This is a peer-reviewed, accepted author manuscript of the following research article: Graham, Duncan. / Can raman spectroscopy be a useful tool in the fight against COVID-19?. In: Spectroscopy (Santa Monica). 2020 ; Vol. 35, No. 6. pp. 44-46.

Can Raman Spectroscopy be a useful tool in the fight against COVID-19?

Duncan Graham, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK, G1 1RD

On the 31st of December 2019 the first cases of what is now known as COVID-19 caused by the SARS-CoV-2 virus appeared on the radar of the World Health Organisation in Wuhan, China. This rapidly escalated into a global pandemic with the virus capable of spreading very rapidly and uncontrollably between individuals, and within groups, where there was unrestricted movement and contact. Due to the rapid spread of the virus and the severe consequences associated with contracting the virus, there was a global response to attempt to combat the effects of the virus. The World Health Organisation were adamant that rigorous and plentiful testing was the key underpinning approach that would provide the data required to control the spread of the virus and inform the models of the extent of the transmission. From the first sequencing of the SARS-CoV-2 virus there emerged a polymerase chain reaction (PCR) based test which relied on reverse transcription of the viral RNA followed by a subsequent PCR. This provides the presence or absence of the virus and has remained the gold standard test to date. Typically, this takes 24 hours before the result is returned from sample acquisition, meaning that it is difficult to rapidly assess a patient presenting with symptoms and whether they are infected or not (although some assays take only 2 hours to run, the process of getting samples to the testing site and obtaining results can take time). Abbott have just had a point of care PCR assay approved by the FDA that takes 5 minutes for a positive result and 13 minutes for a negative result but still relies on nucleic acid detection. In a recent publication (*Nature* (**2020**) https://doi.org/10.1038/s41586-020-2196-x) it was found that the PCR assay responded negatively for serum testing yet patients were still displaying symptoms of COVID-19. When their upper respiratory tracts were tested, positive responses were found beyond the end of symptoms and seroconversion took between 7 and 14 days. This shows that the PCR test is good for respiratory samples but is limited in its ability to provide more information about the progression of the disease and subsequent immunity. This has led to interest in an immunoassay with a number of immunoassays being rolled out by different companies e.g. Biomedomics and Cellex (*Nature Biotechnology*, **2020**, https://doi.org/10.1038/d41587-020-00010-2), however the majority of these test examine the overall immune response and then assume the immune response is coming from SARS-CoV-2 infection. They make use of the fact that the immune system dramatically increases the amounts of IgM protein due to the initial infection by the virus and then subsequently the levels of IgG increase showing that there is an immune response to the virus from the host.(MedRxiv https://doi.org/10.1101/2020.02.28.20029025) Therefore, looking at the IgG and IgM response using an immunoassay (such as a lateral flow test similar to a pregnancy test) gives an indication of whether a presenting patient has an immune response. If they are presenting with symptoms of COVID-19 then the assumption is the immune response is due to that virus. In tests conducted so far, this approach has shown better accuracy for a negative response, however the positive response appears to be less accurate which leaves a considerable gap in determining whether someone has the COVID-19 infection (*Diagnostics*, **2020**, 10, 202). An alternative immunoassay is being developed by Sona Nanotech to detect the S-protein on the coronavirus rather than the immune response of the host which would provide an accurate diagnostic for SARS-CoV-2. All the immunoassays make use of gold nanoparticles and their highly visible colourimetric response whilst the PCR based assays make use of fluorescence detection. The question for this article is whether or not there is role for Raman spectroscopy to play in the fight against COVID-19 and what that role might be?

Raman spectroscopy is a highly information rich technique which provides unique vibrational fingerprints either of individual molecules or complex matrices. Water has a low background signal making it ideal for biological samples which are in aqueous, buffered solutions however the lack of sensitivity can be an issue for Raman spectroscopy. The following are some thoughts about where

Raman spectroscopy and variations of Raman spectroscopy may play a role in the fight against COVID-19 now and in the future.

The first area that Raman spectroscopy can obviously play a role in is the detection and diagnosis of an infected patient sample. There are literature methods reported on the detection of various viruses using Raman spectroscopy and subsequent classification of them, normally based on chemometrics and some form of principal component analysis. Is there an opportunity for Raman spectroscopy to provide direct detection of infected patients? Would this Raman vibrational fingerprint change as the degree of infection either increases or decreases and would this vibrational fingerprint from the patient allow the progression of the disease to be followed and subsequent immunity determined? Would isolation of the viral particles from the sample need to be performed? These are very challenging questions to answer. The virus itself would need to be handled in its live form in category 3 laboratories however there may be opportunities to use some of the deactivated virus in due course. Several groups are working on direct detection of COVID-19 infected patients and one has to remember that unlike other viral detections COVID-19 does not appear to be found in high levels in blood therefore nasal swabs, sputum samples are the preferred option or stool (*Nature* (**2020**). https://doi.org/10.1038/s41586-020-2196-x). There is evidence that the virus replicates in the gastrointestinal tract giving rise to the possibility of detection in the stool as well as sputum. An advantage of Raman spectroscopy coupled with chemometric methodologies is that with the COVID-19 there are huge numbers of samples that can be tested. This would provide a massive training set and the potential for very accurate discrimination between infected and non-infected and also potentially to determine the state of the disease in terms of its lifetime within that specific patient. We know that IgG and IgM antibody levels are already being measured and used as potential biomarkers for infection in the immunoassay format. If these levels are being increased would it make sense to then have a blood based spectroscopic profiling of patients from early infection to later infection which would allow differences and changes in IgM and IgG to be elucidated using Raman spectroscopy and subsequent data analysis to detect global inflammatory responses in the blood? Can Raman spectroscopy be used for surveillance of patients in terms of whether the virus is spreading, how it is spreading, whether they have immunity, whether vibrational fingerprints are changing with time?

Spontaneous Raman spectroscopy may not be sensitive enough to deliver this classification and there are a number of papers in the literature where surface enhanced Raman scattering (SERS) has been used to improve sensitivity. In particular there has been work conducted on classification of measles which again indicates that Raman spectroscopy and SERS could have a vital role to play in terms of classification of patients with the disease. (e.g. *Analyst*, **2010**, 135, 3103-3109). It could also provide a methodology for studying those patients who are asymptomatic but have the vibrational fingerprint of someone who has been infected. This is still one of the unknown questions in the whole COVID-19 pandemic. Another significant question in the COVID-19 pandemic is why do some people display mild symptoms and some display more symptoms? Can Raman spectroscopy have a role to play in determining the answer to that? This may require more high level imaging of the virus and interaction of the virus with different cells where label free imaging with techniques such as stimulated Raman scattering or coherent anti-stokes Raman scattering can play a role. A very recent preprint from Volker Deckert has shown that fast CARS tip enhanced Raman scattering can provide a vibrational fingerprint from single viral particles and could be a very useful methodology to enhance an understanding of the virus and how it interacts with different host cells.(arXiv.org (2020) Laser Spectroscopic Technique for Direct Identification of a Single Virus I: FASTER CARS)

The final area that one can consider where Raman spectroscopy could play a role is in the post infection surveillance and determination of immunity. However, to achieve this the right target needs to be determined through further investigation and immunological studies of those infected with the virus and recovered. There could be an opportunity for a surface enhanced Raman scattering (SERS) immunoassay where sensitive detection of a biomarker is necessary if the right biomarker has been determined. SERS would allow an improvement in sensitivity over conventional immunoassays whilst still retaining the speed of detection however the complexity increases slightly due to the need to produce a Raman reporter and also have a suitable Raman spectrometer available. Many Raman spectrometers have now been designed and published for use specifically with lateral flow immunoassays e.g. *Angew. Chem. Int. Ed*. **2019**, 58, 442 –446 and *Anal. Chem.* **2019**, 91, 19, 12275-12282, therefore the only real barrier to SERS being used for this type of measurement is in determining the right target and having that validated.

Going forward where else can Raman spectroscopy play a role in the fight against COVID-19? We will still need to understand more about the infection pathway and how the virus interacts with specific hosts. This could provide a role for Raman microscopic imaging as a complementary tool to imaging approaches commonly used to visualise virus interactions with host cells which involve fluorescence or mass spectrometry. Until more is known about the nature of the virus and these questions answered it is difficult to say accurately where Raman spectroscopy can play a role. However it is clear that as a label free imaging technique Raman, stimulated Raman scattering (SRS), coherent anti-stokes Raman scattering (CARS) are all techniques which could be used to great effect in this research. More needs to be understood about how repurposed drugs are interacting with the virus and this is an area where label free analysis could be of interest and in particular due to the size of the virus high resolution or super resolution Raman or tip enhanced Raman scattering (TERS) could be techniques that could provide very useful real time information to the researchers trying to understand more about the production of effective anti-virals against COVID-19. This is something which is obviously much longer term but the question has to be asked whether Raman scattering could provide an opportunity to gain information that is useful in completing the picture of analysis required to understand more about COVID-19 infection, its subsequent spread and ultimately control.