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FLUORESCENCE POLARIZATION WITH A CD SPECTROMETER

Fluorescence polarization measurements are possible with any Jasco CD spectropolarimeter *equipped with both LD option and one of the different FDCD accessories* (to collect fluorescence at 90°).
We will try here to explain why and how:

Measuring fluorescence depolarization with a conventional spectrofluorometer, with excitation and emission monochromator, the following equations will apply:

P = polarization = $(I_{vv} - GI_{vh})/(I_{vv}+GI_{vh})$
A = anisotropy = $(I_{vv}-GI_{vh})(I_{vv}+2GI_{vh})$

Where G is a correction factor = I_{hv}/I_{hh} depending from the fact that standard fluorometers monochromators shows different efficiency with linearly polarized light radiation.
So usually G is predetermined experimentally, while I_{vv} and I_{vh} are measured during the actual experiment rotating the polarizer at the emission path. The whole operation may me manual or fully automatic depending on the instrument and accessory you are dealing with.

Other dedicated instruments, such as the Jasco FP-715 features an excitation monochromator, an automatically rotating linear polarizer on the excitation path plus a fixed polarizer + long pass filter + photomultiplier tube in the emission path. Design is in a way similar to our spectropolarimeter set up; here:

P = $(I_{vv}-GI_{hv})/(I_{vv}+GI_{hv})$
A = $(I_{vv}-GI_{hv})/(I_{vv}+2GI_{hv})$

Where G is here I_{vh}/I_{hh}

Now let's try to consider how to carry on same measurement on a Jasco CD spectropolarimeter equipped with the options said above.
In this case (using LD mode) we alter the polarization of the excitation path at 50KHz.

LD conventional transmission mode
In LD (transmission) mode the unit is actually measuring
$LD = (AC/DC) \log_{10} e$
where:
$AC = I_v - I_h$ is the lock-in amplifier output
$DC = I_v + I_h$ is kept constant by dynode feedback mode
This fits very well the real value for ΔOD below 0.2 as in most of the cases

Fluorescence LD mode
When we insert the PM tube at 90° and we collect fluorescence LD keeping the photomultiplier tube high voltage in automatic mode while inserting a linear polarizer in vertical position in front of the PM tube before the long pass filter, we measure
$fLD = (AC/DC) \log_{10} e$
while we would like to measure
$fLD = (AC/DC) = (I_{vv}-I_{hv})/(I_{vv}+I_{hv}) \approx$ fluorescence polarization
where again AC is the lock-in amplifier output, while DC is kept constant by dynode feedback operation.

Therefore in order to get a linear response scale it's mandatory to modify slightly the hardware to output the LD lock-in output directly to the external signal, by-passing in this way the log function inserted in the normal LD acquisition mode.

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If we assume moreover that:
$I_{vv}=I_p$ (parallel)
$I_{hv}=I_{vh}=I_{hh}=I_s$ (perpendicular)
due to the limited aberrations of the layout.

When measurements are carried on without the polarizer we will have
Direct lock-in output = fLD = (AC/DC) = $[(I_{vv}+I_{vh}) – (I_{hv}+I_{hh})]/[(I_{vv}+I_{vh})+(I_{hv}+I_{hh})] = (I_p – I_s)/(I_p + 3I_s)$ which is more similar to anisotropy

The matter has potentially a good interest since in this mode you can carry on fluorescence polarization measurements using hardware already existing. Peltier accessories and stopped-flow cells can be used as well to perform this task, with no redundancy.
The hardware modification is simple and it may be that Jasco would implement fLD direct output (AC/DC with no log conversion) in the future.

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