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STRAY LIGHT IN THE UV REGION

We discussed already on this issue in T.R. n° 20 in August 2000, but we return now on the same subject with more data. Pls refer to previous TR also for related references.

How SRE may influence CD measurement?

CD technique may be sensitive to SRE particularly in the low UV, since in that region in addition to the absorbance of the sample the one of the solvent may play a relevant role.
Let's quantify the phenomena assuming at a certain wavelength:
-1 OD of absorbance of the sample
-1 OD absorbance of the solvent
-little or no absorbance of sample and solvent at higher wavelengths
This situation match the conditions you may often face running CD spectra of proteins.
We may now quantify the relative error induced by SRE on a UV-VIS spectrophotometer (since SRE depress proper Abs reading) and on a CD spectrometer (since SRE will artificially increase the DC component).
Pls use the following table:

% SRE	measured A_t total Abs	measured A_b solvent Abs	A_s ($A_t - A_b$) sample Abs	error in A_s	error in CD
1	1.699	0.958	0.741	- 26%	- 50%
0.1	1.958	0.995	0.953	- 4.7%	- 9.1%
0.01	1.995	0.999	0.994	- 0.6%	- 1%

Measuring SRE of a CD spectrometer in the field

Published specs (if any) are not very detailed. You must also consider that when you buy a monochromator SRE is typically stated (and measured) filling the system with monochromatic source (a laser) and measuring the output at different wavelengths. In a CD spectrometer (as well as in a UV-VIS one) approach is fully different since you feed the monochromator with white source and you measure the *purity* of the output.
We tested a recently installed J-810 according the ASTM E-387 regulations with a 10% NaI aqueous solution in a 1 cm path cell.
Unit has been operated in the %T mode (i.e. measuring the DC mode at constant high voltage applied on the photomultiplier tube) with 1nm SBW.

Fig 1 shows the plot of air (SB_{air}) and of the NaI solution ($SB_{NaI\ 228}$) with 228V on PM tube:

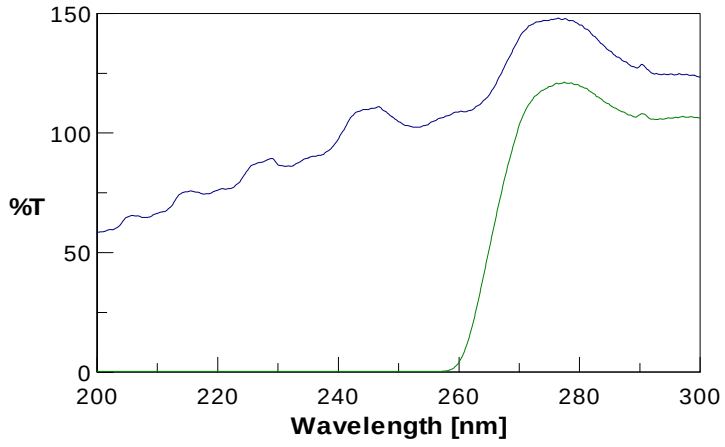

Fig 1

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SRE will be the % of light passing below 245nm.
To quantify this phenomena we must increase sensitivity.
So we rerun the NaI spectra ($SB_{NaI\ 228}$, $SB_{NaI\ 370}$, $SB_{NaI\ 560}$, $SB_{NaI\ 800}$) changing HT from 228 to 370, 560 and 800V at 260, 258 and 248nm, and measuring with care the resultant changes in the amplification (x34.09, x12.67, x16.27 respectively), so when operating at 800V the total gain is 7027.34 times compared to initial 228V.

The resulting spectra are shown in Fig 2:

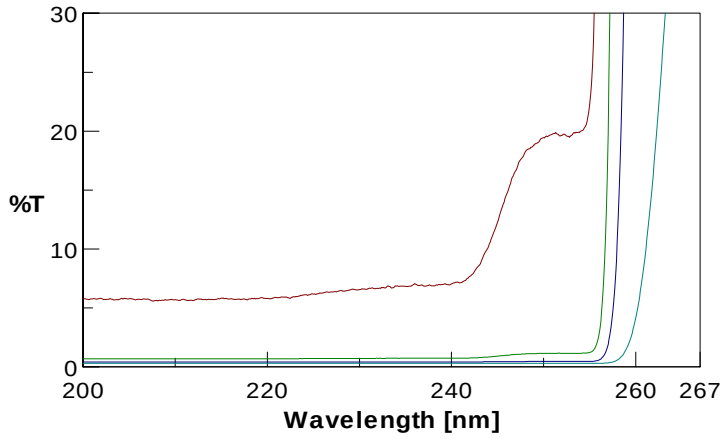

Fig 2

At last we put a dark piece in the cell position and we measured the signal ($SB_{dark\ 800}$) with maximum gain (i.e. HT=800V) and this is compared with $SB_{NaI\ 800}$ in Fig 3:

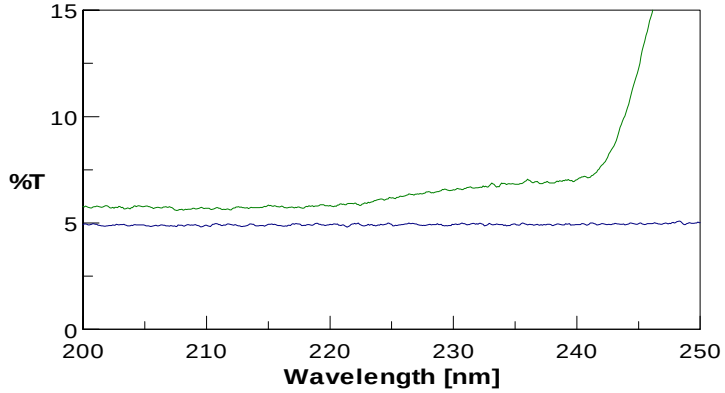

Fig 3

Than we quantified stray light using the formula:
$(SB_{NaI\ 800} - SB_{dark\ 800})$: 7027 x 100 : SB_{air} and we plotted data as from Fig 4:

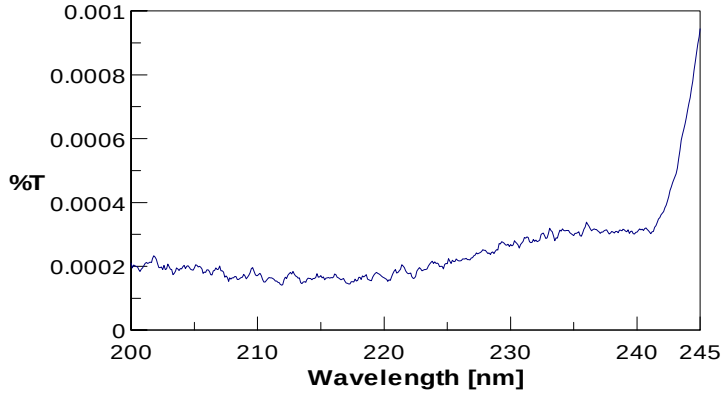

Fig 4

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You'll (correctly) object that approach is not correct since during practical measurement we cannot subtract SB_{dark}
So if we recalculate now on the basis of the formula:
$SB_{NaI\ 800}$: 7027 x 100 : SB_{air} we get the following SRE graph (Fig 5):

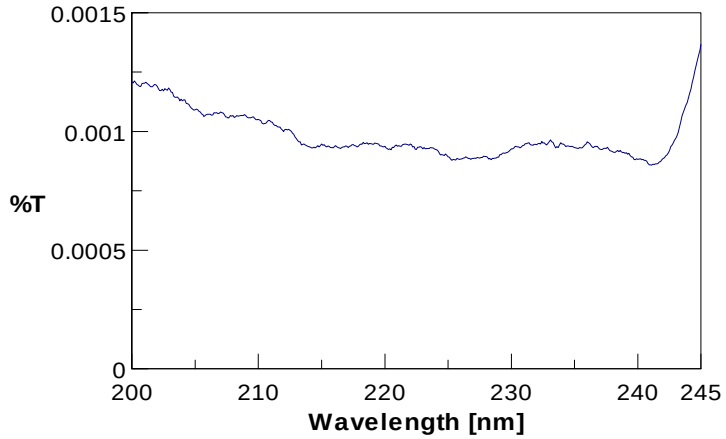

Fig 5

Why SB_{dark} is so high (do degrade sizeably the SRE measurement)?
There are three possible reasons:
-dark current of the photomultiplier tube
-stray light from outside
-DC offset of the PM tube preamplifier

Dark current is hardly the cause since for R376 Hamamatsu specify 3.0nA typical (15nA maximum), while the gain of the preamplifier is relatively low.
DC offset too, while not tested in this case, is typically low.
So we checked first that no light was leaking from sample compartment, while we discovered some leaks between the left sample compartment plate and the PM tube holder (despite the O ring!).
Shielding this part with a piece of tape we got much improvement as from Fig 6:

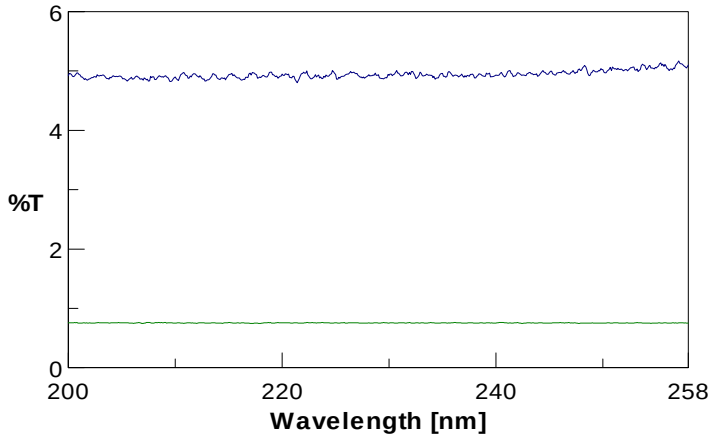

Fig 6

This shows $SB_{dark\ 800}$ without and with shielding tape.
In this mode we confirmed that, on this specific unit, SRE from improper shielding is much higher than SRE coming from the optics.
In practical use you'll hardly notice this phenomena, but it's wise to check and correct if you need to operate with high HT.

To confirm this assumption we run SB_{dark} data with 800, 520 and 228V as HT, as from Fig 7:

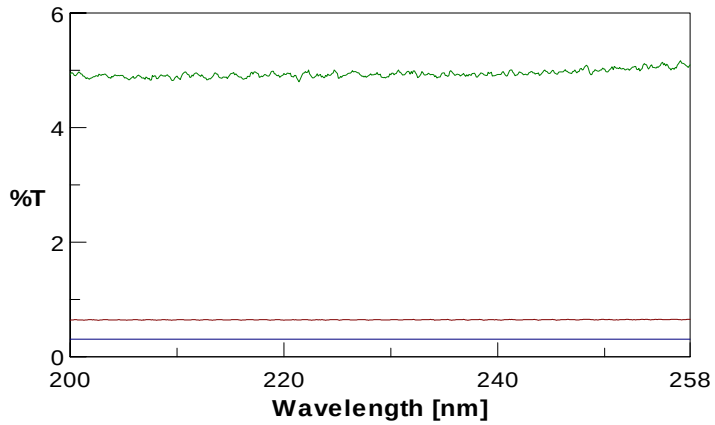

Fig 7

As expected the spurious signal coming from outside light leaking is increasing with the high voltage applied.
When you consider that in normal operation you hardly operate with HT above 500V, the influence of this unwanted external light is negligible.
Otherwise we can conclude that since Jasco J-810 specs for SRE are for less than 0.0003% at 200nm, this specific unit calls for extra tape to meet them (passing i.e. from Fig 5 into Fig 4)!