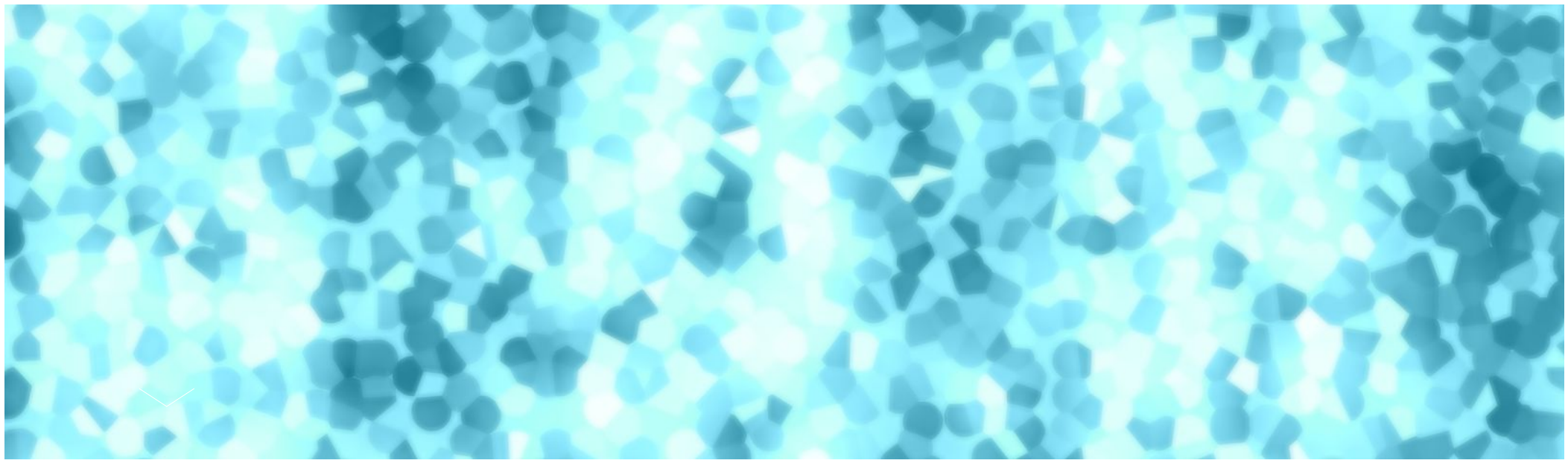


Fluorescence Spectroscopy

What is a Fluorescence Measurement?

Fluorescence

Most molecules occupy the lowest energy state at room temperature, known as the ground state. Within this ground state are vibrational levels. Before becoming excited, many molecules occupy the lowest vibrational level.

The absorbed photon causes the molecule to adopt a higher vibrational energy state when a molecule absorbs a certain wavelength of light. The molecules then collide with other molecules, causing it to lose its vibrational energy and return to the lowest vibrational level of the excited state. The molecule can then return to the ground state vibrational levels.

When the molecule returns to the ground state, it emits a photon of light at a wavelength different to the wavelength that excited it. This is when the molecule exhibits fluorescence. (See Jablonski Diagram)

Fluorescence is measurable by fluorometers. A fluorometer is an instrument designed to measure the various parameters of fluorescence, including its intensity and wavelength distribution of the emission after excitation. Chemists use this to identify properties and the amount of specific molecules in a sample.

Chemists call molecules that can exhibit fluorescence *fluorophores*.

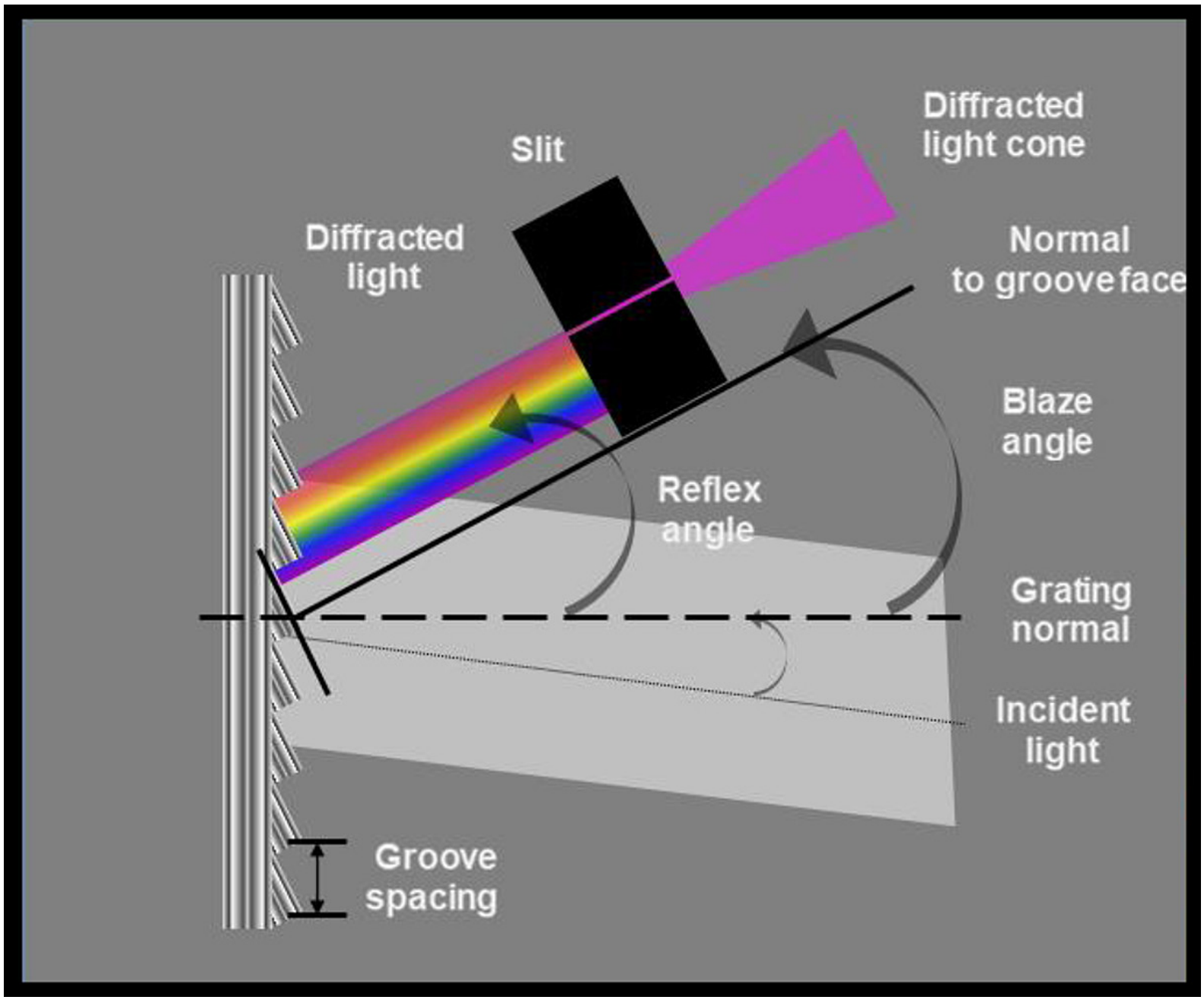
Measuring fluorescence

Scientists use fluorescence spectrometers to excite fluorophore molecules and measure its emitted fluorescence. The spectrometer introduces ultra-violet or visible light using a photon source, like a laser, a xenon lamp or LEDs. The light passes through a monochromator that selects a specific wavelength, often using a diffraction grating. A diffraction grating is a plate of glass or metal ruled with very close parallel lines, producing a spectrum by diffraction and interference of light. The light that exits comes out at a specific angle depending on its wavelength.

The spectrometer focuses the monochromatic wavelength towards the sample. The sample emits a wavelength, which travels to the detector. The detector is usually set at a 90-degree angle to the light source to avoid any interference from the transmitted excitation light.

Photons emitted hit a photo detector. Computer software connected to the detector generates a spectrum, a graphical representation that shows which wavelengths the sample absorbs.

The emission spectrum shows which wavelengths the samples emit.



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