

## Chirascan Circular Dichroism Spectrometer: Technical Description

### Introduction

The Chirascan Circular Dichroism (CD) spectrometer is a totally new instrument designed from scratch in and for the 21<sup>st</sup> century. The instrument has been designed for significantly higher UV light throughput, resulting in higher signal to noise, to increase productivity, and with improved ease of use and lower running costs.

The purpose of this document is to introduce the technical elements of Chirascan which underpin its superior performance, and to describe the overall design of the instrument as a whole.

### Key Technical Features

1. Dual polarising prism optics with digital drive and calibration
2. High throughput F/7 optical coupling
3. Simultaneous CD and Absorbance measurement
4. Rapid and economical nitrogen purge design
5. High performance digital signal processing of raw data
6. Expandable modular electronics

### Optical Train

The Chirascan *light source* is a 150W xenon arc with a optimised ellipsoidal focussing mirror for maximum efficiency and light capture. The light housing accommodates a pre-aligned lamp assembly for easy lamp replacement. The housing must be purged with nitrogen during operation both to allow far-UV light transmission but also to prevent the formation of ozone which is harmful to health and can damage the optical surfaces.

The Chirascan *monochromator* features a dual polarising prism design. This yields pure linearly polarised monochromatic light and allows high spectral bandwidths in the far-UV. Three coupled high precision slits are driven by a stepper motor to maintain a constant (but user selectable) bandpass. A variable shutter/attenuator at the entrance to the monochromator allows control of the level of light entering the monochromator, and prevents degradation of optics when not collecting data by preventing light entering the monochromator.

The entire monochromator assembly is hermetically sealed, with strategically placed purge gas inlets and outlets to create efficient purging of the monochromator. This enables the interior atmosphere to be much more rapidly and effectively replaced with nitrogen (necessary for far-UV transmission) than when using passive purging methods, and also allows the nitrogen atmosphere to be retained for long periods after use.

The contributing factors to signal-to-noise in an optical spectrometer can be written as:

$$S/N \propto (Q \cdot I \cdot \tau)^{1/2}$$

Q= detector performance, I = light intensity,  $\tau$  = time scale of measurement.

Clearly on systems with similar detector performance characteristics, increasing light throughput and the time of measurement are the only ways of improving signal to noise. The Chirascan was designed and optimised using the latest optical modelling and CAD technologies, to maximise light throughput and thus reduce the time needed to obtain quality CD spectra, in the critical UV region using several innovative design features.

F/7 optical coupling is maintained throughout the optical path of the monochromator. This has been chosen to maximize light throughput. Many of the key performance benefits of the

Chirascan are because of this monochromator. The use of dual polarising quartz prism monochromator, with large prism and mirrors optical surfaces allows a large amount of light to be collected and passed through the monochromator. The dual polarising prism arrangement allows large spectral bandwidths in the deep uv without loss of purity of linearly polarised light. While the use of high purity quartz prism dispersive elements and high quality mirrors maximises the efficiency of the optical system in the deep UV.

The linearly polarised monochromatic beam that emerges from the exit slit of the monochromator is then re-focussed down through the photo-elastic modulator (*PEM*). This device, which acts as a dynamic quarter wave plate (1), modulates the beam into alternately left and right circularly polarised states.

The modulated beam then passes into the sample housing. It passes through the sample block/cell holder and then on to the photomultiplier *CD detector*. The standard detector port is in line with the beam but a second port is available at the rear of the housing. This may be used to accommodate a detector for simultaneous fluorescence detection or fluorescence detected CD (FD CD).

Removable sample blocks are provided as standard to accommodate a variety of square and circular sample cells. (A range of cell holders and temperature control options are also available). Up to four temperature sensors can be monitored.

Two bulkhead access plates are fitted to allow other accessories access to the light tight housing. These include Peltier controllers, an optional 4-cell autochanger and the titration accessory as well as any of the user's own devices.

A 'lid open' detector is fitted as standard which will pause an experiment in progress and reduce the photomultiplier high voltage to prevent damage if the lid is raised.

The sample housing has been designed to accommodate quite large specialist cell holders and accessories eg cryostats for low temperature CD, and sample magnets for magnetic CD (MCD) experiments. However in some circumstances the entire housing can be straightforwardly removed and an alternative sample handling system configured, for instance the SF.3 stopped-flow accessory.

### Control and Acquisition Electronics

When the modulated light beam strikes the CD detector it is converted into a photocurrent proportional to the incident light flux. Since the beam polarisation is circularly modulated at 50kHz, if there is a differential transmission of the two states (such as would be caused by CD in the sample), this results in a 50kHz AC component superimposed on a background steady state DC component.

The CD is calculated from the ratio of the AC and DC components since CD (in terms of  $\Delta A$ ) is given by (2,3):

$$\Delta A = (A_L - A_R) = (I_R + I_L) / (I_R + I_L) = V_{AC}/V_{DC}$$

Note CD is also expressed commonly in millidegrees, the unit of molecular ellipticity ( $\theta$ ). The two units can be converted according to the formula:

$$\theta \text{ (millidegrees)} = 32,982 \times \Delta A$$

The AC and DC signal components are first separated using tuned amplification in the detector and passed to the CD acquisition and PEM control module. This uses the PEM drive frequency to synchronise a phase sensitive detector (demodulator) that generates a voltage signal proportional to the rectified AC component. This rectified AC component and the background

DC signal are then digitised using independent high speed 16bit A/D converters. Automatic gain control is applied to the incoming signals so as to match them to the optimum range of the digitisation stage and so maximise resolution. The digital data is then exported via the back-plane data bus to the Comms (communications) module for onward transmission to the PC. Once in the PC the CD is calculated from the digitised AC and DC information according to the formula above (the processing at PC level is discussed in the next section).

The system electronics communicate via a proprietary digital back-plane that can support several acquisition and control modules, all operating independently and, if required, simultaneously with no loss of data throughput (bandwidth). Digital signal processors on all modules are responsible for managing data and decoding and executing commands from the PC.

3 stepper control modules are used to drive the monochromator cam, slits and attenuator. Additional modules can be fitted to allow simultaneous fluorescence acquisition or control of sample handling accessories such as stirrers. Any additional signal acquisition channels will operate in parallel with existing ones so that sampling frequency is not compromised in multi-channel configurations. The comms module, which is the interface of the electronics to the fibre-optic link to the computer, also provides several extra inputs for analogue temperature probes and general purpose digital I/O. Vacant back-plane slots are available for future expansion.

All incoming signals (CD AC, CD DC, fluorescence etc) are digitised at a minimum sampling period of 10  $\mu$ seconds (100kHz). Signals are 1kHz bandwidth limited to prevent noise aliasing but are not otherwise filtered in the analogue domain. This is to prevent the risk of irreversible distortion of spectral features which can arise by the incorrect selection of such smoothing filters when the raw input signal is changing too quickly.

The modular design of the electronics means only required features need be installed and any faults that develop are localised and can be easily repaired by substitution of the appropriate module. All modules have a built in self-test capability which communicates any operational problem to the user, and flash memory is used to allow all onboard DSP firmware to be easily.

### **Simultaneous Absorbance Measurement**

In addition to a record of detector gain (high voltage) during CD scanning Chirascan also provides the simultaneous measurement of absorbance as standard. The absorbance spectrum provides a vital indication of the transmission of the sample and any solvents present over the wavelength range under investigation. This is critical for judging whether a CD measurement is valid, since a lack of light throughput will directly result in an erroneous CD due to the attenuation of AC and DC signals to meaningless levels. This will usually manifest itself as a marked rise in the noise on the CD spectrum but this alone may not, at first glance, indicate the degree of deterioration in the validity of the measurement. This is particularly problematic in the UV region of the spectrum where many buffers rapidly become opaque but CD information is of particular interest (eg during protein scans for secondary structure analysis). The absorbance of many common buffers and salts rises very sharply below 200nm and this 'wall' which is clearly evident in the absorbance spectrum, indicates, to a first approximation, the wavelength limit of detection. The inclusion of this data within the same data file as the CD data allows rapid determination of where in the spectrum the absorbance is low enough to give valid and accurate data, even years after recording the data.

### **Data Filtering and Spectral integrity**

Filtering is both essential in the analogue signal processing electronics of Chirascan and also extremely useful for cosmetic noise reduction of the acquired experimental data. It is essential

however that the latter is applied with caution and reversibly as indiscriminate smoothing can distort underlying spectral features.

Signals derived from photomultipliers inevitably have photon shot noise superimposed which is due to the random nature of photon detection events at the photocathode. This is of high frequency and gaussian in distribution.

Firstly, prior to digitization of the analogue signals, it is important that signals are bandwidth limited to less than half the A/D sampling frequency (the Nyquist critical frequency) to prevent the aliasing of high frequency noise into the frequency spectrum of the measurement. This is well known from sampling theory and is essential in any instrument which digitizes analogue signal at discrete intervals. A 200 $\mu$ sec, 1kHz low pass filter on the CD channels (AC and DC) provides this filtering and also rejects any components of the PEM carrier frequency (50kHz). This fixed time-constant is the only pre-acquisition filter applied to the analogue signal.

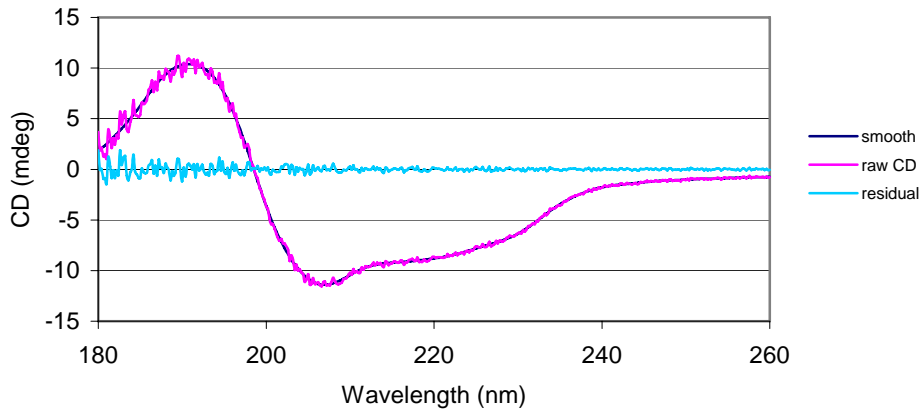
The digitized signals will still exhibit photon shot noise passed by the electronic filtering stage. This noise can often mask spectral features, or at least make them difficult to see, particularly in demanding regions of the spectrum such as the far-UV. Filtering of this noise is carried out primarily for cosmetic reasons

The primary method of noise filtering over which the user has control is sample averaging. This is carried out following digitization but during the acquisition process and simply involves averaging digitized samples together. This yields a signal to noise improvement proportional to the square root of the number of averaged samples. To prevent smearing of spectral information the monochromator moves to each wavelength in the scan and the sampling is carried out before the monochromator moves on to the next wavelength. The averaged measurement is then a true reflection of the input signal at that wavelength. This is in marked contrast to the application of variable time constant filtering during continuous scanning modes found on more traditional spectrometers. Here the smoothed result for a particular wavelength has a decreasing contribution from the signal at earlier wavelengths visited, to the point that spectral features can be irreversibly skewed and attenuated during the acquisition process.

On Chiriscan the average sample size is represented by a time-per-point parameter based on the current sampling period (default setting 25 $\mu$ sec). Because the wavelength drive pauses while the samples are collected, the size of the sample is directly responsible for the time spent per point and therefore the overall scan time. The user can adjust this parameter depending on the signal to noise required for the new spectrum and the time frame desired for the scan. However he or she can be confident that the resulting data are always an accurate representation of the original unfiltered data and that the noise reduction is statistically sound and free from the distortion risks which accompany the use of electronic online filtering methods.

The acquired spectrum may still exhibit a degree of wavelength dependant noise which can be further reduced for enhanced visualization and publication purposes using off-line digital smoothing techniques. The digital smoothing method available in the Chiriscan software is based on the Savitzky-Golay algorithm (4). Because such filtering methods can over-smooth the spectrum, distorting the underlying spectral features, the smoothed results are always accompanied by a display of the residual spectrum which is the difference between the smoothed and raw data. Any systematic deviation of this residual plot is evidence of over-smoothing and the result should be discarded. A random residual indicates a smooth free from distortion (see example below).

Random residual following symmetrical digital smoothing. CD spectrum is not distorted.



The importance of post-acquisition digital smoothing and residual plotting, as opposed to applying preset electronic filtering during data acquisition cannot be over-emphasised, particularly when running samples of unknown character and spectral structure.

### References

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- (4) *Numerical Recipes in C (second edition)*, Press W, Teukolsky S, Vetterling W, Flannery B, Cambridge University Press 1992.