Polarization Measurements: Parallel vs. Non-Parallel Beam Geometry

Polarization (anisotropy) measurements are acquired by using polarizers housed in the fluorimeter. There are two optical design geometries for the collection of fluorescence light in commercial spectrofluorometers: parallel beam and non-parallel beam geometry. In this technical Note we explain the differences between the two approaches.

Light transmission through Glan-Thompson polarizers

Several types of polarizers and approaches are available for selecting the plane of polarization of a light beam: wire-grid polarizers, absorptive, by reflection, birefringent polarizers and film polarizers. Birefringent prism polarizers (Glan-Thompson, Glan-Taylor) are preferred over other types of polarizers for high sensitivity fluorescence measurements requiring high precision. They are made out of right-angle calcite crystals cemented together along the long side; the optical axes of the crystals are parallel between them and perpendicular to the plane of reflection. Light entering the polarizer is split into two rays, the p-polarized and the s-polarized; the s-polarized beam is transmitted.

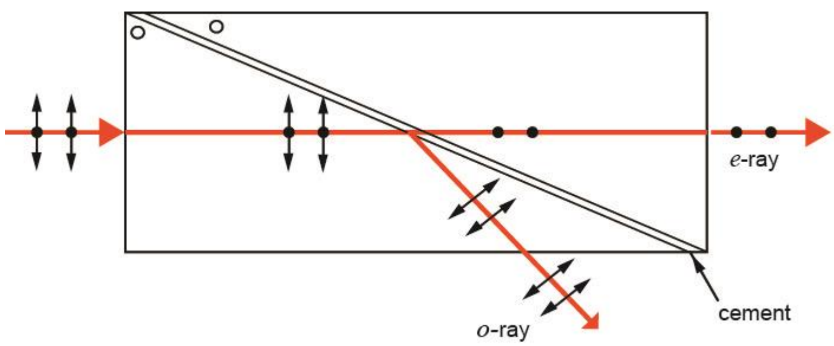

Figure 1. A Glan-Thompson polarizer is made out of two calcite crystals cemented together along the long side. A ray beam entering from left is split into an ordinary beam (p-polarized) and an extraordinary beam (s-polarized). The p-beam is reflected by total internal reflection and deviated; the s-beam is transmitted.

The main parameter characterizing a Glan-Thompson polarizer is the length-to-aperture (L/A) ratio; it determines the usable angular polarized field and its wavelength transmission range:

L/A ratio	Usable angular polarized field	20% transmission
2	15º - 17º	> 260 nm
2.5	14º - 16º	> 330 nm
3	25º - 28º	> 350 nm

For transmission below 300 nm, a polarizer with a L/A = 2 is required; this determines the usable angular aperture of the beam.

Parallel Beam Geometry

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).

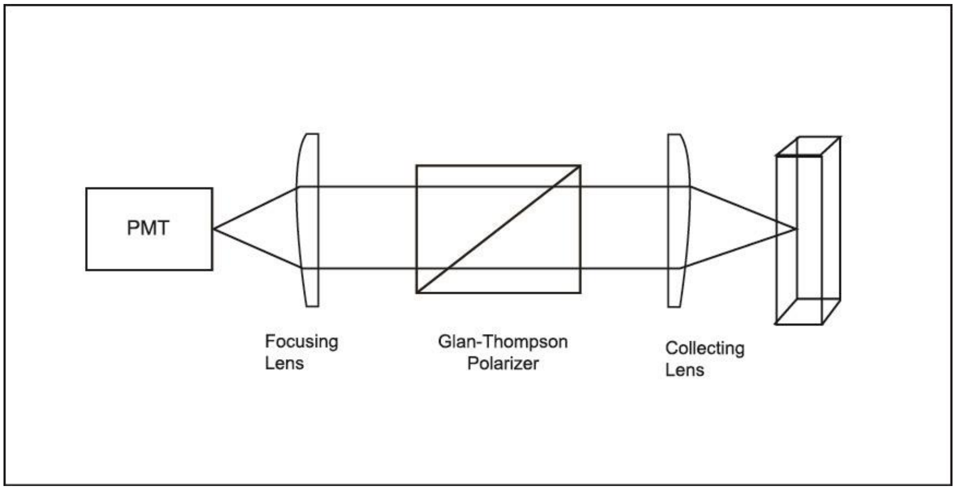

Figure 2. Parallel beam geometry for the collection of fluorescence light in ISS instruments. Fluorescence light rays coming from the sample collected by the lens are parallel to the optical axis; they are transmitted through a Glan-Thompson polarizer and focused onto the light detector (PMT). This set 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; when the angle is above 17º for a polarizer with L/A = 2, the beam is reflected back.

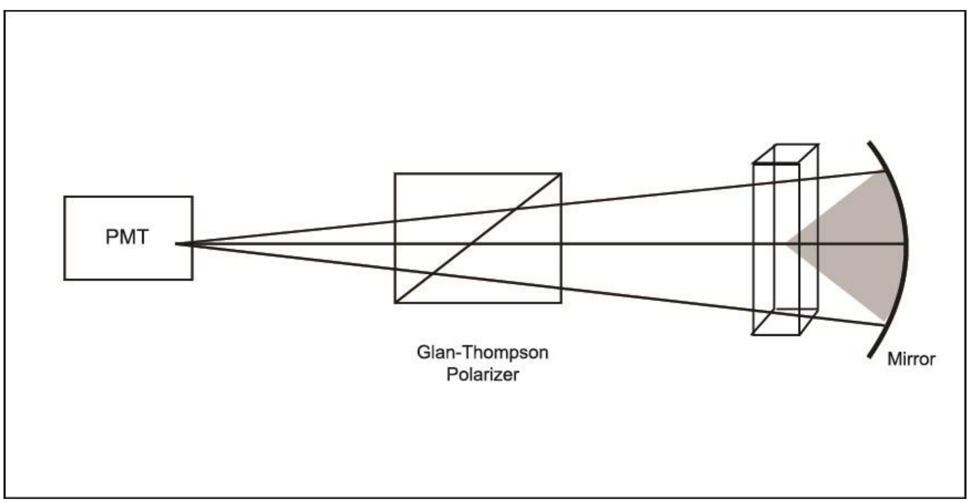

Figure 3. Non-parallel beam geometry for the collection of fluorescence light. Rays at an angle larger than 15-20º (outside gray area) are rejected by the Glan-Thompson polarizer and do not reach the detector. This geometry is not suitable for precise polarization measurements on weak fluorophores

Discussion

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 17° from the optical (with L/A=2) axis cannot pass through a Glan-Thompson polarizer (the angle of transmission depends upon the wavelength and the length-to-aperture ratio (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”. Yet, coating materials undergo oxidation processes, and their performances decrease 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. On the other hand, lenses are not susceptible to these effects and make the overall instrument more reliable over the years.

For more detailed information about the theoretical aspects and applications of fluorescence polarization, the reader is referred to the ISS Technical Note: Fluorescence Polarization.

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