Nota Técnica / Technical Note

# Spectroscopy For Medical Applications
# Grupo Álava S.L.

**ABSTRACT:**

In view of the COVID-19 alarm situation, the following technical note explore the possibilities of spectroscopy in medicine. Biomedical and life sciences professionals require faster and more accurate diagnostic tools. In this sense, spectroscopy is a new powerful tool for automated clinical and laboratory analysis of viruses and bacteria.

**RESUMEN:**

Ante la situación de alarma por COVID-19, la siguiente nota técnica de OPA explora las posibilidades de la espectroscopia en la medicina. Los profesionales de la biomedicina y ciencias de la vida requieren herramientas de diagnóstico más rápidas y precisas. En este sentido, la espectroscopia es una nueva y poderosa herramienta para el análisis automatizado clínico y de laboratorio de virus y bacterias.

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## Promising Diagnostic Tool For Viral Infections

In the face of the coronavirus alarm situation, many resources are being devoted worldwide to research in the fight against Covid-19 and future mutations. Among the most used immunological tests in diagnostic methods are the Polymerase Chain Reaction PCR test and the enzyme-linked immunosorbent assay ELISA combined with photonic and detection technologies such as fluorescence measurement which can be performed with a spectrometer or sensor and UV/VIS reflectance. Fluorescence is a particularly powerful technique for cytometry applications, notably for detection of rare cells and pathogens and to count white blood cells, even at low concentrations. A research team at Simon Fraser University is using fluorescent imaging to sensitively detect RNA molecules within living cells. This is a useful application to understand the functioning of normal cells, which are regulated via RNA, and to understand RNA viruses act in cells.

The Ocean Insight PCR Fluorescence Sensor is an excellent option for quantitative polymerase chain reaction (qPCR) testing. This highly integrated multispectral sensor is tailored to detect common fluorescence in PCR reactions. In this 9 x 9 mm array it is packed the capability to detect 4 different fluorescence signals simultaneously in one optical path allowing optical designers ultimate flexibility in spectral detection, from the choice of optical filter bands to the number of channels, package type, and electronics.

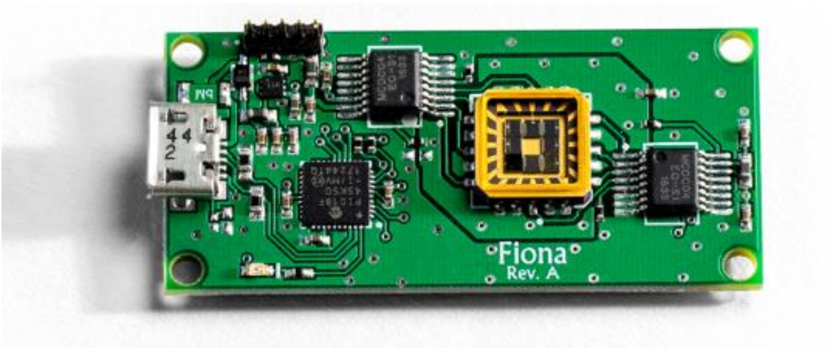


**Fig. 1.** Ocean Insight PCR Fluorescence Sensor

Ocean Insight spectrometers have been used on the detection of Influenza [1]. Since influenza, the virus mainly responsible for the flu, mutates regularly it is important to detect and distinguish the latest virus to give an indication on treatment and danger of the infection. The developed solution contains a multilayer surface. By measuring the spectrum, the researchers could detect changes in the dielectric environment making it possible to selectively recognize the influence virus with buffer and marker.

Even though PCR and ELISA techniques are the gold standard for virus detection none of them are ideal in terms of cost-effectiveness, speed, and accuracy when testing in high volume pandemic situations and sometimes cause false-positive or false-negative results. Currently, the rate of outbreak of emerging viruses is increasing so rapid that diagnosis and quarantine of virus mutant-infected people is critical to contain the fatal viral infection spread given that effective antiviral drugs are normally not available [2]. Therefore, the development of analytical methods for such viral infections are becoming more important.

Near-infrared (NIR) spectroscopy represents a useful new tool in the clinical and virological fields. Studies have been published on how NIR spectroscopy is a promising tool for rapid, accurate and cost-effective diagnosis of viral infections. Moreover, NIR spectroscopy enables non-invasive and non-destructive analysis [3]. Recent research from North Carolina State University outlines how near-infrared (NIR) spectroscopy could be used to make cell-culture-based flu vaccine manufacturing faster and more efficient. By measurement with an NIR probe inserted into the bioreactor, the concentration of the influence virus within cells could be monitored [4]. It has also been proved that NIR measurement can be used for large-scale detection of HIV-1 in human plasma [2].

Raman spectroscopy has advantages for biomedical applications because as a label-free technique it can be used to noninvasively examine a single live cell [5]. Surface enhanced Raman spectroscopy (SERS) is also well suited to biosensing, from blood glucose to diagnosis of diseases like cancer, Alzheimer's and Parkinson's. SERS can be performed on a substrate, or using colloids suspended in solution. While metals like gold, silver and copper are the most common materials used for SERS, novel options like graphene, semiconductors and quantum dots are also being explored. Graphene oxide, a chemically treated version of graphene, has a number of properties particularly suitable to biological applications. It has been demonstrated indeed that viruses such as the coronavirus MERS-CoV can also be detected using Raman Spectroscopy and SERS technique [6].

Researchers at Jackson State University successfully used SERS to detect the human immunodeficiency virus (HIV). HIV was chosen as the first pathogen of interest due to its prevalence as a cause of death in Africa, Asia, and the Middle East. They first tested gold nanoparticles of varying shapes using Rhodamine 6G dye, a well-known Raman-active compound, to determine which would show the greatest electromagnetic enhancement using. Once selected, the popcorn-shaped gold nanoparticles were conjugated to graphene oxide fragments less than 50 nm in diameter to create a hybrid SERS probe [7]. The system used to make the measurements comprised an Ocean Insight QE series spectrometer, a 670 nm excitation laser and an InPhotonics fiber optic Raman probe as shown in Figure 2.

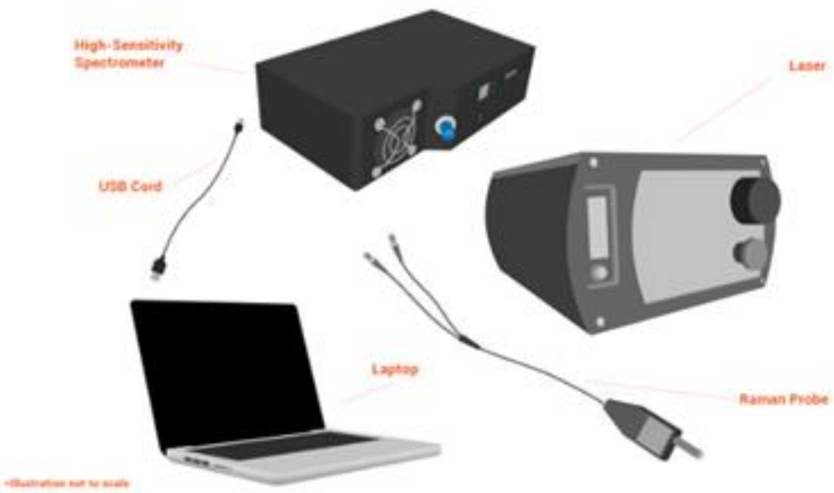


**Fig. 2.** Modular fiber optic Raman spectroscopy system for SERS detection

Each peak in the observed Raman spectra shown in Figure 3 could be assigned to the literature-reported data confirming that the Raman spectra observed were attributable to the presence of the virus in the sample. Moreover, the hybrid probe was found to be ~100x more sensitive than the gold nanoparticle probe alone, showing the power of employing both chemical and electromagnetic enhancement simultaneously in the same SERS probe [7].

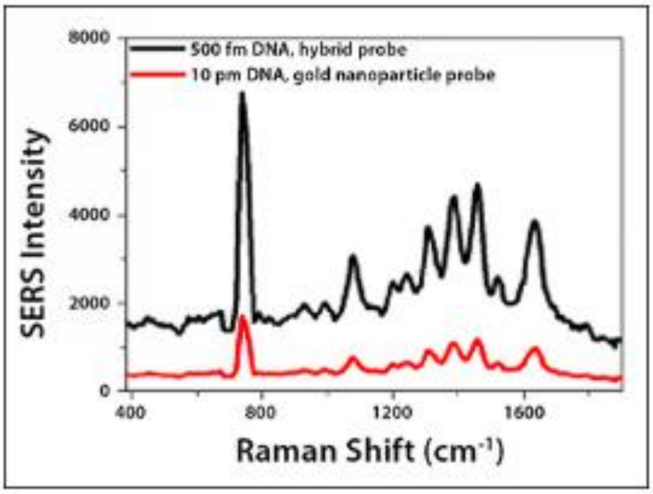


**Fig. 3.** SERS-based detection of HIV using popcorn-shaped gold nanoparticles conjugated with graphene oxide (black), with the gold nanoparticle alone (red)

 

The second pathogen of interest to be studied was MRSA. Approximately 100.000 MRSA infections occur each year in the United States, with 20.000 lives lost. Sensitive detection of this pathogen has the potential to catch infections in the early ages. The researches modified the graphene oxide used in the hybrid probe with the aptamer APTSEB1 to achieve selective detection of Staphylococcus aureus, with MRSA aggregating inside the graphene oxide sheet. SERS data collected compared the enhancement effect of gold nanoparticles and aptamer-modified graphene oxide alone to the hybrid probe (Figure 4). The hybrid probe displayed significantly higher SERS signal than either single-component probe alone, allowing detection of MRSA down to 10 CFU/mL [7].

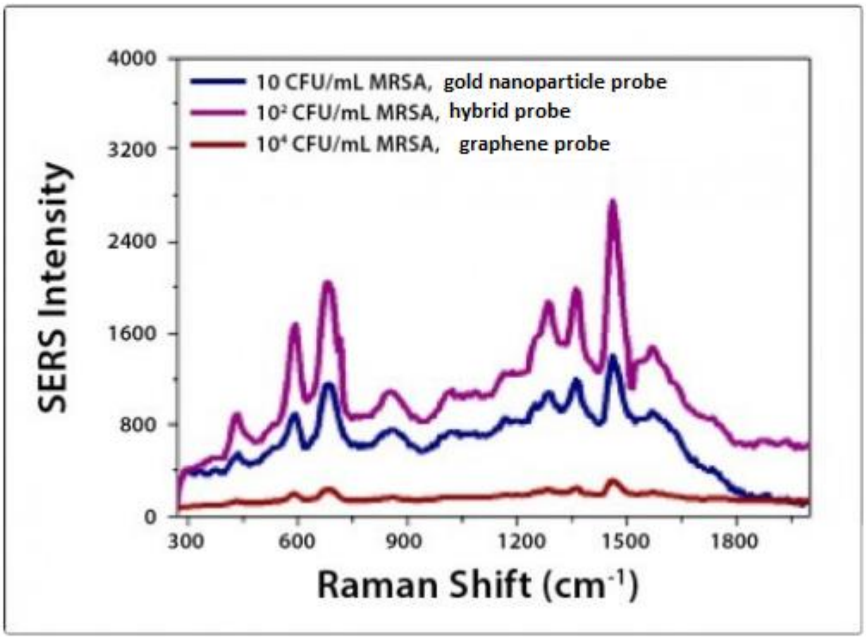


**Fig. 4.** SERS-based detection of MRSA using popcorn-shaped gold nanoparticles conjugated with graphene oxide (purple), with the gold nanoparticle alone (blue) and the graphene oxide alone (red)

Surgical site infections (SSIs) are common post-surgical complications that represent a significant clinical problem. With a view of develop a minimally invasive technique that permit early detection of SSIs, a compact, clinically deployable, bench-top Raman spectrometer coupled to a bifurcated optical fibre probe was used for the study of bacteria in vitro [8]. Figure 5 shows a schematic diagram of the Raman spectrometer set up used in the study.

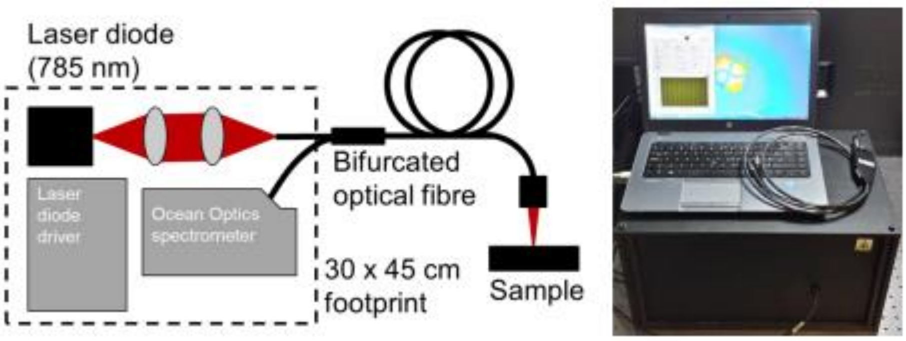


**Fig. 5.** Schematic diagram and photograph of the compact, optical fibre based Raman spectrometer used

 

SERS spectra from *E. coli* is shown in Figure 6. A series of 3-4 Raman peaks are clearly observed in the frequency range 1250-1500 $cm^{-1}$ which are in qualitative agreement with previously published data. Importantly, the characteristic shape of the *E. coli* spectra can be resolved even at acquisition times as low as 1 s which is a clear indicator of the high sensitivity of the system [8].

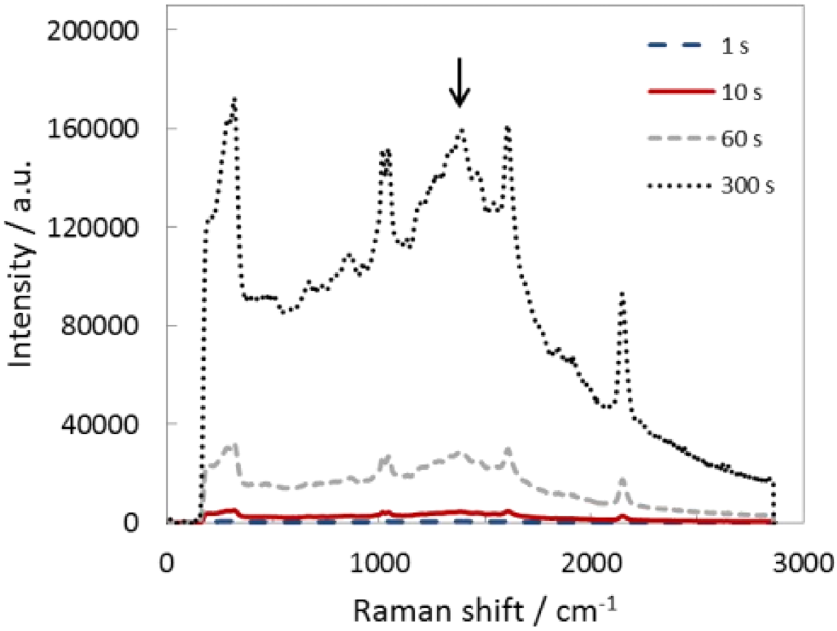


**Fig. 6.** SERS spectra from E. coli at a concentration of $10^9$ cells/ml with acquisition times of 1, 10, 60 and 300 s respectively

Recent research highlights that detection of cells infected with different influenza viruses can be done even faster with SERS technique used in combination with principle component analysis (common statistical analysis within spectroscopy) [9]. A good insight into the mechanism of this technique is offered in the below schematic drawings.

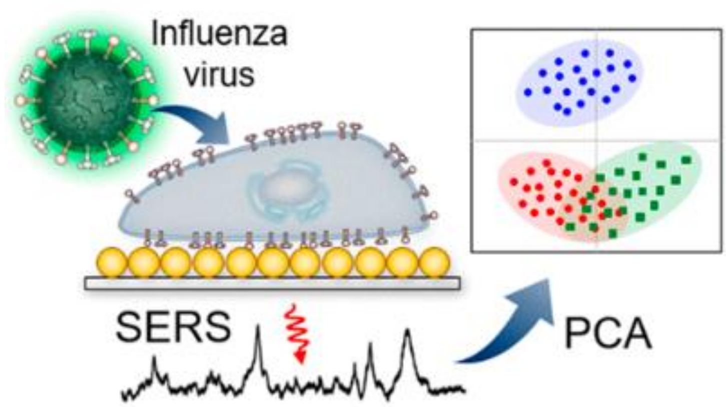


**Fig. 7.** SERS technique used in combination with PCA

In 2016 Ocean Insight SERS specialist ran tests in collaboration with Boston looking at the ability to detect *E. Coli* using specialized nanoparticles. The E. *Coli* bacteria were bound with antibodies and clustered with nanomagnets after which they were dropped on the golden SERS substrates. Results are shown below.

 

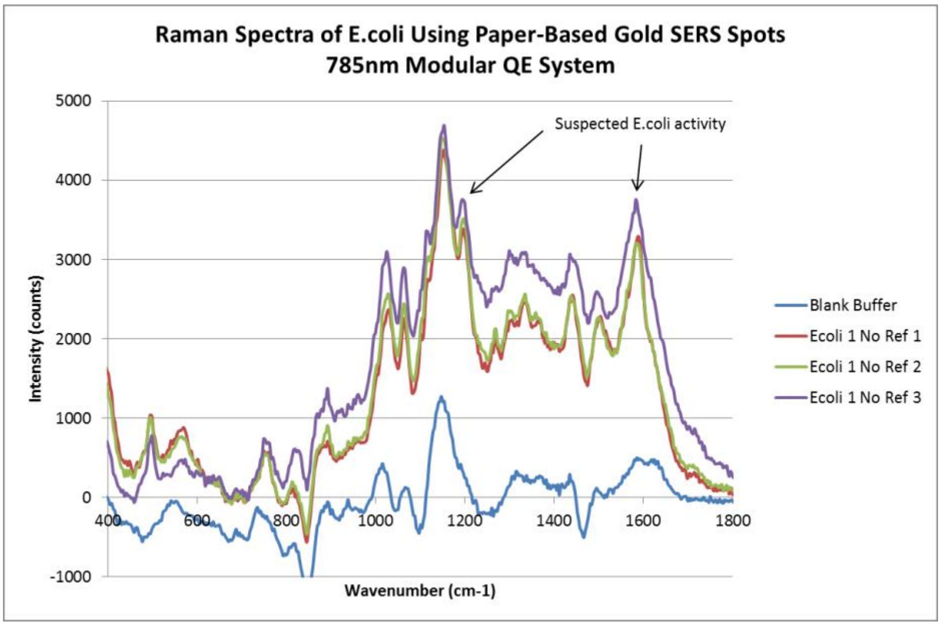


**Fig. 8.** Raman Spectra of E. Coli using paper-based Gold SERS Spots 785 nm Modular QE System, No Magnetic Pulldown

Finally, an amplification-free surface enhanced Raman spectroscopy (SERS) approach for specific detection of DNA point mutations from cancer cells with single-base sensitivity has been also developed. With the help of microfluidic chips, parallel SERS detections were finished in 40 min, much faster than the conventional PCR approaches which take 2–3 hours. Accurate and sensitive identification of DNA mutations in tumor cells is critical to the diagnosis, prognosis, and personalized therapy of cancer. The rapid, sensitive, straightforward, and inexpensive chip-based assay could potentially become a powerful tool for clinical diagnosis and precision medicine [10].

Modular spectroscopy systems are a powerful multivariate and reproducible technique for rapid microbiological diagnosis, showing great potential in today's viral infection research. It is used in systems biology, being a nondestructive, affordable, fast and to some extent, precise approach, allowing vast amounts of information to be obtained in one measurement.

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