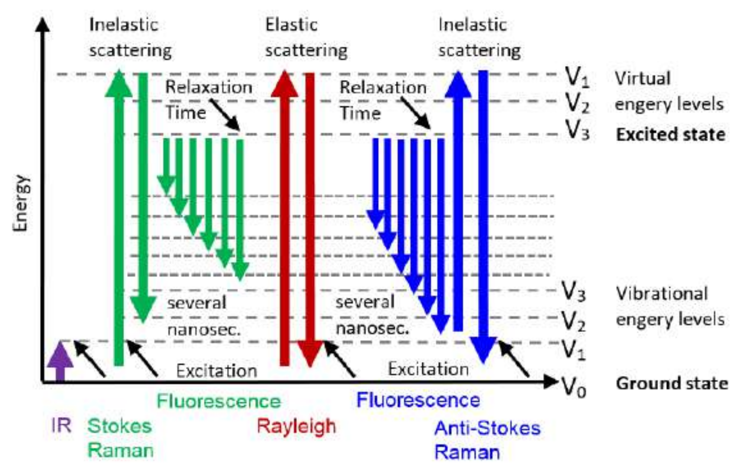
RAMAN SPECTROSCOPY CHEAT SHEET

PHYSICS OF RAMAN AND RAMAN ADVANTAGES

- Optical spectroscopy is a subset of spectroscopy. It is employed as an analysis technique to measure the interactions of different wavelengths of light with matter to determine the physical and chemical properties of the sample with which the light has interacted, hence it provides a unique "fingerprint" for different atomic, molecular, and crystal structures in a sample. Variations in the atomic and molecular structures result in different spectroscopic fingerprints, and optical spectroscopy can be used to identify and quantify these structures.
- When incident light scatters from the material that it interacts with, the vast majority of the photon scattering happens elastically, which is called Rayleigh scattering. **Raman spectroscopy measures the tiny proportion of the photons that are scattered inelastically.** The vibrational state of a molecule can be excited to an energy level above ground state by a photon that loses energy during the Stokes scattering event, or the photon that scatters can gain energy from the drop of vibrational state of the molecule which is called anti-Stokes scattering. These two scattering events are the core of Raman spectroscopy but typically Stokes scattering is used – Rayleigh and Anti-Stokes lines are filtered out.



- Raman spectroscopy is a complementary method to near-infrared spectroscopy (NIRS) as it measures a different spectrum of the molecule. NIRS measures the change in dipole moment during the molecular vibrations causing absorption, whereas Raman spectroscopy measures the change in polarizability during molecular motions. This makes Raman spectroscopy **inherently advantageous to measure analytes/metabolites in aqueous solutions/environment** as the inelastic scattering of polar molecules are less efficient than nonpolar ones and the Raman spectrum of water is weaker compared to NIR absorption of water. **The weakness of the Raman spectrum of polar molecules results in less interference from polar molecules such as water in the band of concern and facilitates the bioprocess monitoring, cell culture media, and protein analysis.**

UPSTREAM APPLICATIONS

- Raman spectroscopy has shown accurate measurements of glucose, lactate, viable cell density, and product concentration leading to adaptive or closed-loop control strategies, such as maintaining glucose concentration at a fixed setpoint or to minimize lactate accumulation by adjusting glucose feed rate. It is also demonstrated that Raman spectroscopy can monitor monoclonal antibodies in real time and distinguish between glycosylated and non-glycosylated molecules. Raman spectroscopy is also shown to have good correlation with traditional offline and at-line metabolite analyzers. In one study, glucose measurements from Mab-expressing cell line culture by Raman spectrum demonstrated good correlation with reference data from Flex2 analyzer from Nova Biomedical. Raman spectrum based predictive models for glucose controlling the cell culture displayed equivalent glucose and viable cell count profiles compared with the reference data. Other studies show good correlation with HPLC, UV/Visible spectroscopy analyzers and various chemistry analyzers.

- The detailed chemical information that is obtained by Raman spectrum, thanks to its sharper bands, enables further ability of transferring models from small scale R&D settings to large scale manufacturing settings. **Raman spectroscopy enables the ability to monitor product quality continuously in real time and the development of predictive and advanced feedback control strategies, and upstream process optimization. It displays scalability of prediction models and methods from minibioreactors to large scale production bioreactors. This results in higher process efficiency and production concentration, better and more consistent product quality, shorter cycle times, and lower costs due to reduced need for calibration efforts for different volumes of bioreactors.**
- Raman spectroscopy, along with appropriate mathematical modeling for multivariate data analysis, is used to measure and predict existence and/or concentration of amino acids, DNA and RNA bases, fatty acids and fats, hormones, organic acids salts, sugars and quantify product titer, viability, antibody titer, total cell density, viable cell density, and osmolality.
- Raman spectroscopy is used in microbial fermentation systems and cell culture systems using CHO, HEK293 and NS0 cell lines.
- Raman analyzers, such as the Tornado, are suitable for use in both laboratory and industrial process environments.

DOWNSTREAM APPLICATIONS

- **Measurement of product concentration and product aggregation:** The product concentration estimation and monitoring of monoclonal antibodies (mAb) is critical in continuous perfusion processes. This part can be accomplished by UV spectroscopy without the need for advanced predictive mathematical modeling that Raman spectroscopy requires. However, Raman spectroscopy achieves one goal that UV spectroscopy cannot achieve. Raman spectroscopy with quantitative modeling can be used for aggregate analysis. High concentrations of aggregate mAb types in the samples can be differentiated from each other due to having different Raman spectra.
- **Glycosylation:** Distinguishing glycosylated and non-glycosylated proteins.
- **Membrane fouling:** The fouling of immersion probes within cross-flow filtration units can be monitored as well. Even though this could be challenging due to complex background and weak signals of proteins, there are promising signs.

HIGH-THROUGHPUT VIRTUAL SLIT (HTVS) AND TORNADO

- Open beam path results in high energy throughput as there is no blockage, but poor resolution. Adding a slit results in good resolution but most of the light is blocked, therefore energy throughput is significantly reduced.
- HTVS reshapes the incoming beam and utilizes 95% of the incoming photons while providing high spectral resolution as a result of “virtual slit” behavior. This results in up to 10x faster measurements (better process responsivity), 3x better measurement quality (better sensitivity for lower detection limits) and good quality and fast measurements with lower laser power.
- No moving parts confer durability, stability and longevity, calibration stability and transferability.
- Tornado Spectral Systems offer HyperFlux PRO Plus Raman Spectrometer or Process Guardian Raman Spectrometer as the Raman analyzer:

HyperFlux PRO Plus

Process Guardian

- Paired with a probe such as Hudson 785 Immersion Raman Probe or Hudson 785 Non-Contact Raman Probe, or SpectroPort Probe specific for Ambr systems from Sartorius.

Hudon 785 Non-Contact Probe

Hudson 785 Immersion Probe

SpectroPort Probe

- Tornado Spectral Systems offer a multiplexer that allow measurements from up to 8 channels (probes) and long fiber optic cables for the probes.