AppliedPhotophysics

Ultrasensitive Spectroscopy for the Life Sciences

User Manual
Chirascan CS/LD

Chirascan Series Linear Dichroism Accessory
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This document contains important safety information. Read this document and the Chirascan series User Manual before attempting to install or use the CS/LD accessory. Failure to do so could result in death or serious injury.

USE OF THIS DOCUMENT

This document is intended to inform the operator of Applied Photophysics' CS/LD linear dichroism accessory on its design, installation and operation. The CS/LD is used with either the Chirascan or Chirascan-plus spectrometer and this document should be used in conjunction with the User Manual applicable those instruments. It is assumed that the user of this document is familiar with the operation of the Chirascan or Chirascan-plus, and with Applied Photophysics Pro-Data software. In particular it is assumed that the user is familiar with the hazards associated with the operation of the spectrometer, and has read the safety information contained in its User Manual.

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HAZARD AND OTHER INDICATORS

HAZARD INDICATORS USED IN THIS DOCUMENT

The sign to the left is used to indicate a hazardous situation, which, if not avoided, could result in death or serious injury.

OTHER INFORMATORY INDICATORS USED IN THIS DOCUMENT

The sign to the left is used to indicate a situation which, if not avoided, could result in damage to the instrument.

ESSENTIAL SAFETY INFORMATION

MAKE SURE THAT YOU HAVE READ AND UNDERSTAND ALL THE SAFETY INFORMATION CONTAINED IN THIS DOCUMENT BEFORE ATTEMPTING TO INSTALL OR OPERATE THE LINEAR DICHROISM ACCESSORY. IF YOU HAVE ANY QUESTIONS REGARDING THE OPERATION OF THE ACCESSORY, PLEASE CONTACT APL TECHNICAL SUPPORT SECTION AT THE ADDRESS SHOWN ON THE FIRST PAGE OF THIS DOCUMENT.

OBSERVE ALL SAFETY LABELS AND NEVER ERASE OR REMOVE SAFETY LABELS.

PERFORMANCE OF INSTALLATION, OPERATION OR MAINTENANCE PROCEDURES OTHER THAN THOSE DESCRIBED IN THIS USER MANUAL MAY RESULT IN A HAZARDOUS SITUATION AND WILL VOID THE MANUFACTURERS WARRANTY.

⚠WARNING The Chirascan spectrometer is powered by the mains electricity supply which can produce a shock leading to serious injury or death. Do not connect or disconnect the instrument from the mains supply unless the supply is powered off at source. Ensure all communications and electrical connections are made before powering on the spectrometer. Exercise care during operation and do not operate units with their covers removed. Operate the spectrometer using only the cables provided. Never operate a spectrometer with damaged cables.

⚠WARNING The Linear Dichroism photomultiplier tube (PMT) and Large Area Photodiode (LAAPD) detectors operate at high voltages can produce a shock leading to serious injury or death. Do not connect or disconnect the detector from the spectrometer unless the spectrometer is powered off.

CS/LD INSTALLATION AND OPERATIONAL REQUIREMENTS

Environmental and electrical requirements

The CS/LD has no environmental requirements additional to those of the Chirascan or Chirascan-plus spectrometer. Operation of the CS/LD is controlled by the Chirascan electronics, and there are no additional electrical requirements.

Bench space

The CS/LD has no bench space requirements additional to those of the Chirascan spectrometer, although some users may choose to use an external device to generate flow in the LD, which may add to the bench space requirement.

Nitrogen purge gas

The CS/LD has no purge gas requirements additional to those of the Chirascan spectrometer.

Servicing

Servicing of the CS/LD should only be undertaken by qualified personnel. If you are in any doubt at all please contact the Applied Photophysics Technical Support Department at the address given on the front of this User Manual.

GLOSSARY

The following abbreviations may be found in this User Manual

APL	Applied Photophysics Ltd.
CD	Circular dichroism
LD	Linear dichroism
PMT	Photomultiplier tube

HYPERLINKS

This document contains hyperlinks between references (for example the Contents tables, or references to Sections or Figures in the text), and sources. To follow a link, place the cursor over the reference and use **CTRL+click**. Hyperlinks in the text are indicated by underlined blue font.

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FIGURES

1 INTRODUCTION

Linear dichroism is the difference in the absorbance of parallel and perpendicular linearly polarized light (Equation 1.1). It is usually produced by preferential orientation of macromolecules, for example by stretching along the streamlines in a flow field.

$$\Delta A = A_{||} - A_{\perp} \qquad 1.1$$

where A is the absorbance of the linearly polarised light, and the subscripts $||$ and $\perp$ indicate parallel and perpendicular respectively.

On older Chirascan and Chirascan-plus spectrometers, linear dichroism measurements required a different detector from that used for circular dichroism measurements. On current instruments (April 2011 onwards), the same detector may be used for both, although it needs to be configured and calibrated for each mode. This should be done by a qualified APL engineer.

Figure 1.1: flow cell of the type commonly used with the CS/LD

Sampling handling and orientation are left to the discretion of the user. Commonly a flow through cell such as that shown in Figure 1.1, available from Hellma UK Ltd., is used. The use of a concentric cylinder (Couette) type flow cell is also well established.

For more information on linear dichroism and its measurement, see B. Norden, A. Rodger and T. Dafforn *"Linear Dichroism and Circular Dichroism"*, RSC Publishing, 2010

2 INSTALLATION AND OPERATION

2.1 *Hardware installation*

WARNING The Linear Dichroism photomultiplier tube (PMT) and Large Area Photoediode (LAAPD) detector operate at high voltages can produce a shock leading to serious injury or death. Do not connect or disconnect the detector from the spectrometer unless the spectrometer is powered off.

The detectors currently (April 2011 onwards) supplied with the Chirascan or Chirascan-plus spectrometers can also be used in CD mode, and their installation is described in the main Chirascan User Manual.

2.2 *Configuration and calibration*

The detector needs to configured and calibrated for LD measurements. If the LD option was purchased with the spectrometer, this will have been performed by APL as part of the production process. If the LD option is purchased as an upgrade, then the configuration and calibration will need to be performed by an APL qualified engineer.

NOTICE Incorrect configuration of the detector can cause damage to the instrument or give incorrect results, and LD calibration of the detector requires a special rig. Configuration and calibration should only be performed by a qualified APL engineer.

Note that the calibration factors are specific to a particular detector. Users upgrading from a single mode CD detector should not use the single mode CD detector and the CD/LD detectors interchangeably.

2.3 *Operation*

From the drop-down list on the **Signal** panel on the Chirascan Pro-Data control page, select **Linear Dichroism** (Figure 2.1). From then on, operation of the instrument is similar to that when making CD measurements, for details of which see the main Chirascan series User Manual.

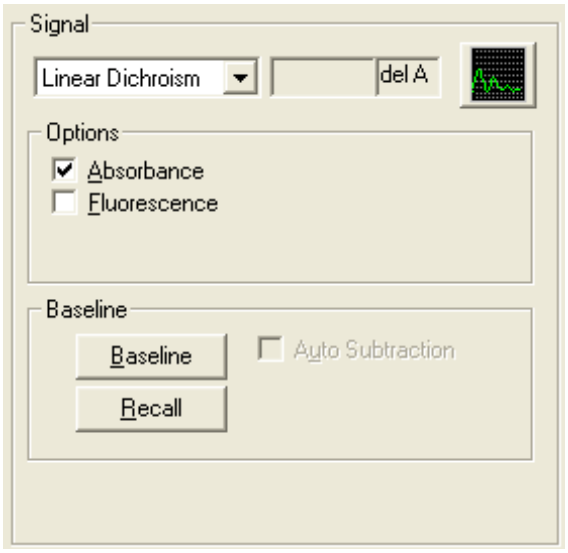

Figure 2.1: the Signal panel

Typically, when making LD measurements, a baseline is taken with no sample present, and LD spectra are then measured with the sample present but no flow, and then at selected flow rates. Background correction is made by subtracting the spectrum measured without flow from those measured with flow.

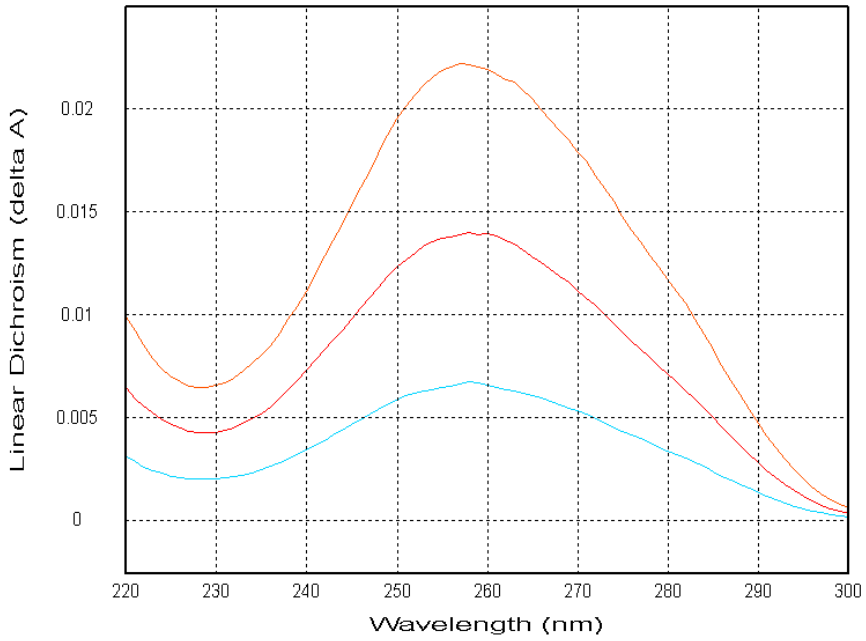

Figure 2.2: LD spectra for calf thymus DNA at three different flow rates

Results for calf thymus DNA at a concentration of 1 mg/ml in phosphate buffered saline are shown in Figure 2.2 at three different pump speeds: 30 ml/min, (orange), 15 ml min (red), and 7.5 ml min (cyan). A 0.5 mm pathlength x 4 mm width flow cell of the type shown in Figure 1.1 was used, with an RH00CTC pump supplied by Fluid-Metering Inc. Syosset, NY, USA .

The spectra have been corrected for background (spectrum with the pump turned off) and a 3-point smooth has been applied to even out the effect of the pulsing pump motion.