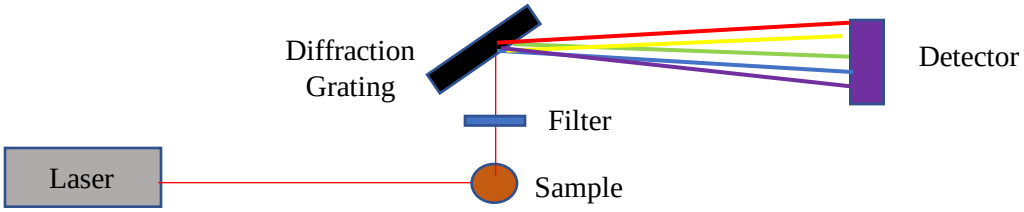
Raman Scattering Spectroscopy Theory, Techniques, and Use in Biology

Most of the time when light interacts with molecules, it exhibits elastic scattering where the energy absorbed by molecules is equal to the energy released [1]. The elastic scattering of light is known as Rayleigh Scattering [1]. However, sometimes light interacts with molecules in an inelastic way where the molecules gain or lose energy [1]. In this case, photons are released from the interaction which are at a different frequency than the ones that were incident [1]. This type of light scattering is known as Raman scattering, and has become useful in the detection and analyses of materials because each molecule has a unique pattern of Raman scattering [1].

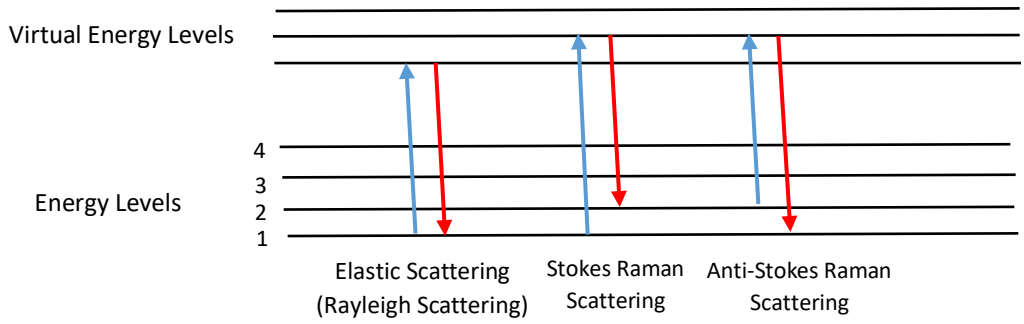
Raman scattering was discovered in 1928 by physicist C.V. Raman who observed Raman scattering for the first time [2]. He understood the concept of Rayleigh Scattering, and its application to the color of the sea; the color of the sea was a result of a reflection of light from the sky [2]. However, he was curious about the accuracy of this relationship and ended up finding that the maximum spectral intensity of the sky and the ocean differed [2]. This would mean that the photons being emitted from light interactions with the sea and the air molecules have a frequency shift, conveying a phenomenon different than Rayleigh Scattering [2]. After a few years of experiments, Raman determined that he discovered a type of light scattering which only depends on the molecules which make up the medium that photons are interacting with [2]. In 1928 Raman published the first journal article about Raman scattering and was given credit for discovering the phenomenon [2]. In 1930 he received the Nobel Prize in Physics for his work [2].

The process of Raman spectroscopy is completed by pointing a monochromatic laser beam at a sample and allowing the sample to scatter the light [5]. The light is scattered towards a filter to remove any other light, and then pointed to a diffraction grating which separates the light of different wavelengths which can then be placed on a detector to be analyzed [5]. A simple visual presentation of this process is shown below.



When the coherent beam of light interacts with the sample, the electrons of the molecule are raised to a virtual, or an unstable, unobservable state which lasts for a very short period of time [5]. The photons which are then emitted from the interaction with the sample will contain more or less energy than the incident photons [5]. When the photons emitted lose energy it is called Stokes Scattering, and when the photons emitted have more energy than the incident

photons it is called anti-Stokes scattering [5]. A representation of the energy levels for anti-Stokes and Stokes scattering is shown below.



In Rayleigh scattering the molecule is excited to a virtual state and relaxes back to the same state it was in previously, releasing a photon that is scattered elastically [6]. In Stokes scattering the molecule is excited into a virtual state but relaxes into a state that has a higher vibrational state than the state it was in originally, which releases an inelastically scattered photon of lower energy [6]. In Anti-Stokes scattering the molecule is in a vibrationally excited state and is excited to a virtual state, then is relaxed to a lower vibrationally state than it was originally in, releasing a photon of higher energy [6]. The frequencies of the induced photon and the emitted photon in Raman scattering is given by the equation [7]:

$$w_{emitted} = w_{induced} \pm w_{vibrational} \quad (1)$$

where $w_{emitted}$ is the frequency of the emitted photon, $w_{induced}$ is the frequency of the initial photon, and $w_{vibrational}$ is the molecular vibrational frequency [7]. The sum results in Anti-Stokes scattering also known as a blue shift, and the difference results in Stokes scattering also known as red shift [8]. Spectroscopy includes measuring the difference in the energy of the incident photons and the photons that have been scattered, which is usually completed by measuring the change in wavenumber between the photons [7].

For a molecule to be able to Raman scatter, it must have an electron cloud that can be distorted by an electric field, to create a dipole moment [5]. The induced dipole moment from an electric field is given by:

$$\mu = \alpha E \quad (2)$$

where μ is the induced dipole, α is the molecule polarizability, and E is the electric field. This equation implies that each electromagnetic interaction of the same frequency will induce a dipole moment that emits radiation at the same frequency, as seen in Rayleigh scattering [8]. However, the ability to indue a dipole on a molecule depends on the location of molecules, so when molecules move, such as through molecular vibration, the polarizability changes [8]. The induced dipole equation with molecular vibration taken into account becomes:

$$\mu = \alpha_0 E_0 \cos(2\pi v t) + \frac{E_0}{2} q_{max} \left(\frac{\partial a}{\partial q}\right) \cos[2\pi(w - w_{vibrational})t] + \frac{E_0}{2} q_{max} \left(\frac{\partial a}{\partial q}\right) \cos[2\pi(w + w_{vibrational})t] \quad (3)$$

where t is the time, w is the excitation frequency q_{max} is the maximum length of molecular bonds from equilibrium, α_0 is the molecular polarizability, $\left(\frac{\partial a}{\partial q}\right)$ is the change in polarization experienced in movement or vibration of molecules, and E_0 is the amplitude of the electromagnetic wave [8]. The first part of equation 3 represents elastic scattering of light while the second and third portions of the equation represents the Raman scattering of light [8]. The measurement that is being taken during Raman spectroscopy which allows molecules to be distinguished is dependent on how easy it is to move the electrons part of a bond in an electric field [6]. So, when there is a bond where the electrons can be easily displaced, the Raman signal will be more intense than when there are bonds that can not be easily displaced [6]. Raman signals can be used to gain information about inorganic and organic compounds [6].

The use of Raman Scattering microscopy allows for 3D visualization of objects without requiring the objects to be labeled with fluorescent proteins [3]. There are over 20 different types of Raman Scattering imaging techniques [4]. The most basic type of Raman Scattering imaging involves random scattering microscopes which incident a light beam on a biological sample and then collect the scattered light from the interaction with a spectrometer [4]. Through the detected scattering, the chemical makeup of the sample can be determined [4]. This simple type of Raman Spectroscopy takes a very long time to create an image [4].

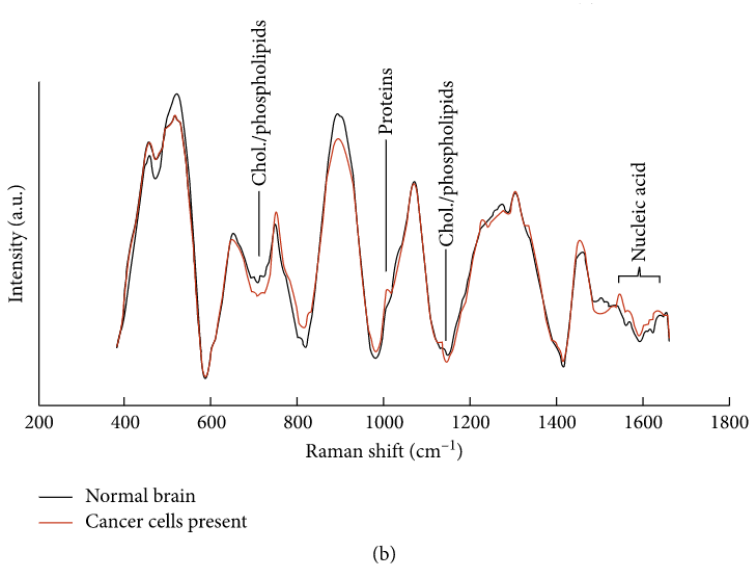
More recent advancements in the use of Raman Scattering for biological imaging have found a more efficient way to create an image by pairing spontaneous Raman scattering with Stokes stimulated emission in a process known as Stimulated Raman Scattering, which is a type of coherent Raman scattering[4]. In this process the rate of vibrational activation is increased by 10^8 [4]. A stokes beam of electromagnetic radiation and a photon from a pump are together forced to interact with the medium being observed [4]. The interaction of the stokes beam, the pumped photons, and the medium results in a vibrational activation of molecules in the medium, an annihilation of a photon from the photon pump and an addition of a photon to the stokes beam [4].

Another technique used to strengthen the Raman signal is Surface-Enhanced Raman Spectroscopy (SERS). In this technique the sample being observed is placed close to a metal surface, such as a surface of gold or silver nanoparticles [6]. This process strengthens the Raman signal because local electric fields are created by plasmon excitation in the nanoparticles [8]. The mechanisms which the enhancement occur are still debated, however the electromagnetic (EM) and chemical enhancement (CE) theories are the most accepted [8]. The EM model depends on the enhancement of an electric field which occurs near the metal surface being used [8]. The CE theory describes the enhancement as a result of a change in molecular polarizability due to interactions with the metal surface which can provide molecular resonance [8]. The two mechanisms work together in the enhancement of Raman scattering [8].

Raman scattering spectroscopy has many different applications, usually to detect the presence of a certain chemical species in a solution [8]. It can be used to determine if a solution has trace chemicals such as dyes, pesticides and hazardous chemicals [8]. Raman scattering spectroscopy is also widely used in bioanalysis and the detection of cancer, and DNA [8]. It is

especially useful in biological diagnosis because it is a technique which does not destroy the sample being observed [9].

When cells are cancerous or are not functioning properly the vibrational nodes of bonds in the molecules of the cell will act differently, allowing the detection of a problem with the cell by Raman Scattering [10]. For the purpose of cancer diagnosis, coherent Raman scattering or surface enhanced Raman scattering are usually used to amplify the Raman signal [10]. In many cancer screenings containing Raman Spectroscopy, a method of two different excitation fields are used where both anti-Stokes Raman Scattering known as CARS, and stimulated Raman scattering will occur simultaneously to amplify the signal even further [10]. Spontaneous Raman Scattering has been shown to be capable of detecting Biofluids, gastrointestinal cancer, skin cancer, breast cancer, brain cancer, cervical cancer and lung cancer [10]. An example of the Raman scattering intensity difference between regular brain cells and cancerous brain cells is shown in the figure below from the journal article "Raman Spectroscopy and Imaging for Cancer Diagnosis" in the *Journal of Healthcare Engineering* [10].



(b)

The sample with cancer cells clearly presents a different intensity of Raman scattering as a result of differing vibrational modes in the cancer cells [10]. If a sample Raman signal of body cells is compared to a known normal cell's Raman signal of the same cell type it can be determined if or if not cancer cells are present [10].The use of Raman scattering in cancer detection is still being researched, specifically how to differentiate and find unique markers for different types of cancers [10].

Since the first discovery and presentation of Raman Scattering in 1928, it has become a large component of molecule detection and analysis. Advancements in the use of Raman scattering microscopy are constantly being made and current techniques are being improved, which is necessary for Raman scattering techniques to become leading techniques in diagnosis and detection in biology.

Citations

[1] “Introduction to Raman Spectroscopy.” *YouTube*, YouTube, 15 Mar. 2020, www.youtube.com/watch?v=M1v_77kswqg.

[2] Masters, Barry R. “C.V. Raman and the Raman Effect.” *Optics & Photonics News (OPN)*, Optics & Photonics News, 16 Jan. 2016, www.osa-opn.org/home/articles/volume_20/issue_3/features/c_v_raman_and_the_raman_effect/.

[3] Ji-Xin Cheng, Y. Kevin Jia, Gengfeng Zheng, X. Sunney Xie, Laser-Scanning Coherent Anti-Stokes Raman Scattering Microscopy and Applications to Cell Biology, Biophysical Journal, Volume 83, Issue 1, 2002, Pages 502-509, ISSN 0006-3495, https://doi.org/10.1016/S0006-3495(02)75186-2. (https://www.sciencedirect.com/science/article/pii/S0006349502751862)

[4] Jones, R.R., Hooper, D.C., Zhang, L. *et al.* Raman Techniques: Fundamentals and Frontiers. *Nanoscale Res Lett* **14,** 231 (2019). https://doi.org/10.1186/s11671-019-3039-2

[5] Other Types of Raman Spectroscopy. (2017, September 18). Retrieved May 6, 2021, from https://chem.libretexts.org/@go/page/74391

[6] Theory of Raman Spectroscopy. (2019, May 10). Retrieved May 6, 2021, from https://chem.libretexts.org/@go/page/74388

[7] Raja, P. M. V., & Barron, A. R. (2021, March 21). Raman Spectroscopy. Retrieved May 6, 2021, from https://chem.libretexts.org/@go/page/55879

[8] Resonant vs. Nonresonant Raman Spectroscopy. (2020, August 15). Retrieved May 6, 2021, from https://chem.libretexts.org/@go/page/1851

[9] “Raman Spectroscopy Applications.” *Avantes*, 16 Apr. 2021, www.avantes.com/applications/cases/raman-spectroscopy-applications/.

[10] Sishan Cui, Shuo Zhang, Shuhua Yue, "Raman Spectroscopy and Imaging for Cancer Diagnosis", Journal of Healthcare Engineering, vol. 2018, Article ID 8619342, 11 pages, 2018. https://doi.org/10.1155/2018/8619342