CCMX-ScopeM Advanced Course
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Analytical Optical Spectroscopy & Microscopy

Renato Zenobi
Laboratorium für Organische Chemie, ETH Zürich

Abstract
Optical spectroscopy methods offer detailed insight into the composition of matter, in particular for the determination of the molecular composition. Molecular systems absorb or scatter light, and depending on the wavelength range, we can learn about different properties:

• In the visible and UV spectral range, the presence of chromophores can be determined. In the simplest case, these are fuctional groups like double bonds, although conjugated □ electron systems have higher absorption cross sections and absorb closer to or in the visible range.

• Some chromophores re-emit light in the form of fluorescence. Strong fluorophores can be used to label non-fluorescing molecules for specific detection in a complex environment.

• In the infrared part of the spectrum, molecular vibrations are excited. This allows one to recognize the presence of functional groups in molecular systems. For example, C–H, C–D, C–F, and C–O bonds (and so on) all have different characteristic absorption frequencies.

• Another way to measure a vibrational spectrum is via Raman scattering. As opposed to IR spectroscopy, Raman spectroscopy is not based on absorption of photons, but on inelastic scattering.

• Although not really a variant of “optical” spectroscopy, irradiation of molecular systems with microwaves will result in excitation of rotational motions, which are also characteristic.

When combined with microscopy, high-resolution spectroscopic images can be generated by tightly focussing an irradiating beam and scanning the sample under the beam. This is particularly interesting and widely practiced for fluorescence and Raman spectroscopy.

In terms of spatial resolution, all optical spectroscopies – even in the form of microspectroscopy – are diffraction limited, i.e., a resolution around λ/2 is the best that can be achieved by focussing an illumination beam or collecting light from a sample with a microscope objective. There are, however, modern methods that break the diffraction limit: superresolving optical microscopy using special fluorescent dyes, near-field optical methods, and local field enhancement by plasmonic nanostructures (e.g. tip-enhanced Raman spectroscopy, TERS). In the last part of the lecture, a short glimpse into the world of nanospectroscopy will be given.

Contact details

HCI E 329, ETH Zürich
CH-8093 Zürich, Switzerland
E-mail: zenobi@org.chem.ethz.ch
Phone: +41 44 632 4376
Web: www.zenobi.ethz.ch