Technical Overview

This guide provides a technical description of the Chirascan-plus CD Spectrometer.

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Content

1.0 Introduction

The Chirascan-plus CD spectrometer is a development of the successful Chirascan CD spectrophotometer, introducing a new large area avalanche photodiode (LAAPD) based CD detector. The increased quantum efficiency of this detector, along with the high (UV) light throughput of the Chirascan monochromator, means greater productivity and improved ease of use accompanied by greater operational confidence.

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

1.0.1 Key technical features

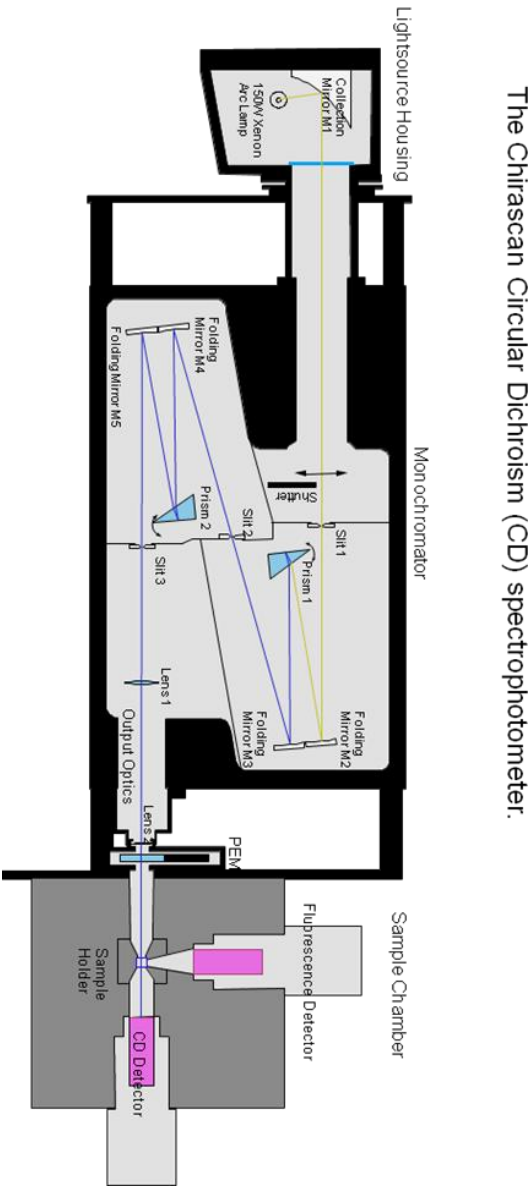
1. Dual polarising prism optics with digital drive and calibration
2. High throughput F/7 optical coupling
3. LAAPD based detector system
4. Rapid and economical nitrogen purge design
5. High performance digital signal processing of raw data
6. Expandable modular electronics
7. High speed real-time control
8. Client server windows software supports remote monitoring of experiments in progress

The schematic diagram of Chirascan-plus on page 4.9 illustrates the three key subsystems of the instrument – the optical train, the control and acquisition electronics, and the Chirascan Windows™ software.

1.0.2 Chirascan-plus specifications

Light source	*150W air-cooled Xe lamp*
Monochromator	*F/7 split-Wollaston prism, dual polarising, dual dispersive optics; wavelength limits 160nm – 1360nm*
Wavelength accuracy	*±0.2nm (170nm – 400nm)* *±0.5nm (>400nm)*
Wavelength precision	*±0.05nm (170nm – 400nm)* *±0.1nm (>400nm)*
Wavelength resolution	*0.1nm all wavelengths*
Bandwidth	*0nm < BW ≤ 2nm at 160nm* *0nm < BW ≤ 4nm at 180nm* *0nm < BW ≤ 7.5nm at 200nm* *0nm < BW ≤ 12nm (software limited) above 222nm*
Stray light	*< 5ppm at 200nm* *< 8ppm at 180nm*
Baseline stability (290 nm)	*±0.02mdeg/hr*
CD / absorption detector	*High-performance UV-visible-IR avalanche photo-diode*
Wavelength range	*Practical limits with sample in place: 170nm ≤ λ ≤ 1150nm*
*RMS noise. All measurements at **1nm** BW and **2s** integration*	*0.02m^o @180nm* *0.02m^o @500nm* *0.02m^o @200nm* *0.04m^o @800nm* *0.02m^o @250nm* *0.05m^o @1000nm*
CD scale and resolution	*Standard ±1500m^o with automatic scaling* *Resolution better than 0.025m^o in 1500m^o*
Standard detection modes	
Spectroscopic probes	***Simultaneous** circular dichroism, absorption and fluorescence. Configurable for FDCD*

1.1 Optical train



The Chirascan Circular Dichroism (CD) spectrophotometer.

The Chirascan-plus light source is a 150W xenon arc with an an 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-plus monochromator features a dual polarising prism design. This yields pure linearly polarised monochromatic light and allows high spectral bandwidths in the far-UV. The digital drive system uses a stepper driven cam with digital calibration and with active temperature compensation. Three coupled high precision slits are driven by a second stepper motor and a third is used to control a variable aperture/shutter.

The entire monochromator assembly is sealed with strategically placed nitrogen purge inlets and outlets. This enables the interior atmosphere to be rapidly and efficiently purged with nitrogen (necessary for far-UV transmission), and also allows the nitrogen atmosphere to be retained for long periods after use.

F/7 optical coupling is maintained throughout the optical path. This has been chosen to maximize light throughput without compromising focussing performance.

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 LAAPD 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 (FDCD).

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 and magnetic stirrer support can be added as an option.

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 turn off the LAAPD bias.

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

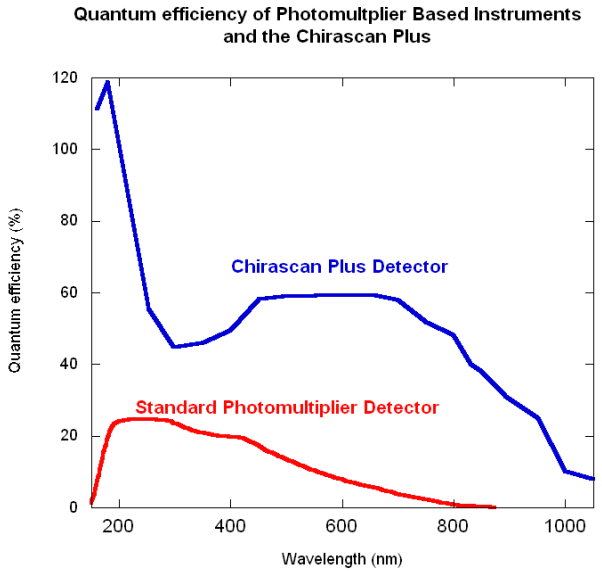
1.2 LAAPD detector and associated electronics

The distinguishing feature between the Chirascan and Chirascan -plus is the latter’s use of a solid-state large area avalanche photodiode or LAAPD, over the traditional vacuum photomultiplier tube used on the Chirascan.

Avalanche photodiodes are in many ways the solid-state equivalents of photomultiplier tubes. Using a high reverse bias voltage, the detector exhibit a high internal gain, in the same way high voltages to photomultiplier anodes boost the internal gain of photomultipliers.

Large area avalanche photodiodes are a new generation of avalanche photodiodes with many features that make them high performance replacements for photomultiplers in many applications. These feature include: a large light collection area similar in size to photomultiplier photocathodes; higher quantum efficiency compared to photomultiplers; extended wavelength range sensitivity; rugged construction and high internal gain.

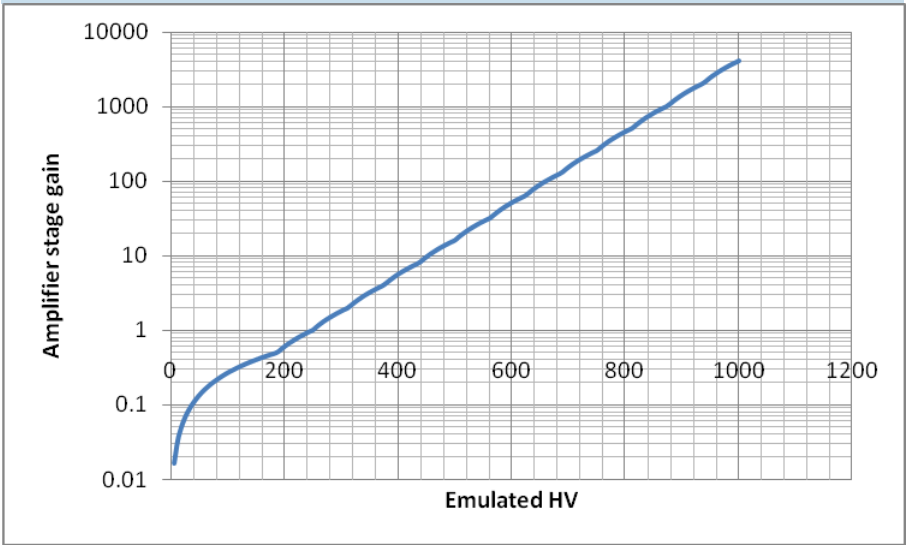
The LAAPD detector in Chirascan-plus is a 1cm diameter round detector, windowless silicon APD. The detector shows a higher quantum efficiency throughout the wavelength range (figure below). As CD is a photon shot noise limited (2) measurement, the increase in quantum efficiency of Chirascan-plus provides a 2 fold signal to noise gain over the Chirascan in the UV region. This extends to several order of magnitude in the near IR region, effectively extending the wavelength range of the instrument into the near IR.



Quantum efficiency of the Chirascan-plus LAAPD detector, and the quantum efficiency of the standard Chirascan PMT detector.

The windowless design removes a possible source of birefringence, reflective light loss, and absorption losses of light in the deep UV. Another benefit of the detector is the insensitivity

The LAAPD detector has a maximum internal gain of 250. This is in comparison to the $1x10^6$ maximum internal gain of the Chirascan PMT. To provide a similar level of total gain and fine grained gain control to the Chirascan, the Chirascan-plus detector has an output stage of multiple cascading low noise programmable gain amplifiers, they provide 16bit control providing amplification gain from 0-4096 in 65536 gain levels. Combined with the internal gain of the LAAPD of 200-250, provides a maximum gain of ~ $1x10^6$. The amplifier gain is still recorded and reported by the software as an emulated HV, providing consistency and transparency in the user interface between the Chirascan and Chirascan plus. The relationship between the Chirascan-plus amplifier stage gain and the emulated HV is shown on the next page.



Gain of the Chirascan-plus LAAPD detector amplifier stage and the emulated HV value.

Dark-current in the LAAPD detector is autocorreted within the electronics and software. At the start of data acquisitions when the autoshutter is closed, a dark current measurement is made and a correction factor set. This backoff is autmoatically adjusted by monitoring changes in the temperature of the photodiode, and using a temperature correction factor to adjust the dark current correction factor.

High levels of light hitting the detector will produce a high signal current. Sustained high levels of light producing a large current that can overload the photodiode element. To prevent this the internal gain of the detector can be reduced by lowering the reverse bias voltage. The reverse bias voltage has been set at a level to allow safe operation of the instrument at a 2nm spectral bandwidth at the factory. As a precaution, the electronics can detect an excess light condition, warning the user and turns off the detector reverse bias voltage reducing the internal gain.

1.3 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 ΔA) is given by (2,3):

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

Note CD is also expressed commonly in millidegrees, the unit of molecular ellipticity (θ). 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 and incorporating appropriate gain factors (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.

Two twin 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 μseconds (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 upgraded via the computer.

1.4 Chirascan Windows™ software

Communications between the computer and instrument is carried out over a high-speed fibre-optic link and a purpose designed PCI interface card and driver handles the bi-directional communications and data capture.

1.3.1 Signal processing

Data smoothing during spectral acquistion is provided by user variable digital signal averaging. This is applied to the incoming digitised signals at each wavelength of measurement. Each resulting averaged data point (which may be the result of many thousand individual samples) is guaranteed to accurately represent the raw input signal value, since all samples are collected at a fixed wavelength. A dedicated high-speed averaging algorithm in the driver software carries out the necessary accumulations and computes the averaged result for all monitored signals. The operator is able to select the overall sample size. This is most usefully presented as the time spent at each spectral wavelength (time per point). This time will have a direct bearing on the overall scan time and the signal to noise of the result but will never cause a systematic distortion of the kind that can be caused by the use of analogue filtering.

1.3.2 Application software architecture

The schematic shows the layered architecture of the Chirascan application software. The object-oriented design has several benefits particularly from the points of view of maintenance, reliability and future expansion. It also allows the same overall architecture to be tailored to different APL products providing a consistent look and feel across the range.

A feature central to the whole instrument control and acquisition process is the datastore. This ‘object’ provides a blueprint for experimental data collection and

also becomes the data storage object after acquisition. In its simplest form it may simply be constructed for a CD spectrum, containing the wavelength range required and a time per point parameter etc, but in CD titrations it will contain a dimension describing the concentration range to target or for a temperature ramp the temperature set points for each measurement. It will also indicate whether simultaneous fluorescence or absorption is to be measured.

The device sequencer (level 3) is responsible for taking a datastore blueprint and executing the experimental cycle that it describes. This is achieved by sending commands to the individual virtual devices which represent the instrument (in the level 2 software). These are then translated and passed to the physical instrument modules themselves via the driver (level 1) and PCI fibre-optic interface. In our simple example the monochromator will move to each point in the wavelength dimension and the CD will be measured and stored. The datastore is designed to be multidimensional and expandable so that if new measurement variables arise it can expand to accommodate them. If new devices are added, eg a fluorescence emission monochromator for example, this can be integrated quite easily.

At the top level (level 5) the Chirascan Control Panel provides the GUI for setting up and executing an experiment. It is this part of the software with which the Chirascan operator interacts. During the set up process a new datastore object is created which describes the experiment, which then drives the acquisition and is used to accommodate the captured data.

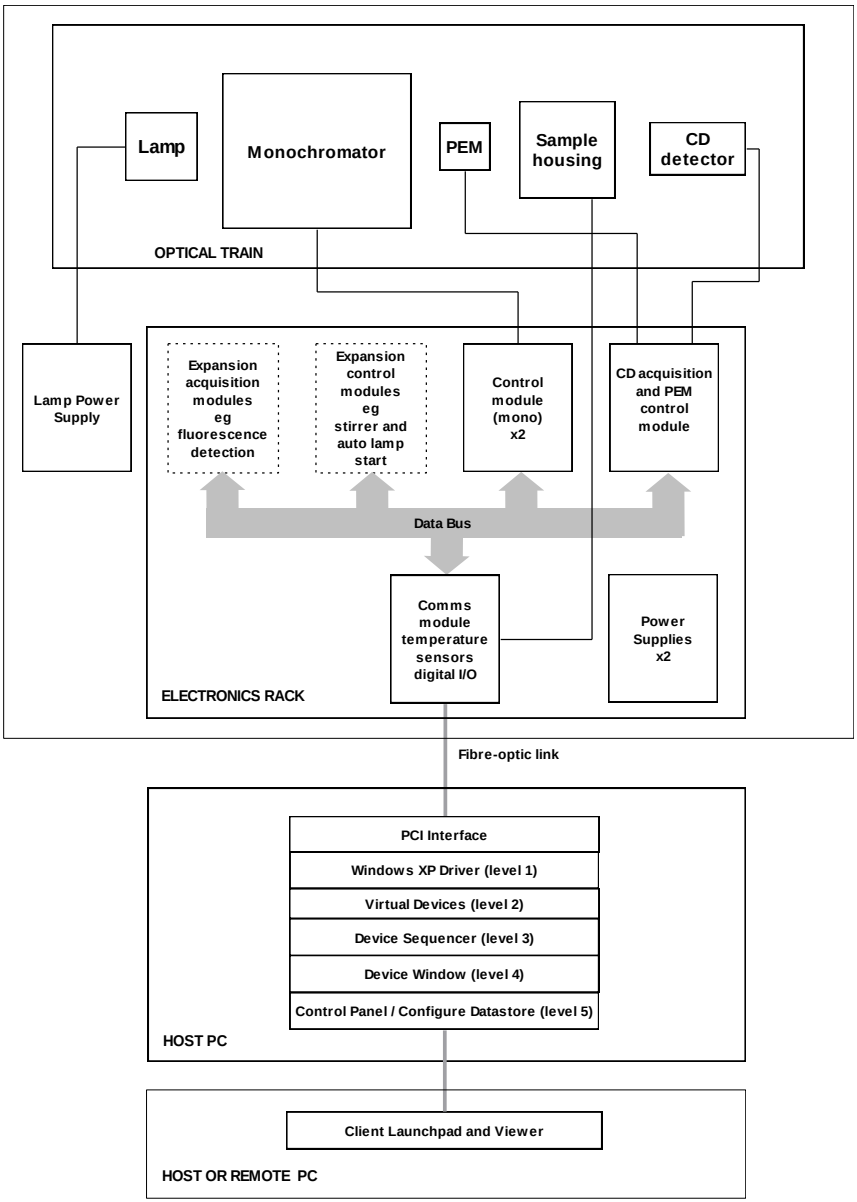
The resulting datastore object is self-contained and forms the basis for the data storage on disc. Since the datastore drives the acquisition a datastore filed on disc can itself be trivially used to initiate a new and identical acquisition, by dragging it onto the Chirascan control panel. All the settings derived from the datastore are automatically reflected in the control panel configuration.

Data visualisation and management is carried out with the Chirascan Launchpad and Viewer (referred to in abbreviated form as CSViewer). Client-server architecture means this can operate either on the host computer alongside the control panel or on a remote networked computer. This allows experiments in progress to be monitored remotely from the instrument, at another location. The data is transferred to the launchpad and viewer automatically during acquisition so that the experimental measurement appears in real-time. On completion the new data can be saved on the PC running the linked viewer, though a backup is always stored on the host PC as well.

Once collected the launchpad and viewer can be operated independently of the control panel software for navigating amongst data files and performing a range of visualisation and data processing options (see software guides).

Finally a range of detailed device windows (level 4) can be accessed on the host PC for diagnostic purposes. These are not usually required during normal operation and in some cases are password protected to prevent unauthorised interference (as calibration data and motor drive parameters can be modified). They are provided to allow complete instrument control on site by APL personnel and to allow tests to be run by customers under APL supervision.

CHIRASCAN SCHEMATIC



Appendix A: Calculation and importance of absorbance measurement with CD

A.1 Introduction

The simultaneous and accurate measurement of absorbance during a CD measurement is a useful and important facility on Chirascan-plus. The absorbance spectrum provides an indication of the transmission of the sample and any solvents present over the wavelength range under investigation. This is helpful in 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 absorbance spectrum of the sample is obviously a useful measurement in its own right and is a useful supplement to the CD spectrum. As a rule of thumb, optimum CD measurements are commonly obtained where the sample absorbance in the cell is about 0.8-1.0 a.u. (2). Much lower and the CD signal will be weak and much higher will seriously limit the light reaching the detector. The absorption spectrum also provides a measurement of the sample concentration and indeed can be used to calculate sample concentration given the observation cell pathlength. This will enable accurate molar ellipticity, and molar extinction values to be calculated.

An important ratio known as the g-factor, or disymmetry factor, which is the ratio of the CD to the absorbance, can also be obtained:

$$g = \frac{\Delta A}{A} = \frac{\Delta \varepsilon}{\varepsilon_M}$$

ΔA is the measured CD, A the absorbance measured under the same conditions, $\Delta\varepsilon$ is the molar CD and ε_M the molar extinction coefficient, ie

$$\Delta A = \Delta\varepsilon.c.l$$
$$A = \varepsilon_M.c.l$$

l is the cell pathlength in cm and c the sample concentration in moles dm^{-3}.
The g-factor is a particularly useful quantity when estimating or assessing enantiomeric excess (1).

A.2 Absorbance measurement on Chirascan-plus

The simultaneous measurement of absorbance and CD is complicated by the mechanism by which CD signals are optimized on Chirascan-plus. Absorbance is normally calculated according to:

$$A = \log \frac{T_{REF}}{T_{SAMPLE}} \qquad \text{(i)}$$

T_{REF} is the light transmission with no sample, only solvent, in the observation cell (this is often referred to as 100% transmission or T_{100}) . T_{SAMPLE} is the transmission with sample in place. For an absorption spectrum, first the solvent transmission is scanned (the baseline) followed by the sample to yield corresponding reference and sample transmissions at each wavelength. The absorbance spectrum is then simply calculated as the log-ratio for each data pair.

It is essential for this calculation to be valid that the two separate measurements are taken under identical conditions of detector gain (constant photomultiplier high voltage or LAAPD amplifier stage gain).

However this constraint does not apply to CD baselines and spectra because CD is a self-referencing measurement (the AC/DC ratio is available from a single scan). The means the detector gain can be automatically adjusted on-the-fly to optimize the signal levels and allow the most accurate and low-noise CD measurements, whether baselines or spectra, to be made.

This is done as the wavelength range is scanned, and involves continuously adjusting the the detector gain to deliver the optimum photometric DC voltage via

the electronics to the analogue to digital convertors. This high voltage and gain control to maintain the photometric DC voltage is referred to as AutoPM.

Because of this process the detector gain will routinely vary during and between reference (baseline) and sample CD measurements so that the conditions for the normal absorbance calculation are not met.

In order for Chirascan-plus to deliver accurate absorbance spectra the following method has been developed which accounts for the variable gain element in the calculation.

For the LAAPD detector, the internal gain of the detector is constant, while an amplifier stage is used to control the gain. The absolute gain, and the change in gain between two measurements is always known. This is unlike the PMT detector where the gain has to be estimated.

From equation (i) the reference and sample DC signals can be expressed as:

$$DC_{REF} = f.\mu_{REF}T_{REF} \qquad \text{(ii)}$$

$$DC_{SAMP} = f.\mu_{SAMP}T_{SAMP} \qquad \text{(iii)}$$

where μ_{REF} and μ_{SAMP} are the two gains necessary to obtain DC_{REF} and DC_{SAMP} from the light transmitted by the reference and sample respectively (f represents an appropriate conversion constant dependent on the units of T).

If, however, DC_{REF} and DC_{SAMP} are the same (which is the purpose of the AutoPM function) then the following holds:

$$\mu_{REF}.T_{REF} = \mu_{SAMP}T_{SAMP}$$

and, $$\frac{T_{REF}}{T_{SAMP}} = \frac{\mu_{SAMP}}{\mu_{REF}}$$

therefore, $$A = \log \frac{\mu_{SAMP}}{\mu_{REF}} \qquad \text{(iv)}$$

Therefore under conditions where the same target DC level is achieved using the AutoPM function, equation (iv) can be used to accurately calculate the absorbance from the detector output amplifier gain used for the reference and sample measurements.

However, in practice, the AutoPM function returns when a signal DC level is within a tolerance window close to the target value. This lack of accuracy would introduce errors into the calculation above. However this error can be corrected by re-introducing a term based on the classical calculation in equation (i).

From (i) (ii) and (iii),

$$A = \log \frac{T_{REF}}{T_{SAMP}} = \log \frac{\mu_{SAMP} DC_{REF}}{\mu_{REF} DC_{SAMP}} \qquad \text{(v)}$$

Here we retain the individual DC terms for sample and reference to account for the AutoPM discrepancy . This formula allows an accurate absorption to be calculated when the individual DC signal levels are recorded together with the detector high voltages.

It can be seen that in the event of the gain being constant and the AutoPM function is not applied equation (v) simplifies to the classical Absorbance calculation in equation (i). If the AutoPM function is perfect and the target DC signals are the same then only the left hand term applies and the calculation is the same as in equation (iv). Any intermediate situation is accommodated through application of the full expression (v).

Appendix B: Adaptive sampling

B.1 Introduction

Incoming signal data on the Chirascan and Chirascan-plus is digitally averaged to reduce measurement noise. The raw signal is bandwidth limited before digitisation at a default frequency on all channels of 40kHz (max 100kHz). The signal to noise (s/n) improvement yielded by this averaging follows a square-root relationship to sample size (N) ie;

$$s/n \propto \sqrt{N} \qquad \text{(i)}$$

The averaging is carried out in the Windows™ driver and can be controlled by the operator from the Chirascan control panel. The sample size for each average is more usefully expressed as a resulting time per point e.g. if N=40000 the time-per-point will be 1sec at a sample period of 25μsec. Chirascan-plus moves to, and stops at, each wavelength in the spectrum to carry out this sample average before proceeding to the next wavelength. The overall scan time is given by the following relationship:

Scan-time = (Time-per-point + step-time) x total wavelength-steps

The step-time is a time overhead comprising a contribution from the time taken for the wavelength drive to move to the next wavelength and a short settling time prior to signal acquisition. It only becomes significant during rapid scans with minimal signal averaging. Total wavelength-steps is also equal to the product of the scan range and scan resolution (in steps/nm) plus 1.

CD is a demanding technique due to the commonly very small magnitude of ΔA to be measured (typically 10^{-4}-10^{-5} AU) and the high degree of photon shot noise in regions of the spectrum where light throughput is limited. This means signal integration (averaging) over time is essential and scan times are typically of the order of several minutes, particularly when extending into the far–UV. The selection of a time-per-point is almost always going to be dictated by the adequacy of the noise reduction in this region of the spectrum and not by the less demanding requirements of regions of the spectrum where there is plenty of light (almost everywhere else).

The result is that in some circumstances, in order to have adequate noise improvement in the far-UV much time will be spent averaging data elsewhere in the spectrum where it is not necessary.

The purpose of the adaptive sampling option is to increase scanning efficiency by dynamically altering the data averaging sample size according to the light throughput as the scan proceeds.

This unique feature of Chirascan and Chirascan-plus is made possible by variable real-time digital signal averaging.

B.2 The AutoPM function

During a normal CD scan, the monochromator moves to successive points in the wavelength range of the scan. At each position the CD signal is optimised, by tuning the gain on the detector. In the Chirascan software the AutoPM function performs this feedback control. It operates by increasing or reducing the gain on the output of the detector until the optimum photometric DC voltage is achieved regardless of incident light intensity. This best matches the digitisation stage and results in the best quality CD resolution. In regions of the spectrum where there is a high photon flux (high light level) the required gain, and emulate HV will be relatively low, whereas in regions of the spectrum where there is a low photon flux (for example in the far-UV) the emulate HV and associated gain will be increased. Note at the very lowest light levels the emulated HV will reach 1000 volts, which is a gain of 4096 over the internal detector gain, and the maximum permitted.

It should be noted that when the emulate HV has reached its maximum level, and the incident light continues to fall, the CD signal can no longer be further optimised. However, even with reduced signal levels a good CD signal can still be measured. A very high emulated HV is not necessarily an indication that there is no CD signal.

B.3 The adaptive sampling algorithm

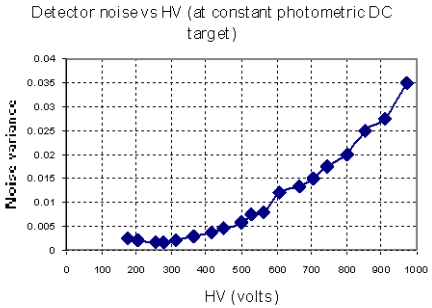
As explained in the introduction, adaptive sampling aims to optimise the overall scan time by spending more time collecting data where it is most needed, which means in regions of low light intensity, and to scan more quickly where light is plentiful. This function has been optimised to maximise the signal to noise of the PMT detector of the Chirascan. The Chirascan-plus has been designed to have a similar relationship of emulated HV, gain and photon shot noise, and so the function will work equally as effectively.

By *reducing* the number of samples (reducing the sampling time per point) in regions of high light flux, a spectrum can be measured in a shorter time. Put another way, the use of adaptive sampling can lead to better spectra being measured in the

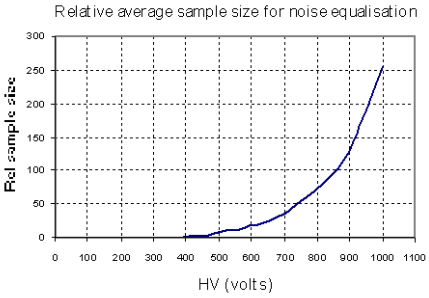
same time. With careful experimentation, using adaptive sampling can produce better CD spectra in a shorter period of time.

Although the relationships between HV, gain and photon noise are quite complex it is also true that, for a given detector, the observed photon noise amplitude is a direct function of the applied HV, and to a large extent independent of other instrumental parameters such as wavelength or bandwidth. By using the relationship in equation (i) it is possible at any HV to equalise the noise in averaged data by a suitable adjustment in the sample size i.e. as the light flux reduces and the HT rises, when a doubling of photon noise occurs this can be compensated for by a 4-fold increase in the average sample size. This is the basis of Chirascan's adaptive sampling mechanism.

Implementation of the approach required making measurements of the noise variance of a typical detector as a function of HV. Some sample results are shown below. A clear non-linear increase in signal variance with HT can be seen between 200 and 1000 volts (the usual operating range of the detector).



The data were then used to construct a plot of required relative average sample size required to maintain a constant noise level versus HV. Fitting an empirical formula to this data (ii) yields the continuous curve below enabling a relative sample size to be calculated for any HV.



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To make use of this function we first define a default sample size and reference HV which act as the starting point for the adaptive algorithm. These values determine a default time-per-point and noise level which is broadly acceptable for typical spectral measurements into the far-UV, but which can be dynamically adapted by the algorithm to improve the scan efficiency as the light throughput varies. These values are the default sample size (20000 or 0.5 seconds time-per-point) and an HV of 800 volts.

If adaptive sampling is not active and the scan proceeds, a 20000-point average will be calculated at every wavelength in the scan range.

If adaptive sampling is selected then whenever the AutoPM function returns an HV of greater or less than 800 volts the sample size and therefore the sample time is increased or reduced according to the relative sample size function:

$$Y = e^{(-.003.(800-HV))} \qquad \text{(ii)}$$

Thus at the reference HV of 800 volts, the adaptive sampling scaling factor Y is 1.0; at an HV of 500 volts, the sampling scaling factor is 0.407 and at an HV of 1000 volts, the scaling factor is 1.82.

So, at 1000V there would be roughly twice as much time spent acquiring each point as compared to the default time-per-point. At HV levels of 500V, each point would be measured in roughly 40% of the default time.

The net effect for a typical sample is that a scan should be faster with adaptive sampling than without for the same default sample size, particularly if the scan extends into high light throughput regions of the spectrum. This will be offset to some extent by increased time spent where light is limited – in the far-UV and in the

presence of absorbing solvents - but here there will be a small enhancement to the signal to noise.

It is also possible for the user to change the default number of samples and therefore the default time-per-point used during normal and adaptive sampling. Minimum and maximum sample sizes can also be modified to limit the sampling extremes during adaptive sampling.

In conclusion, the adaptive sampling option will not always offer significant benefits, but with experimentation will result in scan time improvements for certain samples and scan wavelength ranges.

Appendix C: Data filtering

C.1 Introduction

Signals recorded for CD measurements have photon shot noise superimposed which is due to the random nature of photon detection events of the detector. This is of high frequency and gaussian in distribution and is usually the most significant type of random noise contributing to the overall noise in a CD measurment. Other sources of noise include electronic noise and noise which arises from the PEM modulation frequency 50kHz .

There are three distinct forms of signal filtering which are implemented or available on Chirascan:

- Signal bandwidth limiting and noise rejection in the analogue electronics
- Post-acquisition signal averaging for shot noise reduction where signals are weak
- Cosmetic smoothing of experimental results for presentation

The first of these is an essential and optimized part of the processing of the raw analogue signals prior to digitization, whereas the second two are flexible and under operator control.

C.2 Electronic filtering

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 (4) and is essential in any instrument which digitizes analogue signals at discreet intervals. A 200μsec, 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 and is not user adjustable.

C.3 Signal Averaging

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 acquistion 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 (see equation (i) in Appendix II). 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 on earlier 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 skewed and attenuated during the process (see APL technical note).

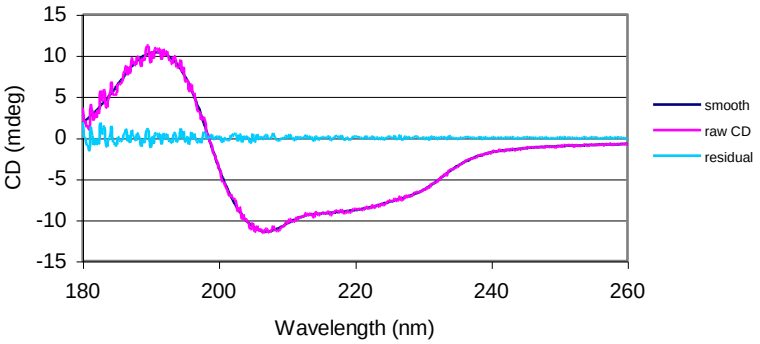
On Chirascan the average sample size is represented by a time-per-point parameter based on the current sampling period (default setting 25μsec). Because the wavelength drive pauses while the samples are collected, the size of the sample is directly responsible for the time spent per point and therefore 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 other online filtering methods.

C.4 Cosmetic smoothing

The acquired spectrum will probably 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 Chirascan 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 below).

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



Appendix D. References

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(2) Drake A.F. 1994. Circular Dichroism in Methods in Molecular Biology 22, p 219.
(3) Velluz L, Legrand M, Grosjean M, 1965. Optical Circular Dichroism, Principles, Measurements and Applications, Academic, New York.
(4) Numerical Recipes in C (second edition), Press W, Teukolsky S, Vetterling W, Flannery B, Cambridge University Press 1992.