is still at an acceptable value. In particular, the capability for the proposed system to identify even a small perturbation in the sugar concentration (1%) is highlighted by the differences between the orange and the blue curves in Figure 3 (bottom panel).

Finally, it is worthwhile noticing that here only results on the amplitude of the transmission coefficient have been presented. However, in the full data-reduction procedure, the phase information of the transmission coefficient as well as the complex reflection coefficient will be used to extract the spectral permittivity information. This will increase the sensitivity to detect small features in the media under test.

4.2. Visible band

The measurement method is based on the detection of a fluorescence signal using a customized instrumental set-up arranged in a right-angle geometry configuration. Figure 4 shows a diagram of the measurement setup.

The biological sample under test is prepared in a commercial plastic micro-cuvette and located in a dedicated holder. A laser-source with an opportune wavelength in the visible band illuminates the sample. The fluorescence (and scattering) radiation is then collected by means of an optical fiber-bundle that directs the signal through a spectrograph instrument. Several spectra are acquired and combined together after removing the background noise of the detector. This process allows to improve the SNR of the final results. Final step of data-processing is the comparison with a buffer spectrum (the solvent).

Preliminary tests have been conducted using solutions with various fluorophores at different levels of concentration. Figure 5 shows

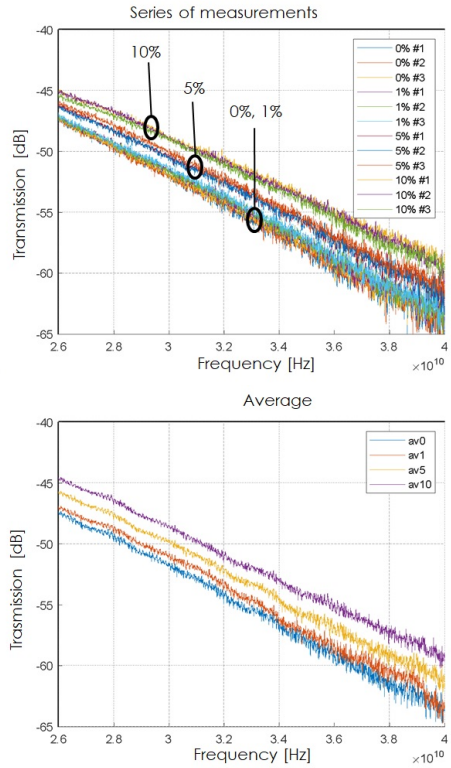

Fig. 3. Top panel: Transmission coefficient in dB for a sequence of measurement with isotonic solution and different concentration of sugar. Bottom panel: Average of transmission coefficient in dB computed from the three consecutive measurements with identical sugar concentration. The four curves refer to the following cases: no sugar, 1%, 5% and 10% sugar dissolved in the isotonic solution.

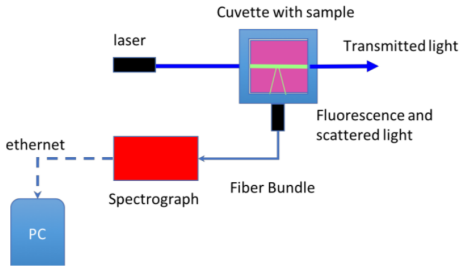

Fig. 4. Overview of the measurement setup based on the LIF spectroscopy in the visible band.

typical fluorescence spectra of solutions including only fluorescein or rhodamine (top panel), and a mix solution between the two fluorophores (bottom panel). The presented spectra are obtained illuminating the sample with a laser-diode at a wavelength lower than 500nm and averaging a series of subsequent acquisitions with exposure time of the order of tens of milliseconds. Background noise and buffer (solvent) spectrum have been removed. Molecules such as rhodamine and fluorescein present very bright emissions and are easy to detect. This behavior is derived by a very high values of the quantum yield (QY). QY is defined as the number of emitted photons relative to the number of absorbed photons and represents together with fluorescence lifetime one of the most important characteristics for a fluorophore, connected to the limit of detection in LIF spectroscopy.

In addition, other fundamental parameters to be taken under control in the preparation of the instrumental setup based on LIF spectroscopy are the choice of a proper light source in relation with the fluorophore used, an appropriate selection of the illumination exposure time to prevent the photo-bleaching phenomenon (Lakowicz, 2006) and the sensitivity of the instrumental apparatus.

5. Conclusions

One of the strengths of this research project is the interdisciplinarity of the proposing group. The skills in the field of infectious diseases of ISS, the theoretical and applicative work of the group of UniRoma1 in dielectric spectroscopy in biology and medicine, and finally the expertise of INAF and CNR in the characterization of dielectric materials and in fluorescence spectroscopy represent a “unicum” able to fully cover the perimeter of the research project.

The current status of the project is the implementation in laboratory of the methods to reach the ambitious goal of this project.

New measurements are now in progress to mimic with higher fidelity the real experiment’s objectives. For this purpose, we are

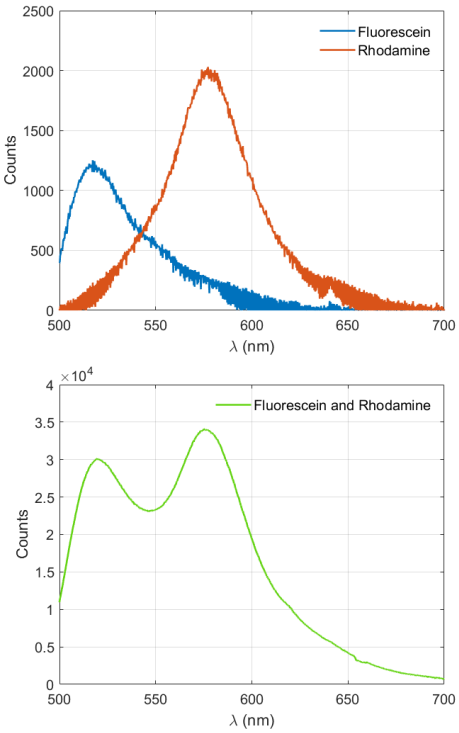

Fig. 5. Top panel: Fluorescence spectrum of a fluorescein solution (red line) and a rhodamine solution (blue line). Background noise and buffer (solvent) spectrum have been removed. Bottom panel: Fluorescence spectrum of a mix solution between fluorescein and rhodamine.

performing a differential spectroscopic analysis by using virus-like particles.

Finally, a patent application on both techniques is currently in progress.

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