Single-Molecule Spectroscopy
Every Molecule is Different!

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Three scientists (William E Moerner, Eric Betzig and Stefan W Hell) have been awarded the 2014 Nobel Prize in Chemistry for their contributions to single-molecule spectroscopy (SMS). This new branch of spectroscopy was invented 25 years ago in 1989. In this article, we give a brief outline of the history of this technique and some of the exciting applications.

1. Introduction

Molecules of the same chemical composition are often assumed to have identical properties. In reality though, every molecule is different. For instance, from the kinetic theory of gases, we know that different molecules move with different velocities. As a result of this, different molecules absorb at different external frequencies because of the Doppler shift (see Box 1). Further, in a heterogeneous medium the local environment of each molecule may be different. This gives rise to large variations of those properties which depend on the medium (e.g., local polarity or viscosity). For instance, in a biological cell the local environment at the membrane may be drastically different from that in the cytoplasm or in the nucleus. Even within the cytoplasm, different organelles (mitochondria, lysosome or endosome, etc.) may have different properties.

For several decades, many scientists were excited by the prospect of recording high-resolution spectra free from Doppler broadening. One popular strategy of eliminating Doppler effect is cooling of gases by adiabatic expansion and then selecting only one group of ultra-cold atoms/molecules having identical velocity.

1 High-resolution spectrum refers to a spectrum consisting of very sharp lines. The sharp lines clearly display transitions to individual rotational, vibrational and electronic states.

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Box 1. Doppler Broadening

The condition of absorption of a photon is

$$\Delta E = E_2 - E_1 = h\nu_{\text{obs}},$$  

(i)

where $\Delta E$ represents the energy gap and $\nu_{\text{obs}}$, the frequency of a photon observed by a molecule. Even if $\Delta E$ is the same for all the molecules, the observed frequency may vary from one molecule to another because of the Doppler shift. If a molecule moving with a velocity $u$ is irradiated by an external frequency $\nu_{\text{ext}}$, the frequency observed by a molecule is

$$\nu_{\text{obs}} = \nu_{\text{ext}}[1 \pm (u/c)].$$  

(ii)

In (ii) ‘+’ corresponds to the situation when the molecule is moving towards the photon (source) and ‘−’ corresponds to molecule moving away from the photon (source). Since the velocity of the molecules exhibits a broad distribution, different molecules absorb at different $\nu_{\text{ext}}$. For each value of a velocity, there is a narrow absorption line. But the overall absorption spectrum of a molecule is a superposition of many such sharp lines corresponding to different velocities. The superposition gives rise to a broad spectrum (Figure A) which resembles the Maxwell–Boltzmann velocity distribution curve. Since the molecules have different absorption frequencies, the gas is inhomogeneous. Thus Doppler effect is an inhomogeneous broadening effect.

This technique is widely used in atomic or molecular beams and has attracted the 1986 Nobel Prize in Chemistry. The other strategy is to completely eliminate motion by trapping a single ion in a quadrupole electric field (1989 Nobel Prize in Physics) or by momentum transfer from photon (laser cooling, 1997 Nobel Prize in Physics).

2. History of SMS: The Pioneers

In the 1980’s, there were several attempts to record absorption spectra of individual molecules at different sites (or defect centers) of a solid crystal, under Doppler-free condition. In a solid, the translational motion of the