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DETECTION OF COVID-19 USING LIGHT

INTRODUCTION:

Earlier, mass spectrometry-based assays for identification of microbial pathogens were limited to detection of microbes from pure cultures and not capable of detecting pathogens directly from clinical specimens such as nasopharyngeal swabs. Viruses range in size from 10-300nm.Spectroscopy signatures of laser properties can be used to identify single viral structural features. Size of the virus is identified by observing the amount of light scattering.

Photonics technology can be used to achieve remote monitoring of COVID-19 propagation through a multi-sensor intelligent network and contribute to diagnosing COVID-19. Fiber lasers are used to process data efficiently and reliably, are stable and scalable, and are a highly accurate tool in the diagnostics of viruses.

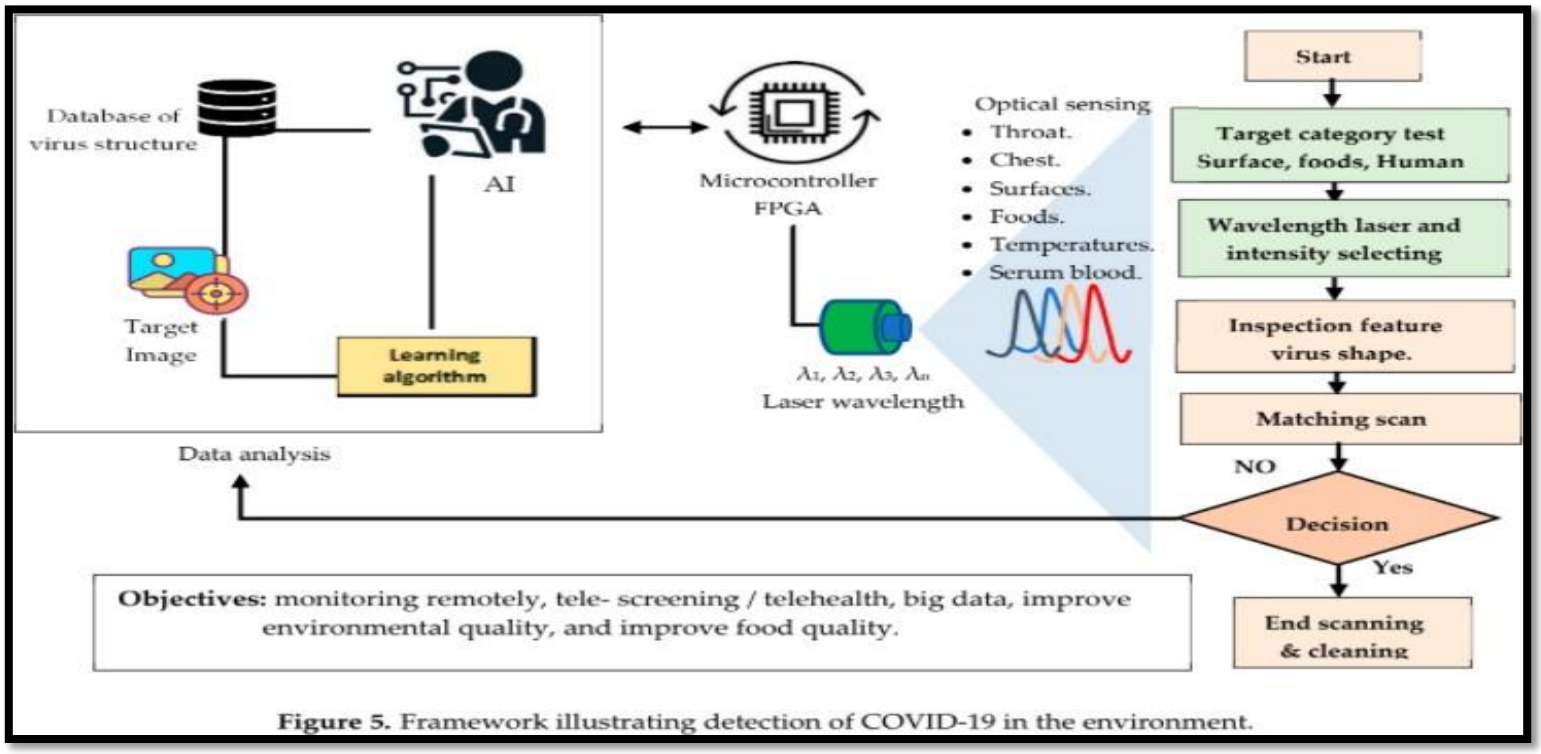


Figure 5. Framework illustrating detection of COVID-19 in the environment.

Surface-Enhanced Raman Scattering

Raman scattering is a mechanism in which a sample's physical and chemical properties are determined by the recognizable spectral signatures associated with them, including light–molecule interactions. The tool is used to detect viruses from various biological fluids, such as saliva, nasopharyngeal secretions, and tears.

Advantages: High selectivity, no signal interference from the analyte medium, single

molecule detection, the ability to conduct multiplex sensing with a single laser beam and high throughput.

Problems: weak signal and low sensitivity with a low protein concentration.

LASER INDUCED FLUORESCENCE

Fluorescence spectroscopy with powerful emitters is a unique technique for identification and imaging down to the single molecule size, which is attributed to its ultra-high cation and imaging down to the single molecule size, which is attributed to its ultra-high sensitivity.

1. This method employs fluorescence techniques to determine the presence of COVID-19 RNA, techniques roughly determine the presence of COVID-19 RNA.
2. A laser multiwavelength excitation and receiving system which collects the imitated signals such as scattered light and fluorescence from the laser-interrogated target.
3. Recently, detection of the SARS coronavirus protein in human serum samples was achieved using a fiber-optic biosensor based on localized surface plasmon coupled fluorescence (LSPCF) at the limit of detection 1 pg/mL.
4. This technique reached 100 percent sensitivities and 99 percent specificities with both forms of sampling
5. The threshold for the fluorescence signal is obtained by the standard divergence of cycles 3–15 of the average baseline fluorescence is called the cycle threshold(CT).
6. It is established by the number of PCR cycles needed to report quantifiable fluorescence, fluorescence is greater than the threshold of the fluorescence signal.
7. A lower CT value implies there is a higher viral RNA load.

SPR: SURFACE PLASMON RESONANCE

It is an optical sensing technique used to diagnose biomolecular interactions in real time. The photon energy excitation needs a coupling medium through the interface.

1. variations in the optical properties of the metal plate and the dielectric/sensing layer affect the depth, the direction (angle or wavelength), and the phase of the observed SPR change.
2. Optical biosensors such as SPR and LSPR have been extensively used in lab conditions to identify virus strains associated with SARS and MERS.
3. SPR was used to measure and characterize the kinetics of severe acute respiratory syndrome (SARS).
4. LSPR–sidelong flow, the LSPR–DNA selection approach and the LSPR–PCR model were also reported to detect covid-19. For stability, it is mixed two different effects: optical and thermal.
5. The biosensor artificially created DNA receptor sequences complementing the RNA genome parts of COVID-19 based on nanoparticle gold constructs on a glass substratum. These unique sequences were grafted onto the gold nanoparticles,

detecting COVID-19 with a high sensitivity but low detection limit of 0.22pm.

6. $\sqrt{\epsilon p}\sin(\theta res) = \sqrt{\frac{\epsilon m\,\epsilon d}{\epsilon m+\epsilon d}}$ is a necessary condition. Where,

ε_p: dielectric constants of the substrate (prism, optical fiber backbone, etc.),
ε_m : a plasmonic material (metals)
ε_d : a dielectric layer (analyte medium) respectively
θ_{res} : the incident resonance angle.

Plasmonic sensors: These sensors exploit simultaneously the enhancement and the localization of electromagnetic fields close to the interface between a metal and a dielectric.

RAMAN SCATTERING WITH SPR INTEGRATED

Similar to fluorescence, the process begins with the absorption of a photon and finish with the emission of a dispersed or fluorescent photon.The method employed was to deposit silver nanoparticles on 3D cellulose paper in order to produce a large number of inter-particles for enhancing the Raman signal. The factors while detecting analytes with SERS techniques:

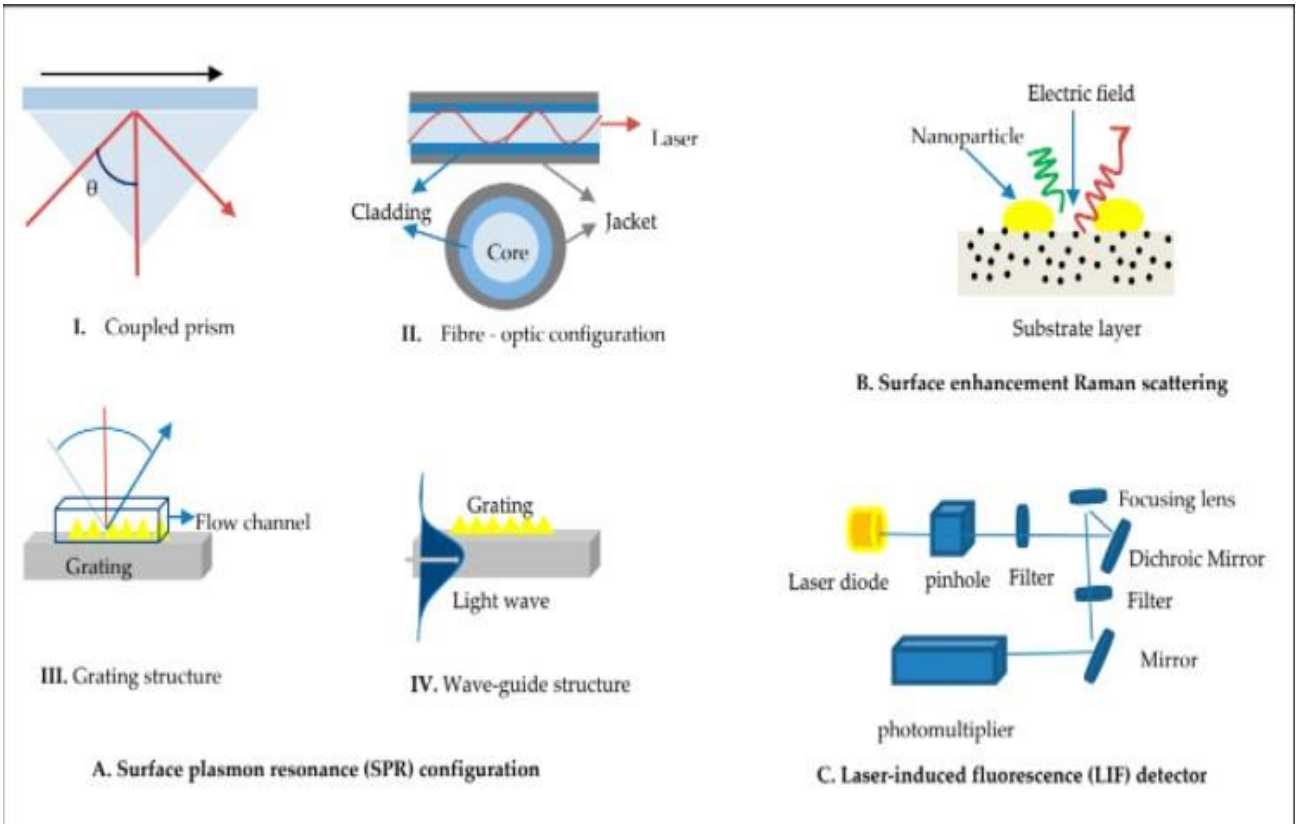
nanostructure geometry	substrate design	viral sizes wavelength
hot spot generation	laser type	power

1. Changing the refractive index can reduce the velocity wavelength of the laser in the optic structure.

2. The quantity of light reflected must be managed when reaching the interface and the critical angle for total internal reflection.

3. Limitations:

    a. Raman signals are often weak, can only characterize materials in their solid-state, and have trouble detecting analytes in liquids.
    b. Time consuming for sample collection from patients and analysis.
    c. Although the assays' high specificity and theoretical sensitivity are approved for COVID-19 molecular detection, false negatives appear to be reported a very high rate.



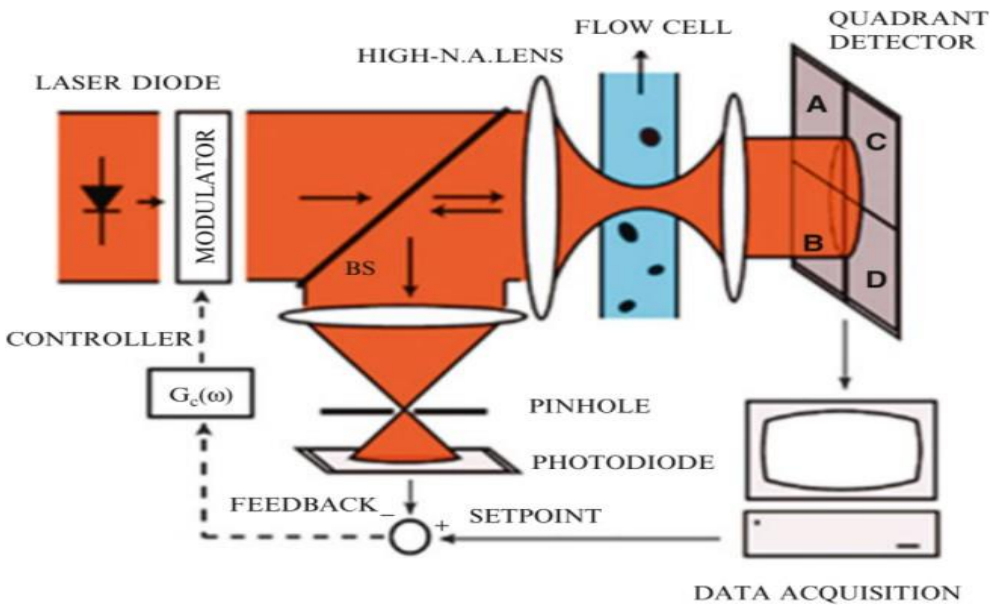
Raman Scattering SPR

OPTICAL SPECTROSCOPY:

In the technique, size and shape of a nanometer-size particle (virus particles) are measured by detecting the optical gradient force.Current optical detection methods which are well developed for single micrometer size particles, cannot be applied to nanoparticles due to a strong signal dependence on particle size.

1. Back-scattered light is detected by a photodiode which is integrated into a feedback loop with the modulator, which prevents clumps of viruses or other large particles from being trapped and thus from blocking the detector.

2. The light scattered in forward direction is used to track particle position with respect to the focus.



3. An off-axis detector measures power of scattered light.
4. The latter is a function of particle properties such as size, concentration, and optical density.
5. In the tens of nanometers size regime particles act as dipoles and the power of scattered light is proportional to the sixth power of particle size.
6. Lowering the detection size limits for the existing detectors places an impossible requirement on noise optimization.
7. Unlike the traditional PCR, IEM techniques in which analysis of a sample involves several tedious steps (several hours),this enables quick and accurate characterization of nanoparticle and virus samples; several nanofluidic flow-through schemes have been recently developed.
8. The minimum concentration is low (10^{8}particles/ml) . Ideal assessment needs 10^{6}particles/ml.
9. Ultracentrifugation may be required before processing.
10. IR Spectroscopy (of various forms, e.g. RAIRS, MIR) and Electron Energy Loss Spectroscopy (EELS) are the commonly used techniques.

11. Advantages:

high sensitivity, variable selection rules, spectral acquisition to below 400 cm^{-1}.

12. Limitations:

requirement for flat, preferably conducting, substrates, lower resolution.

LIMITATIONS

All these optical techniques limit at:

- Reduced performance of sensitivity and accuracy.
- Time-consuming preparation and purification of samples
- The devices complex process
- A need for highly skilled professional staff.

Fluorescence method	Surface-enhanced Raman scattering	Surface plasmon resonance	Raman scattering with SPR integrated
•Low sample size. • Fluorophores have a short lifetime. • Interference is possible. •Collection of samples consumes time.	•Weak signal relative to background. • Low sensitivity with low protein concentration. • Laser wavelength is unstable. • Consumes time to collect samples. • Noise signal interference.	• Low selectivity. • A small perception depth. • Mass transport challenge. • Heterogeneity of surface. • Misinterpretation of data. • Collection of samples is time-consuming.	• Collection of sample consumes time .• Weak signal. • At high analyte concentrations, there is failure to identify the virus, and identification is nonlinear. • Nonuniform absorption of the molecules onto the nanoparticle surface leads to a decrease in signal intensity.

Hence,

After going through different light technologies that are developed for diagnosing Covid-19, we understood that covid-19 shows various optical properties. Some of the viruses like influenza, previous variants of SARS showed some optical properties, which was useful for comparative study of present SARS Cov-2. So, we wanted to detect covid-19 under light using simple equipment and if there are any significant differences of optical properties of it under light.

(This content is extracted from the research paper of mass spectrometry for covid detection)

for having better detection of the virus

1-we have to remove other particles intervention in the process

2-we have to increase the concentration of the virus sufficient enough for the detection.

3-we have to make a chemical composition for better observation at that wavelength as we didn't know the actual properties of particles in swab composition we can't make exact analysis.so we did a comparative based study and listed observations below:

In order to improve the sensitivity of the detection, we evaluated

Step 1: Briefly, the antibody would be biotinylated using a biotinylating kit. Biotinylated antibody (1 mg) will be coated on streptavidin MSIA tips in 0.1% BSA containing 1X PBS on the Versette automated liquid handler.

Step 2: Now Nasopharyngeal swab samples (750 ml) will be mixed with zwitterion S. Reuse et al. / Biomedicine 69 (2021) 103465 3 Z3-16 at a final concentration of 0.002% in 96 well plates and were inactivated at 70 °C for 30 min.

Step 3: Inactivated samples are subjected to enrichment using mass spectrometry immunoassay (MSIA)-based enrichment using a biotinylated antibody, which will be washed two times with 200 ml 1X PBS and eluted in 100 ml of 50% ACN/0.002% Z3-16 in 0.1%TFA.

Step 4: Sample eluent will be mixed with 300 ml of rapid trypsin digestion buffer (2) and subjected to in-solution trypsin digestion at 70 °C for 1 h on a shaker incubator. The digest will acidify using TFA to a final concentration of 1% TFA.

Step 5: The acidified digests are spiked-in with synthetic isotope labelled heavy peptides as retention time monitoring standards and the samples are loaded on Evo Tips (The Evo tip serves as **an integrated purification step**, where contaminants are left on the tip, and analytically relevant peptides are eluted). Quality control samples include recombinant nucleocapsid protein spiked into a pool of negative samples, negative pooled samples, positive pooled samples .

Step 6: The C18 Evo Tips were activated using 20 ml of 100% acetonitrile followed by equilibration with 20 ml of 0.1% formic acid in water. Activation and equilibration were carried out at 700 x g for 1 min. The sample will be loaded at 500 x g for 5 min followed by washing using 0.1% formic acid once. At last, the tips are loaded with 100 ml of 0.1% formic acid and processed for targeted analysis. A detailed explanation of Step 4: Processing of nasopharyngeal swab samples and in-solution trypsin digestion 1) Nasopharyngeal swab samples should be collected in PBS (phosphate-buffered saline) and digested directly. Now 100 ml of sample to be diluted three-fold with a rapid digest buffer and 1.5 mg of trypsin was added. The samples were to be placed on a thermomixer at 70 °C for 1 h with rotation at 1,150 rpm for trypsin digestion.

2) To terminate the digestion, the samples were acidified to 0.2% TFA (Trifluoroacetic acid). The samples were desalted using C18 solid-phase extraction spin columns, dried down and reconstituted in 20 ml of 0.2% formic acid.

3) Recombinant proteins and inactivated purified SARS-CoV-2 were digested using the method described above for the swab samples with the following modifications: protein content of the purified SARS-CoV-2 was estimated using a BCA assay (Bicinchoninic acid) and the samples were concurrently reduced using 5 mM TCEP and in-solution trypsin digested at a 1:10 enzyme to substrate ratio.

4) Following digestion, alkylation was carried out using 5 mM IAA. Samples were incubated in dark for 30 min at room temperature prior to sample acidification and cleanup

By this way we can have better chances of detection.
After getting the required composition for better analysis we now proceed to the main part of the experiment.

Requirements for the experiment:

- Wavelength of light:

We need to find a range of wavelengths where we can observe the optical properties of virus to a better extent, so that we can have more accuracy in establishing the range of transmitted wavelengths.

- Optical properties of the virus:

We need to take care of possible optical properties of virus under a given wavelength, so that we can predict the property changes of virus which can reduce the range of transmitted wavelengths which gives accuracy to the experiment.

<u>To find wavelength</u>:

Wavelength is very important as well as useful for detecting at what range some element or a substance can be identified. So, first we started to check for some relative study on various technologies and some properties of different viruses which relate to covid-19 under light. We found resemblance to 1-2 viruses which can be useful up to some extent whose diameter was nearly 110 nm (which was near to 125 nm- diameter of covid-19). Then, eliminating some domain of range of wavelength was possible using analysis mentioned below:

Behavior of SARS Cov-2(Covid-19) in the Electromagnetic spectrum. Conclusions obtained from them are:

- When light of 100 nm wavelength interacts with a substance, it will cause the atoms to ionize.
- Solar UV radiation near the Earth's surface, primarily UVB at 280-315 nm, can inactivate SARS Cov-2.

Blue light with a wavelength of 400-420 nm can inactivate enveloped viruses like severe acute respiratory syndrome coronavirus (SARS Cov-2).

- The visible light (400 to 700 nm) wavelength is used to image targets in light microscopes and sometimes to visualize viruses because they lack enough magnification.
- **LIGHT DETECTION AND RANGING (LIDAR) :**

A technology in which a laser-pulse is transmitted, the reflected (light) signal will be detected using a UV Xenon Fluoride excimer laser source used to identify and characterize biogenic materials for which the wavelength range used is between 315-550 nm.

For testing simpler samples such as inorganic molecules or smaller organic molecules, it is best to use a visible laser such as 532 nm because by moving to shorter wavelengths, the scattering efficiency increases, but good resolution is not possible to obtain.

To mention the wavelength, there is a requirement of other optical properties of light as well which can be obtained from the spectroscopy technique.

Refractive index:

Refractive indices of the family of CoVID-19 are investigated with respect to EID (Electronic Infusion device) concentration of viruses, showing that the refractive index ranges from "-0.96725 to -0.999998" corresponds to '0.01 to 10000' EID virus concentration.

Transmittance and Absorbance by Spectroscopy:

UV/ Visible/ IR spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light). Each spectrum is background corrected using a 'blank'- a cuvette filled with only the dispersing medium - to guarantee that spectral features from the solvent are not included in the simple extinction spectrum.

The transmittance of a sample (T) is defined as the fraction of photons that passed through the sample over the incident no.of photons.

Transmittance(T) = I/Io

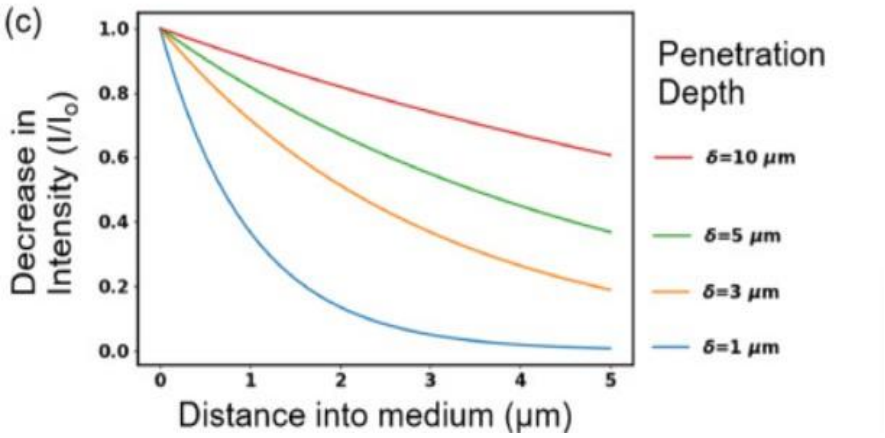
Absorbance(A) = -log(T) / log10

When we draw a graph between wavelength and absorbance, an isosbestic point can also be observed. An isosbestic point is the wavelength in which the absorbance of two or more species are the same. From this graph, we can detect the wavelength required.

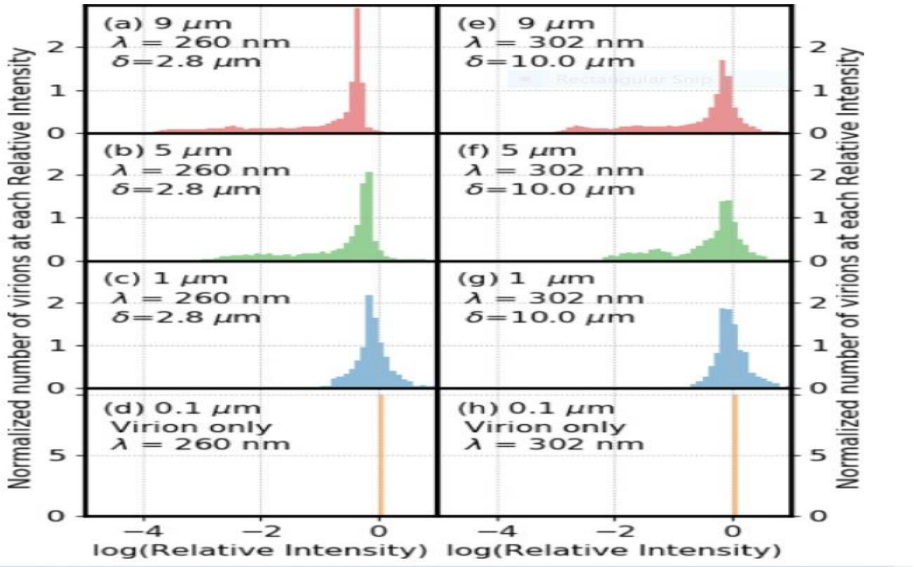
Properties under UV:

The model virions and dried particles have sizes and optical properties to approximate SARS-CoV-2 and dried particles formed from respiratory droplets, respectively. In 1-, 5- and 9-μm diameter particles on a surface, illuminated by 260-nm and 302 nm UV light from a direction perpendicular to the surface and an individual virion is taken and all these particles are observed under wavelengths mentioned above.

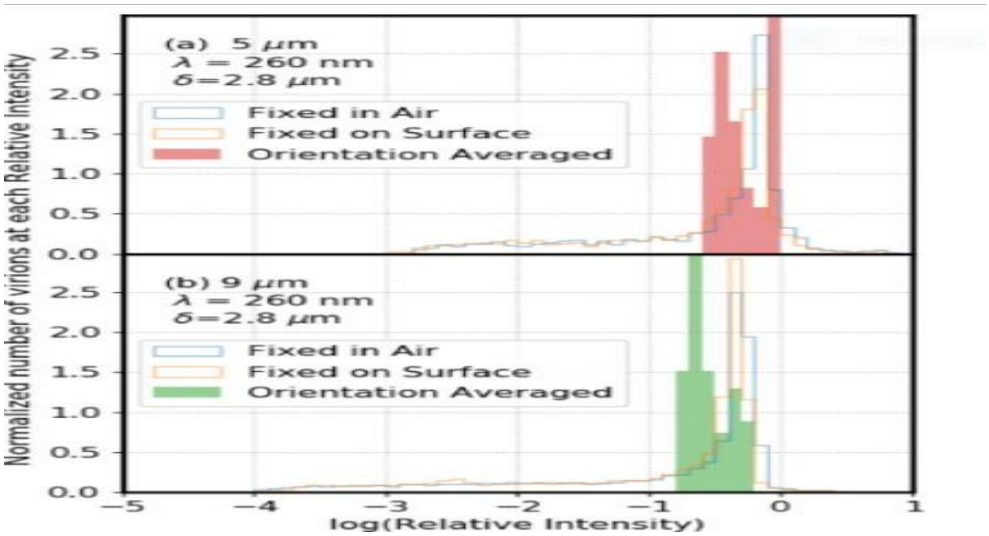
- **Decrease in intensity with distance into a medium as a function of penetration depth for δ for particles of respective diameters.**



- **Distributions of virion numbers vs UV intensity for particle diameters of 0.1, 1, 5 and 9 μm** Either 260- or 302-nm illumination the extent of shielding decreases as particle size decreases. At 260 nm, in the 9-μm particle, 29.6% of virions have Ri (Relative intensity of ith viroin) < 0.1 and 6.4% have Ri < 0.001; in the 5-μm particle, 24.2% of virions have Ri < 0.1 and 10% have Ri < 0.01, but none have Ri < 0.001; in the 1-μm particle over 31% of the virions have Ri > 1.0, and 0.2% have Ri < 0.1. In the limiting case of the 0.1 μm naked virion there is no shielding.

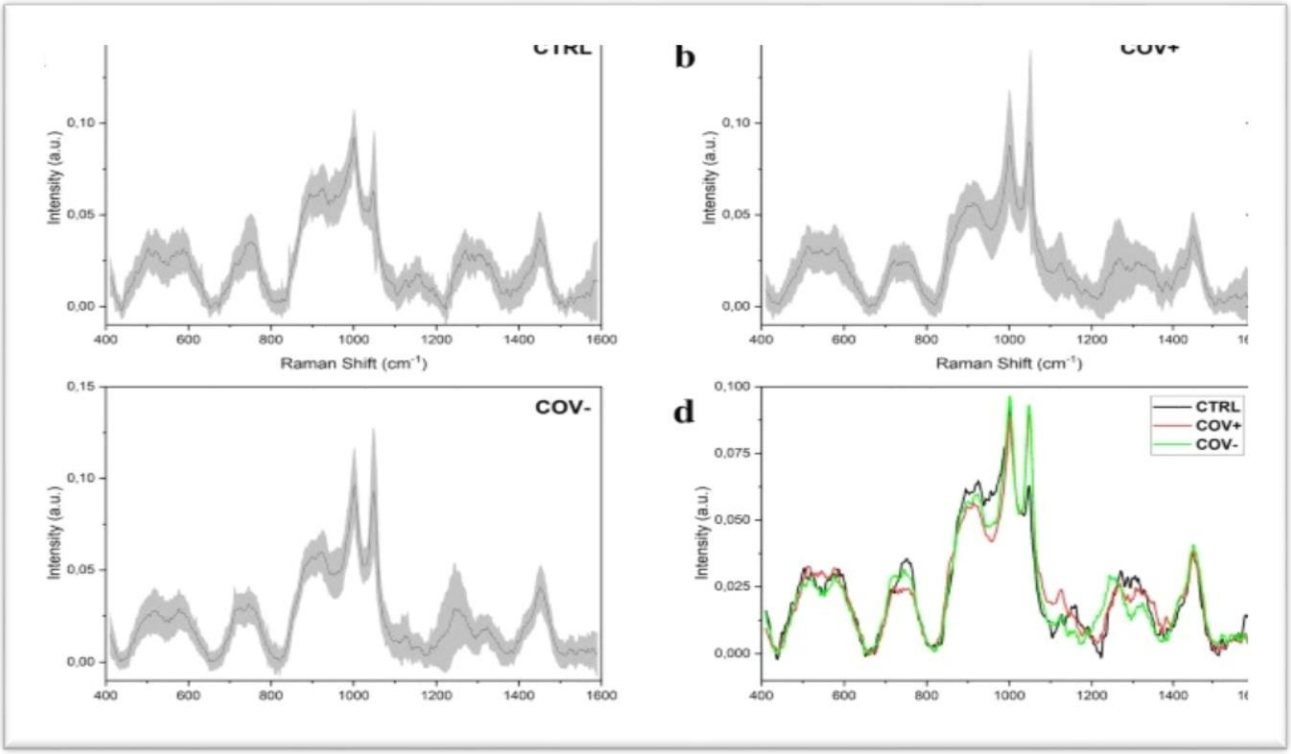


- **The UV distributions for the fixed-orientation cases, on a surface and in air.**



From above, we can conclude that shielding happens least in the particle of largest size considered. We think this aspect of UV light can also be used to some extent in detecting Covid-19 in case it does not completely inactivate the virus.

<u>COVID-19 RAMAN SPECTROGRAPHY</u>:



Average Raman spectra obtained from (a) healthy subjects (CTRL), (b) patients affected by COVID-19 (COV+) and (c) subjects with at least two negative SARS-CoV-2 tests after being positive (COV−). (d) Overlapped average spectra ofthe three experimental groups highlighting spectral differences. The grey bands represent the standard deviations.

Offline requirements:

We did not get an exact information regarding the how properties of covid-19 changes in the presence of light. We would like to perform the spectroscopy experiment to get an idea of how it's properties change under light.

We want to gain knowledge about how scattering, reflection, absorption and transmission of light through various particles change under different conditions.

Conclusion:

Range of wavelength that can be used (470 - 550) nm.

References:

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   2. **https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Kinetics/02%3A_Reaction_Rates/2.01%3A_Experimental_Determination_of_Kinetics/2.1.05%3A_Spectrophotometry**