How does OLIS achieve a single spectrophotometer which optimizes for CD, CPL, and anisotropy?

We call the answer our "Polarization Toolbox."

The **Polarization Toolbox** is a sample compartment developed to allow immediate and fail-safe positioning of polarizer(s) and a modulator before or after the sample. Let's call this positionable polarization hardware a "Polarization Modulator Assembly" or PMA.

If the PMA is before the sample, incoming light is switched between L&R. The Toolbox is measuring CD. If the PMA is after the sample, the polarized emission light is being analyzed for CPL and anisotropy.

This document focuses on the emission measurements.

With a vertically polarized excitation and where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the vertically & horizontally polarized emission.

Fluorescence anisotropy (r) : $r = (I_{\parallel} - I_{\perp})/ (I_{\parallel} + 2I_{\perp})$

Polarization of fluorescence (P): $P = (I_{\parallel} - I_{\perp})/ (I_{\parallel} + I_{\perp})$

The method for emission data collection is described in the 1974 Analytical Chemistry paper, "Recording polarization of fluorescence spectrometer: Unique application of piezoelectric birefringence modulation," https://pubs.acs.org/doi/abs/10.1021/ac60340a004

DeSa realized that the use of "piezoelectric birefringence modulation" would eliminate the need for two detectors, rotation of polarizers at each moncohromator,

and the associated four G-factor[1] corrections. His colleague and co-author, Wampler, wrote the math for this novel advance in polarization measurements.

With their '74 method, a single detector is used, no movement of the polarizer is needed, and no G-factor correction is required for anisotropy or CPL measurements.

The '74 Method simplifies the hardware and collects the desired data directly and thus perfectly.

Richard DeSa's motto: Why do manually what can be done electronically? Electronically is failsafe, easy, perfect.

Very commonly, the modulator is a photoelastic modulator (PEM), as is used for circular dichroism. This 50 kHz modulation rate switches the polarization 50,000 time per second. A PEM is always used for CD mode and anisotropy stopped-flow.

The same PMA can be moved to a 90-degree position and used for CPL. For CPL, we commonly choose a quarter waveplate instead of the PEM, because this frees data collection from the 50 kHz modulation rate. The freedom to collect Lum(L) and Lum(R) at slow speed has considerable advantages.

The following figures show PMA positioning for the measurements. The software does the rest:

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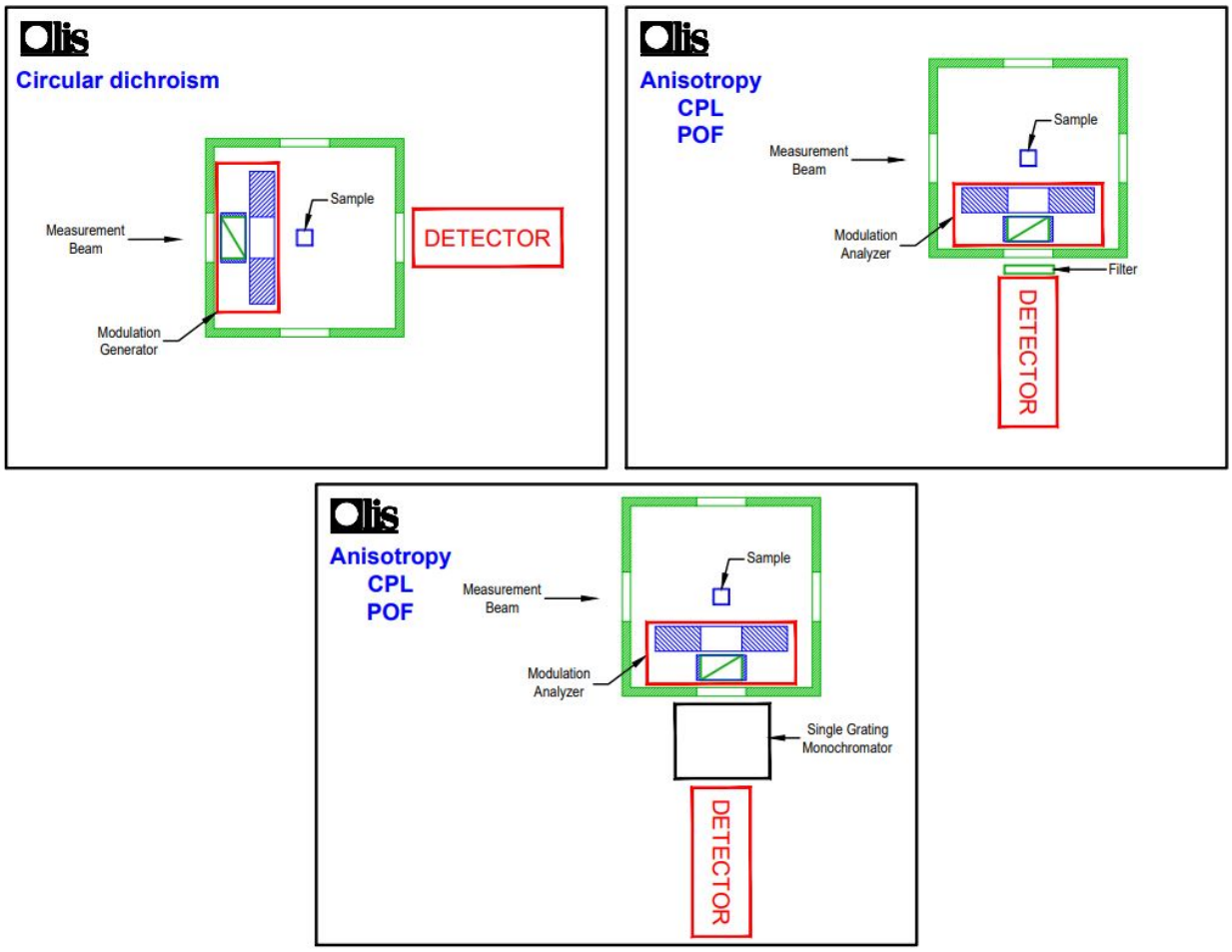
[1] A G-factor is the correction to the effect of the inherent bias of monochromators to the orientation of a polarizer. Every monochromator has some polarization bias. This bias causes changes in the transmitted intensities between the two orientations of the polarizer. When a measurement requires a change in the orientation of the polarizer, a correction factor must be applied to cancel this effect, i.e., the G-factor correction.



For Anisotropy, the excitation light ("measurement beam") passes through a vertically oriented linear polarizer before striking the sample.

The light emitted by the sample is captured at 90-degrees after it passes through a fixed polarizer and either a filter (shown) or scanning monochromator, and then to the detector.

Unlike L-format polarization systems which other manufacturers are limited to -- in which the polarizer must be physically rotated between the vertical and horizontal positions – the DeSa-Wampler method is more direct, simple, and perfect.

DeSa's utilization of a modulator – a 50 kHz photoelastic modular (PEM), liquid crystal variable retarder (LCVR), or waveplate -- **rotates the plane of polarization of the emitted light rather than moving the polarizers.**

One of the beneficial consequences is that no G-factor correction is needed to correct for the different intensity responses of the monochromators to the two states of polarization. The OLIS method has the sample between fixed and stationary polarizers.

In the case of the LCVR and waveplate, switching between L and R (or parallel and perpendicular) can be made at an arbitrary speed, liberating data collection from the 50 kHz rate of the PEM.

So, if the emission polarizer has been fixed in the horizontal position, and the modulator is off so that no alteration of the emission light is occurring, I_H can be measured. When the modulator is turn on (to a level that the polarization of the emission light is rotated 90 degrees) the detector will measure I_v.

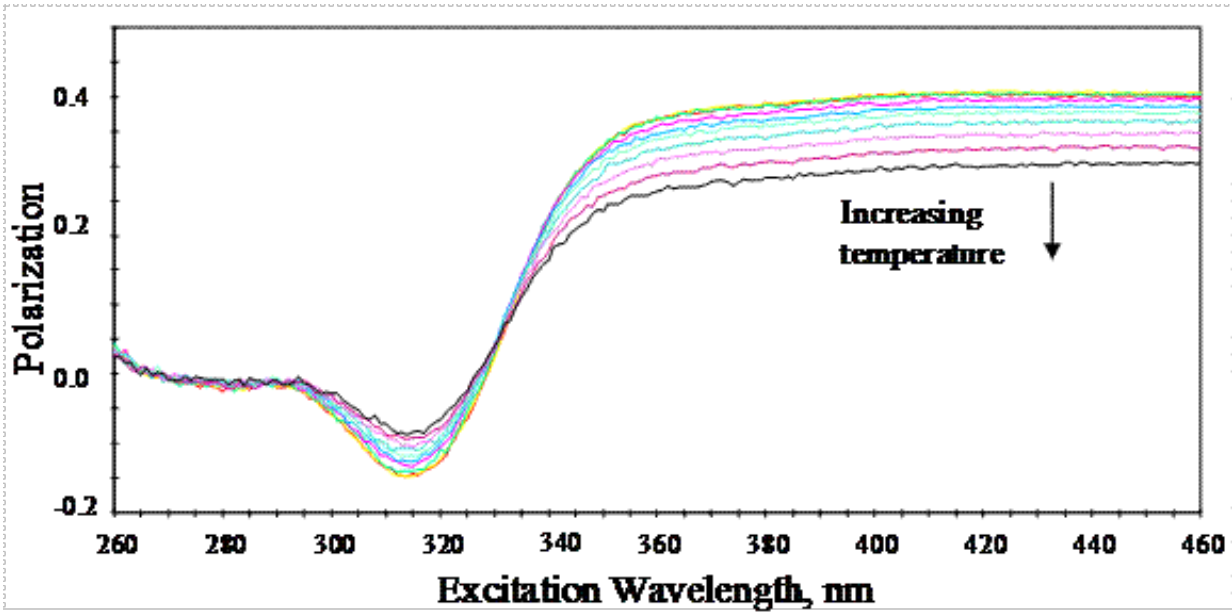
That is, the polarization of the left and right will have some Horizontal character since the modulator rotated 90 degrees. Vertical has become horizontal and passes through the fixed horizonal polarizer

Detection is handled by the exquisitely sensitive gated photon counting PMT for maximum sensitivity.

By synchronizing the detection with the modulator, the appropriate Ivv and Ivh values can be obtained and used to calculate anisotropy or CPL.

Because the polarization state of detected light is always horizontal, there is no effect of polarization (including Wood's anomalies in the monochromator) on the intensity. There are no moving parts; neither polarizer is rotated.

The following figure shows an example of a dilute fluorescein solution in glycerol. The temperature was increased from 20°C to 80ºC as measured in a Peltier temperature-controlled cell. The recorded polarization decreases as a function of temperature, reflecting the increased motion of the fluorescein molecule.



The choice of the modulator -- PEM, LCVR, or waveplate -- should be based on two aspects of the measurement. The first is the switching rate required. Applications such as stopped flow and CD, which require rapid switching, require the PEM. Slower anisotropy and all CPL measurements can be done with the PEM, LCVR, or waveplate.

In addition to speed of modulation, one must also consider the wavelength range required. The respective spectral ranges are 170 nm – 1400 nm for the PEM, 340 nm -2000 nm for the LCVR, and 270-2700 nm for the waveplate.

Circularly polarized luminescence (CPL) is the differential emission of left and right circularly polarized light from a chiral fluorophore. CPL is extremely sensitive to the environment of the excited state. CPL has been used to characterize transition metal ligand complexes (a signal is seen only when coordinated to a chiral ligand), trivalent lanthanide complexes, and chiral lactones. In addition, bio macromolecular molecules have been probed by using Tb3+[ref], dansyl, acridine, and intrinsic tryptophan. These probes, particularly tryptophan, are sensitive to tertiary structure.

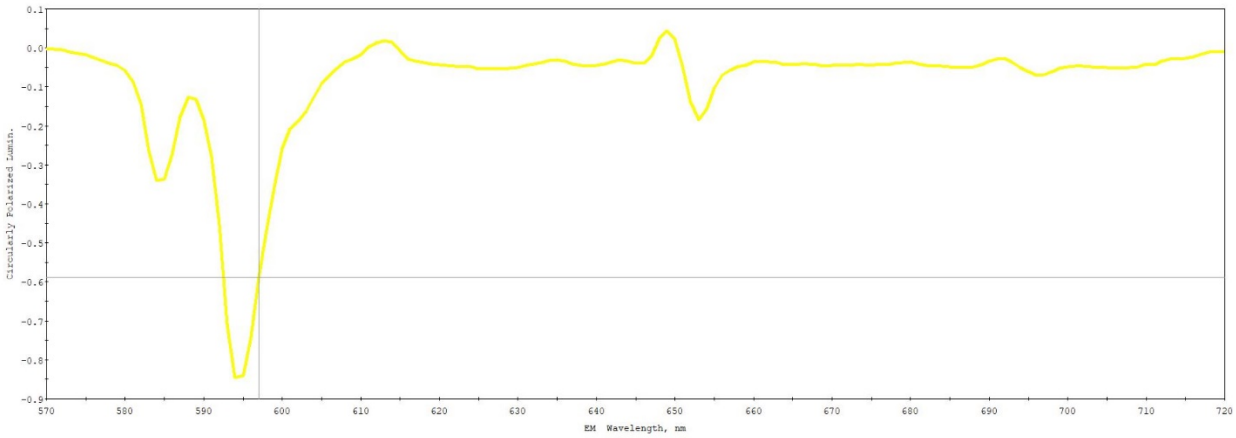
The units for CPL are $G_{(LUM}{}^{2})$, where

$$G_{(LUM)} = 2\,[(I_L - I_R)/I_L + I_R)]$$

Interesting fact: Unfolded proteins, exhibit zero CPL signal.

A CPL spectrum of Europium(III) tris[3-(trifluoromethylhydroxymethylene)-d-camphorate in DMSO.

X-axis = 570-720 nm; Y-axis = -0.9 - 0.1 $G_{(LUM)}$



The Polarization Toolbox is a self-contained sample compartment which we offer on three OLIS instruments:

1. The CPL Solo is the Polarization Toolbox, emission monochromator and emission detector, and one or more LEDs.
2. The OLIS DSM 172 is the Polarization Toolbox, double prism-grating monochromator, emission monochromator, 150-watt xenon arc lamp, and detectors for both CD (PMT) and CPL (photon counting PMT). This model has both UV/Vis and NIR: CD is 185-1700 nm and CPL is 230-850 nm.

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[2] Strangely, Jasco returns CPL as "mdegs," which is utterly incorrect. Millidegrees is the unit used for circular dichroism, the absorbance analogue to CPL.

3. The OLIS DSM 245 is the Polarization Toolbox, double grating monochromator, emission monochromator, 150-watt xenon arc lamp, and detectors for both CD (PMT) and CPL (photon counting PMT). This model supports 170-700 nm for CD and 230-850 nm for CPL.

References

Recording Polarization of Fluorescence Spectrometer -- A Unique Application of Piezoelectric Birefringence Modulation
John E. Wampler and Richard J. DeSa, *Analytical Chemistry*, 46,563 (1974)

Modulator polarization technique:
Wampler, J. E. and DeSa, R. J. (1974) *Anal. Chem.* 46, 563.

Older, but good review of CPL:
Richardson, F. S. and Riehl (1977) *Chemical Reviews 6*, 773-792.