Calibration and quality assurance procedures at the far UV linear and circular dichroism experimental station DISCO



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# Calibration and quality assurance procedures at the far UV linear and circular dichroism experimental station DISCO

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**Abstract**. Circular and Linear dichroism spectroscopy used in biophysics, are both differential absorption techniques, which explore the chirality of complex macromolecular structures such as proteins and nucleic acids in solution. In the past two decades synchrotron radiation facilities throughout the world, have accommodated circular dichroism (SRCD) experiments. These intense VUV light sources have greatly expanded the wavelength range exploitable (down to 120nm) at high constant photon flux improving signal to noise ratio and data acquisition speed. Here we present the calibration procedure for the circular and linear dichroism experiments explored on the SRCD beam line DISCO at the synchrotron SOLEIL as well as the specially designed automated sample rotation chamber.

## 1. Introduction:

Circular Dichroism (CD), the difference of circular left and right polarized light, is routinely explored to provide qualitative information of the folding state of macromolecules such as proteins DNA and sugars. Therefore CD is routinely explored in pharmaceutical research, providing insights into protein folding patterns including drug binding, protein-protein interactions as well as membrane protein studies. The differential absorption of left and right circular polarized light provides namely information of electronic transitions within a three-dimensional macromolecule, ultimately indicating the secondary structure content (1,2).

Linear Dichroism (LD), the difference of horizontal and vertical linear polarized light of oriented molecules gives insights into the polarization of charge transition. Therefore LD is a probe for molecular orientation typically used for orientation and conformation assays of orientable macromolecules such as DNA or membrane proteins (3). Over the past decades several reviews have been published on the concept, design and standardization of CD and LD spectroscopies applicable for conventional as well as for synchrotron light sources (4).

On DISCO care has been taken to provide the users with a calibrated and standardized beamline compatible with other synchrotron light sources as well as conventional CD machines (5,6).

## 2. Sample Chamber and environment

For SRCD, samples in solution are generally loaded on VUV transparent CaF2 cells (Fig 1), with very low loading volumes or Suprasil Quartz cells [7]. For SRLD measurements, inherently aligned samples are used. These have to be orientated in respect to the incident beam (Fig 1). Aligned samples are revealed using polarization filters. Samples are mounted in the rotation chamber, allowing 360° rotation at 0.5° steps.

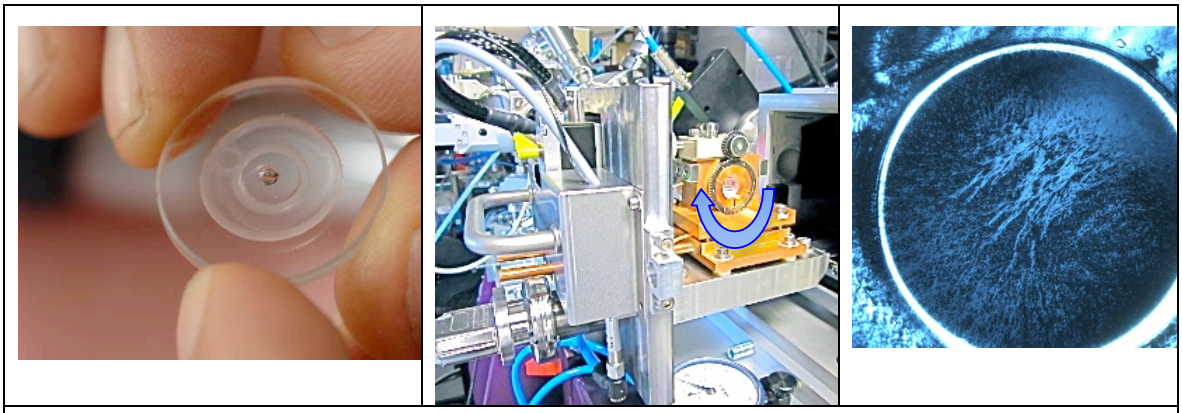

**Fig 1.** Left: 2µl sample loaded on CaF2 window. Centre: Sample drawer with motorized and Peltier controlled sample holder, blue arrow indicates rotation movement. Right: polarized light visualisation of an exemplary aligned sample (Lanreotide) squeezed in between two CaF2 windows of 5µm pathlength.

## 3. Calibration

The calibration and standardization of the CD experiment has been treated extensively in previous publications (7) (8). For the quality of the monochromatic light, stray light measurements (9) using KCl (Fig.2) with the 1st order of the monochromator reflection, proved that CD and LD spectra on DISCO are not affected by the diffusion of zero and higher order light. This was also shown by the additional spectra of Vitamin B (Fig3). Diffusion would otherwise be detected due to re-occurrences of absorption bands at multiples and therefore impact the signal and the high tension (HT), corresponding to the light transmission.

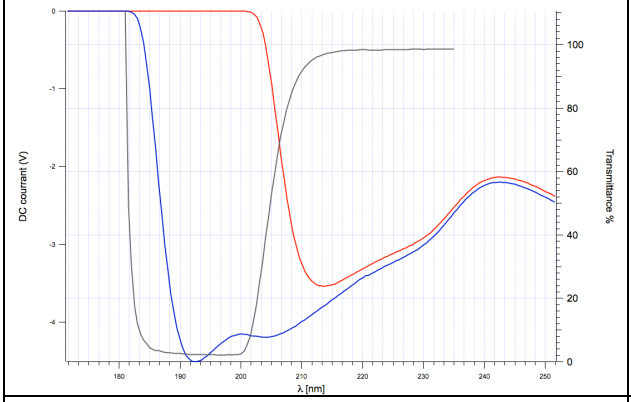


**Fig 2.** Stray light measurement: 1.2%aqueous KCl, 1cm pathlength intensity measurements (red ) versus water baseline (blue). The DC current was measured keeping the high tension (HT) of the PMT constant at 570V, using 0.5nm step size at 1nm bandwidth with 1.2s integration time. Transmittance at the KCl cutoff was 2.4%.

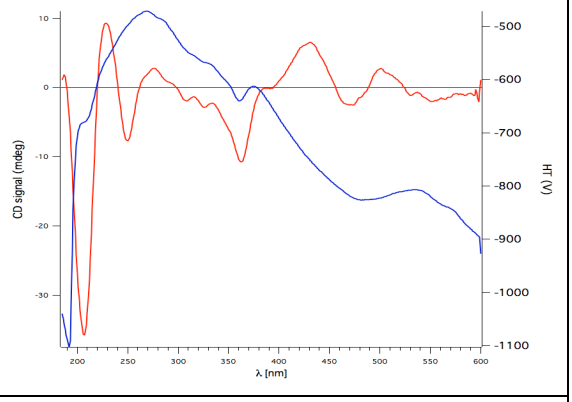


**Fig 3.** SRCD spectrum of Vitamin B12 (red) at 0.5mg/ml in 500micron pathlength, recorded at 1s time constant, 4s integration. In blue the high tension of the photomultiplier showing no interference of higher orders originating from the monochromator. Spectra are identical to in-house spectra recorded on a conventional CD spectrometer.

Wavelength calibrations are routinely carried out with nitrogen, holmium and benzene absorption peaks. Calibration of the Photoelastic Modulator (PEM) retardation down to the far VUV region, important for optimizing accuracy of SRCD and SRLD measurements, has been carried out during commissioning (10). For SRCD PEM modulation phase is set to traditional 0.567 π,radians (90°); amplitudes and wavelengths positions are verified with each beam-fill using camphor sulfonic acid (11). Additionally, spectral quality assessment is obtained through regular comparisons of standard protein spectra (Fig 4). For SRLD the PEM modulation phase is set to 0.765 π radians (138°) and the lockin-amplifier analyzing frequency set to twice the PEM frequency, minimizing any SRCD contribution while maximizing SRLD signal(12). LD signal quality is assessed with naphthalene,

aligned on a stretched cling film, dried and mounted in a rectangular slide (13,14). The proportion of parallel and perpendicular absorption strongly depends on initial alignment of the naphthalene molecules,as demonstrated in Fig 5.

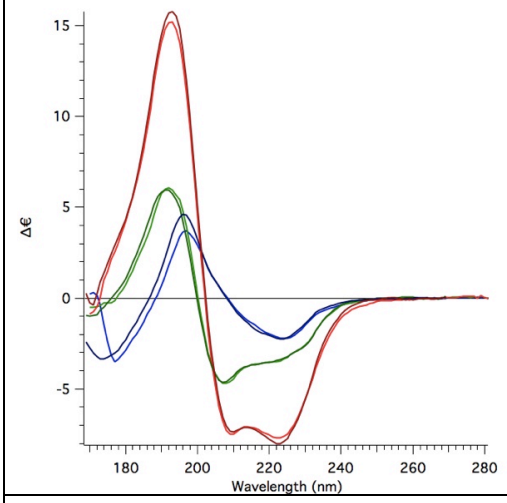


**Fig 4.** SRCD Comparison of three standard protein spectra with reference spectra (SP175): Myoglobin (red), Lysozyme (green), ConcanavilinA (blue). Differences are due to sample preparation and buffer conditions, in dark reference spectra in light DISCO spectra.

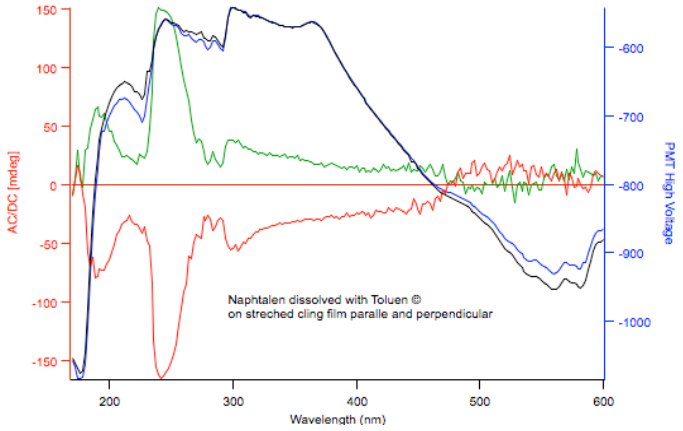


**Fig 5. SRLD:** Linear Dichroism of Naphatlene stretched out and aligned on cling film. The electronic transitions observed, correspond to the orientation of the molecules in the film. The film has been rotated by 90°; in green and red parallel and perpendicular sample orientations respectively.

## 4. Conclusion and Outlook

The DISCO beamline at SOLEIL provides users of the biochemical, pharmaceutical and structural biology with VUV light down to 120nm for dried samples in films and to 168nm for samples in solution. Circular dichroism and linear dichroism can now routinely be measured. Standardizations protocols have been applied to proof the validity, accuracy and compatibility with compatible benchtop spectrometers and synchrotron facilities. Currently we inspect with great interest the impact of circular differential scattering in weakly scattering material with small constant values of refraction indeces, nR – nL (15).

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