Optical Biosensors: Implementation of Raman Spectroscopy

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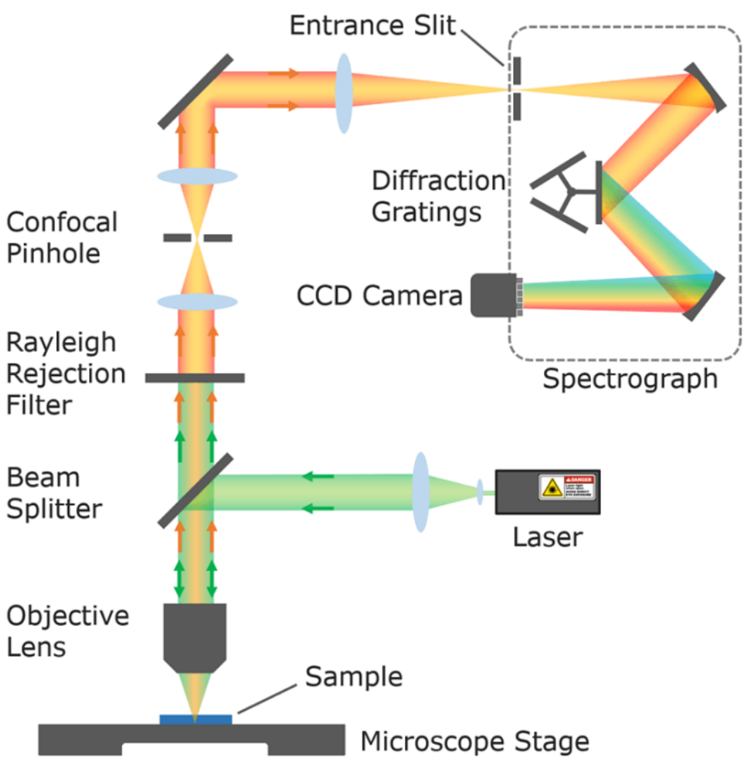
Content

- Raman spectrometer
- Optical microscope and objective lens
- Detectors of low light intensity
- Optical filters
- Raman spectra of target molecules

Raman Spectrometer / Microscope



https://www.edinst.com/resource/what-is-confocal-raman-microscopy/

Raman scattering probability ~ λ^{-4}

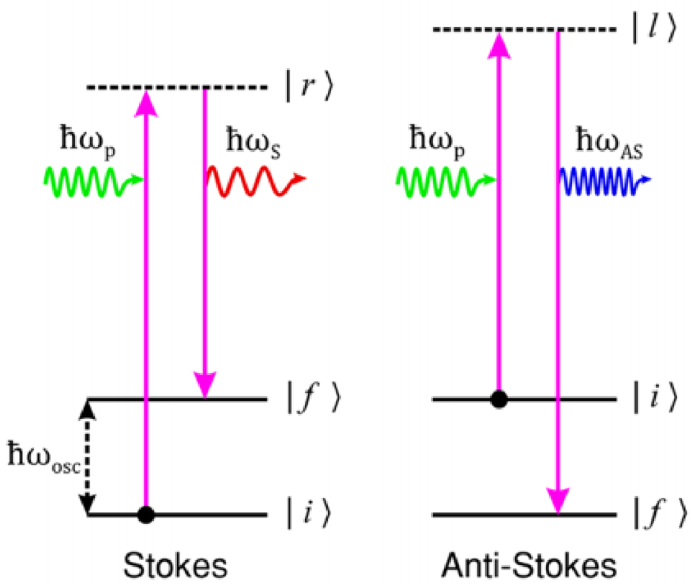
Weak intensity – using of single photon detectors like cooled CCD.

Efficient filtering of the background signal – using of notch filters, confocal configuration.

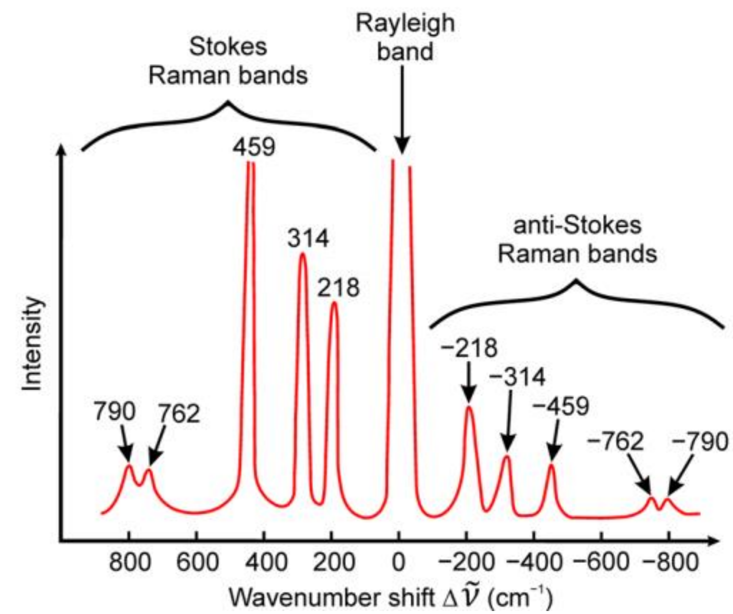
Typically long integration times are needed to collect a signal in counts per second, operation in a dark room…

Hole – confocal configuration
Slit – resolution of spectrometer

Raman Spectra



https://doi.org/10.1186/s11671-019-3039-2



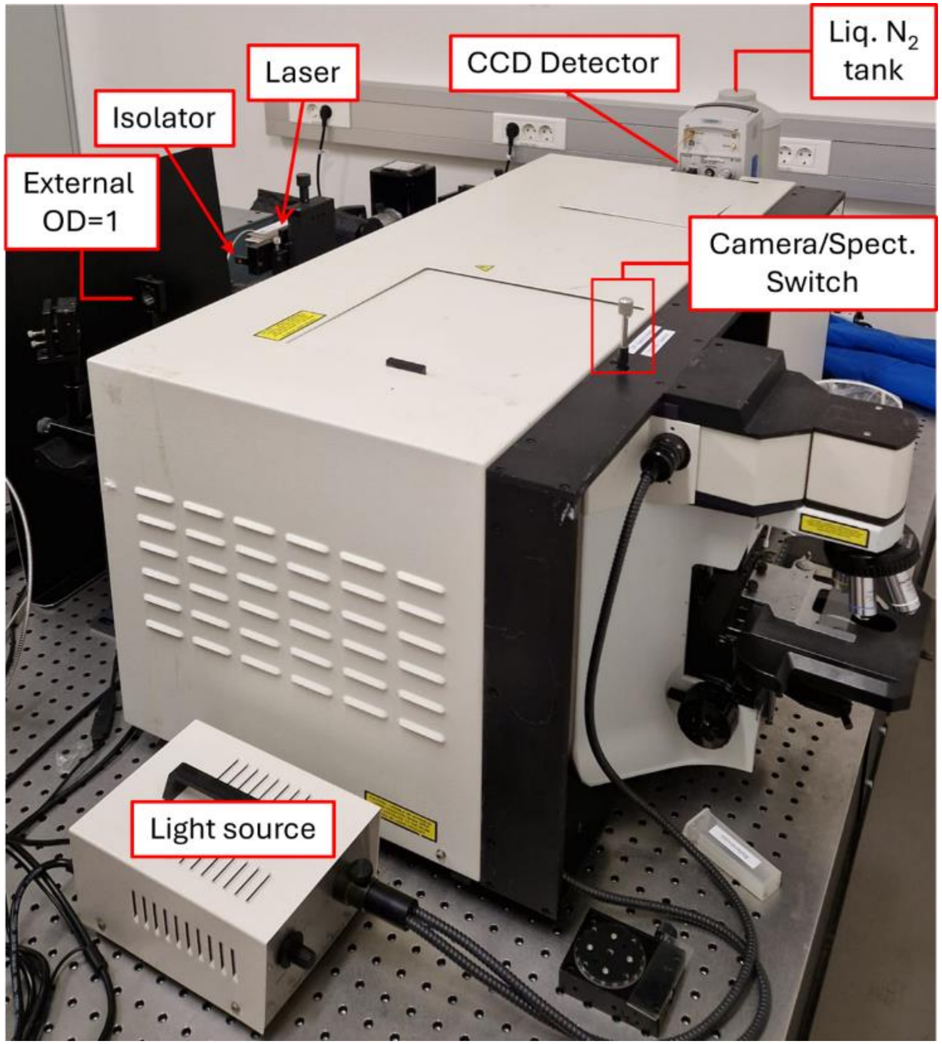
Ratio of Stokes and anti-Stokes band intensity:

$$\frac{I_{AS}}{I_S} = \left(\frac{\omega_p + \omega_{osc}}{\omega_p - \omega_{osc}}\right)^4 e^{\left(-\frac{\hbar\omega_{osc}}{kT}\right)}$$

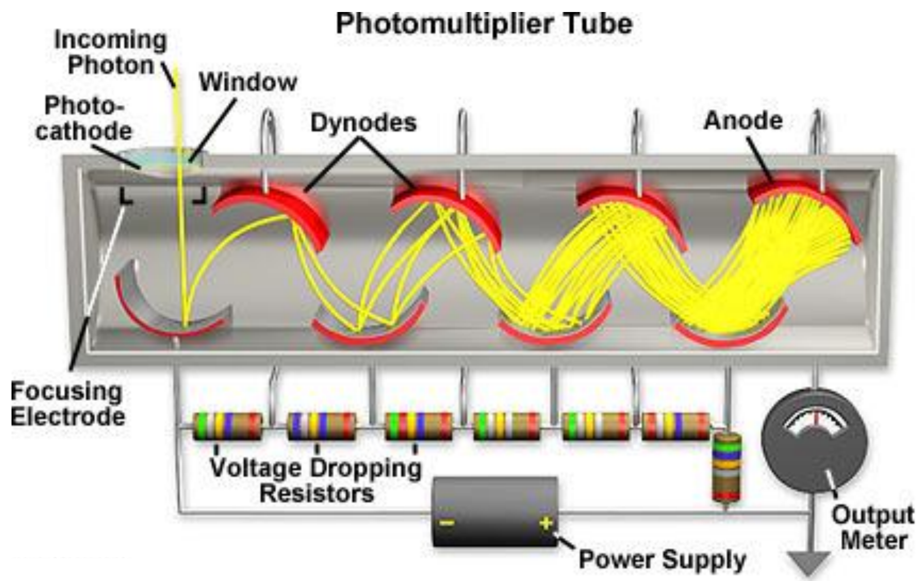
The spectra of plotted as depending on energy shift in cm^{-1}:

$$E = \hbar\omega = h\nu = h\frac{c}{\lambda} \qquad \Delta E \sim \frac{1}{\lambda_1} - \frac{1}{\lambda_2}$$

Horiba Raman Spectromenter



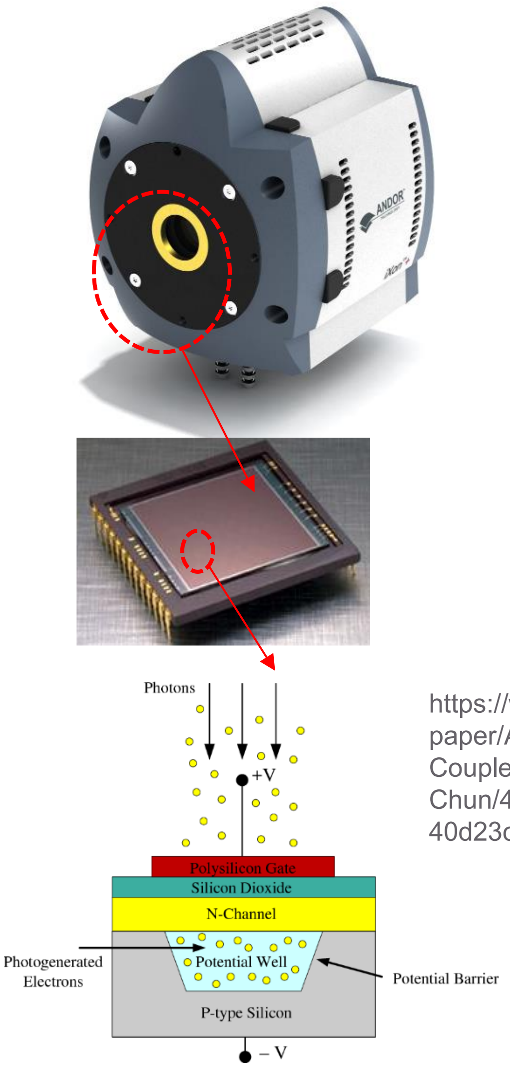
Photomultiplier



- Detector designed for the measurement of low intensity of light.
- Allows for counting of individual photons (e.g. counts per second – cps, fluorescence rate in Hz)

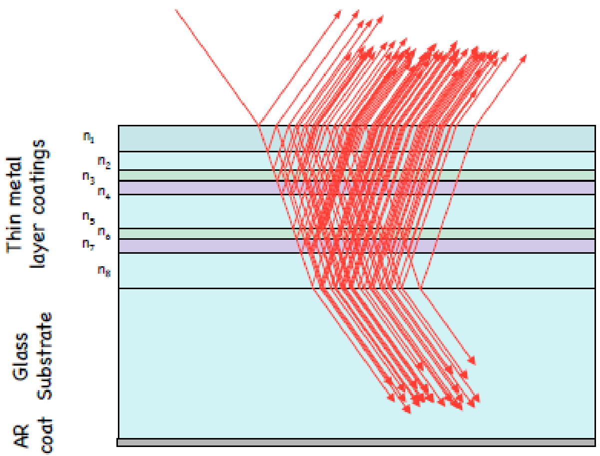
Charged Coupled Device - CCD

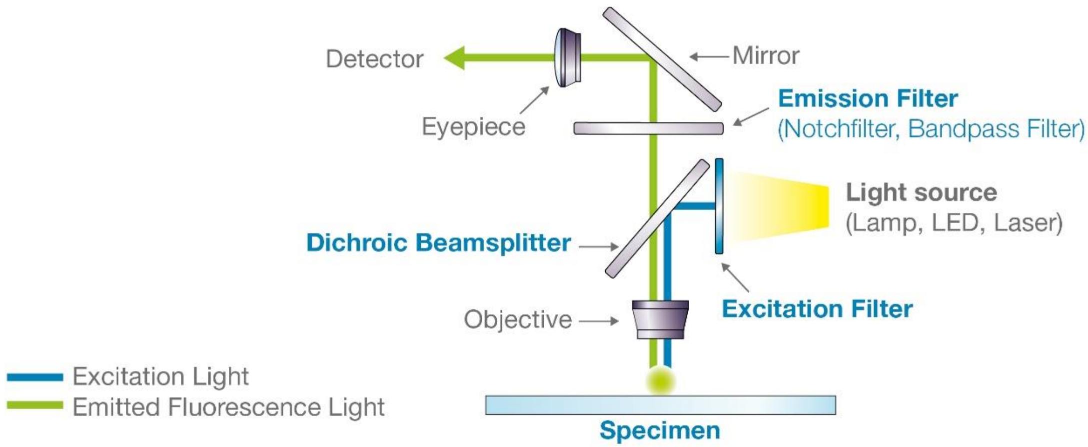
- Allows for acquisition of the whole image without scanning the beam or the sample.
- Sensitivity can reach single photon counting for each pixel.
- Recently, there are available (comparable) electron multiplying charge-coupled device (EMCCD) or complementary metal oxide semiconductor (CMOS)



https://www.semanticscholar.org/paper/A-High-Voltage-Charge-Coupled-Device-(CCD)-ASIC-for-Chun/4bceb2f351025c1f4688f56640d23cc0493654e5

Fluorescence Filters



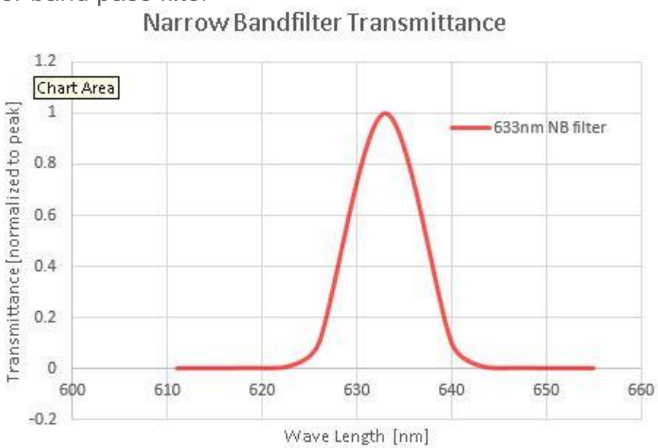


- Sensitive fluorescence measurement relies heavily on optical filters to spectrally filter the excitation and emission light:

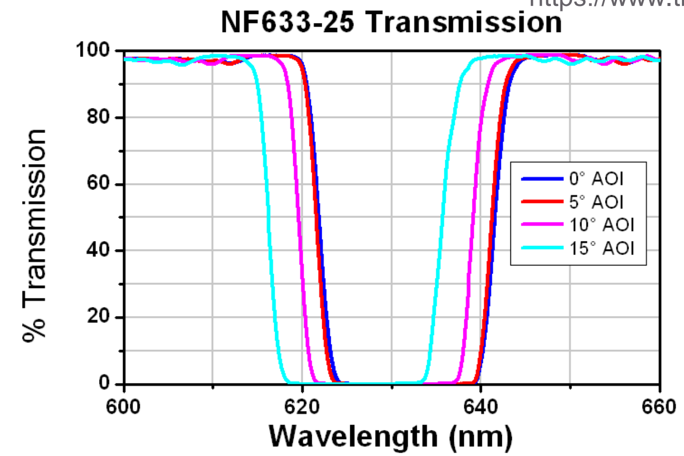
- Laser band pass filter – transmission of only λ_{ex}
- Emission band pass filter – transmission of only λ_{em}
- Notch filter – used to stop λ_{ex}
- Dichroic mirror – high reflectivity for λex and high transmission for λ_{em} at tilted angle.

Optical Filters

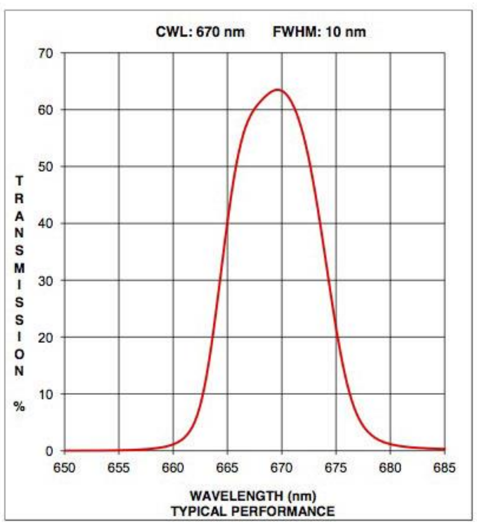
Laser band pass filter



https://www.thorlabs.com/



Emission band pass filter

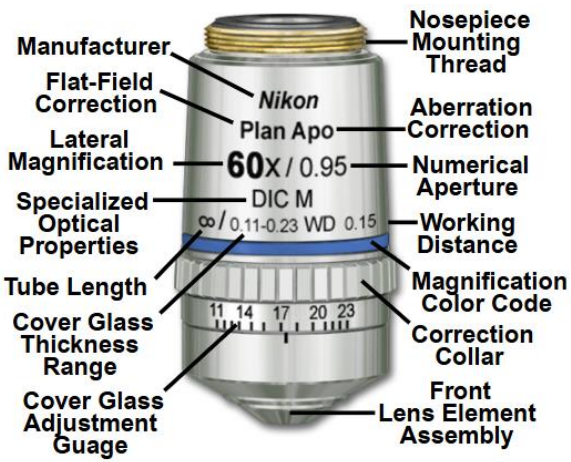


- Filters needs to be selected with respect the used fluorophore and the excitation wavelength.
- Due to the fact that are based on interference, they are sensitive to angle of incidence.
- The suppression of transmission is quantified by optical density (3-7 orders of magnitude):

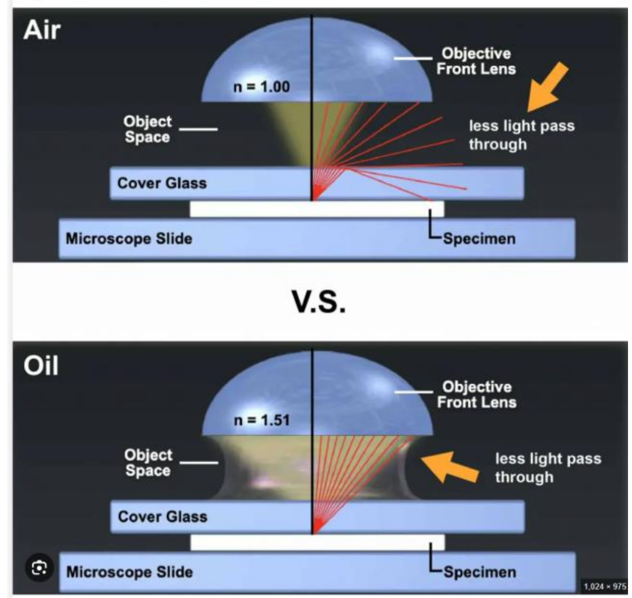
$$\mathrm{OD} = \log_{10}\left(\frac{1}{T}\right), \text{ or } \; T = 10^{-\mathrm{OD}}$$

Objective Lens

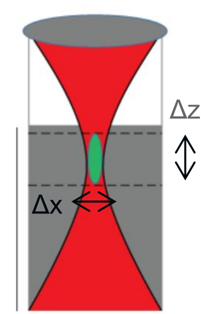
https://www.microscopyu.com/microscopy-basics/microscope-objective-specifications



https://rsscience.com/how-to-use-microscope-immersion-oil-to-get-higher-resolution-images/



- Immersion oil objective used for high magnification and NA
- Focal spot is elliptical with a characteristic volume down < fL



$$\Delta z = \frac{2\lambda}{NA^2}$$

$$\Delta x = \frac{0.61\lambda}{NA}$$

https://fluorescencecorrelation.com/Confocal%20volume.html