Polarization Measurements: Parallel vs. Non-Parallel Beam Geometry

As shown in Figures 1 and 2, there are two optical design geometries for the collection of fluorescence light in commercial spectrofluorometers: parallel beam or non-parallel beam geometry.

Parallel Beam Geometry

ISS instruments use parallel beam geometry for the collection of light. When the parallel beam geometry set up is used, the fluorescence emitted by the sample is collected by a lens producing a beam parallel to the optical axis; the beam passes the Glan-Thompson polarizer and is focused by a second lens onto the light detector (usually a photomultiplier tube (PMT).

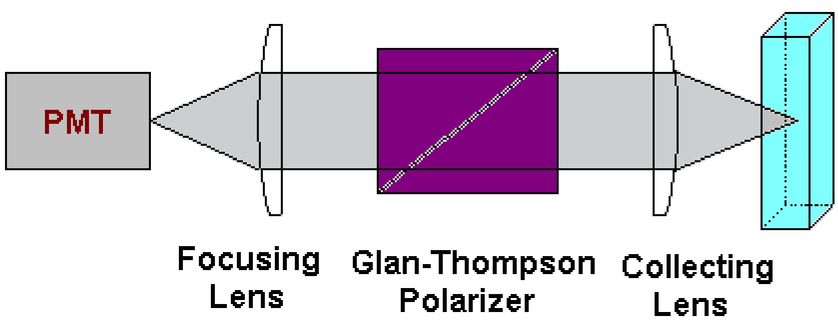

Figure 1. Parallel beam geometry for the collection of fluorescence light in ISS instruments. Fluorescence light rays coming from the sample collected by the lens are parallel to the optical axis; they are transmitted through a Glan-Thompson polarizer and focused onto the light detector (PMT). This set up allows high precision polarization measurements even on weakly emitting samples.

Non-parallel beam geometry

Instruments that are based on non-parallel beam geometry utilize mirrors to focus the fluorescence onto the light detector; usually this mirror is placed behind the sample for larger collection of the fluorescence light. In this case the beams crossing the polarizer are not parallel.

Although instruments with a non-parallel beam geometry design are more effective in collecting fluorescence light (emitted by the sample), fluorescence polarization measurements tend to be a serious handicap with these instruments: beams at an angle larger than about 15-20° from the optical axis cannot pass through a Glan-Thompson polarizer (the angle of transmission depends upon the wavelength and the ratio length-to-aperture (L/A)).

The focusing mirrors can also cause problems for the spectrofluorometer. State-of-the-art mirrors are coated to maximize the transmission over a wavelength range and to make the transmission "wavelength-free". Coating materials undergo oxidation processes whose rates increase rapidly when light with a wavelength below 400-nm hits the material. Damage of the mirror coating results in a decrease of reflected light: replacement of the mirrors may be required every 6-12 months. Lenses are not susceptible to these effects and make the overall instrument more reliable over the years.

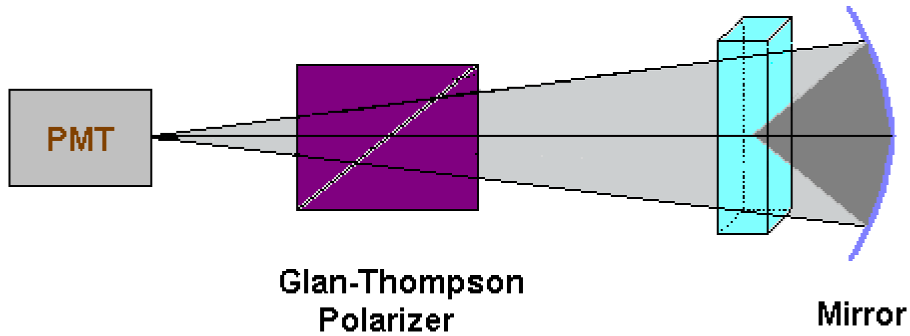

Figure 2. Non-parallel beam geometry for the collection of fluorescence light. Rays at an angle larger than 15-20º (outside gray area) are rejected by the Glan-Thompson polarizer and do not reach the detector. This geometry is not suitable for precise polarization measurements on weak fluorophores.