Processing CD wavelength data using the AVIV software



1) After data collection, navigate to the Data Review & Wavelength window:

Navigate to the multi-data set view:

🔁 Da	ta Review					-	-		×
File	Configure Experimen	t Displays	Math Operations	Control Panels	Axis Definitions	Help			
2		Data Review Di	splay : Wavelength		Left Axis Defi	nition			
•	biometical, Inc.				Left- Multi-D	ata Set			
E	Experiment Type	_			Right Axis De	finition			
	Wavelength				Right- Multi-	Data Set			
	CD - PMT	1			Clear Left Axi	s Definition			
Si	ignal : 196.91 m deg	-			Clear Right A	xis Definition			
Dyr	node : 402.25 v				Data Review	Average			
PMT	FDC : 1.045 v	1							- 11
EI.	DMT	_			Data Collecti	on Display Se	lection	1	
	uorescence i- wit				Data Collecti	on Average			- 11
Si	gnal: N/A	-			Save Data Op	tions			
Dyr	10de : IN/A				Trace Color C	onfiguration			
		1					_		_
	Monochromator	_						Ļ	
Way	elength : 574.10 nm								
Bar	ndwidth: 1.00 nm	-						+	
S	litwidth : CLOSED							L	
	Sample								
	25.00 deg C	+	1	i	i i	1		t	
DII									
NU								0.55	
Experii	ment is IDLE	Ready					Stirri	ng: OFF	

Select the "CD Signal" scans for averaging, for the sample and the buffer:

🔁 Data Review	v						-		\times
File Confi	gure Experiment	Displays	Math Operations	Control Panels	Axis Definitions	Help			
	Multi-Data Set Sele	ection	enlav : Mavalanath	×					
Experim Wavel CD - Signal : 1	Available Data Sets AC22S 10mMF Scan #1 CD S Scan #1 Error Scan #1 CD D	904 250511 #13 ignal	19	^				-	
Dynode : 4 PMT DC : 1 Fluoresce Signal : 1	Scan #2 CD S Scan #2 Error Scan #2 CD D Scan #3 CD S Scan #3 Error Scan #3 CD D	ignal ynode ignal ynode							
Monocl	Data Review A 10 mMPO4 25 Scan #1 CD S Scan #1 Eror Scan #1 CD D	lverage Ave Re 0511 #138 Ignal ynode	sults	•					
Bandwidth Slitwidth		ок –	Cancel						
Samp 25.00	le deg C	+				-		+	
RUN EXPE	RIMENT								
Experiment is ID	DLE	Ready					Stirr	ing: OFF	

Click "OK". They should now be displayed:



2) These sets of three scans need averaging to produce a single, mean scan for each of the samples, and the buffer. To do this, navigate as follows:



Select the three scans for the sample:

Data Average		-		×
Left Axis Traces □ = AC225 10mMPO4 250511 #139:Scan #1:CD Sig □ = AC225 10mMPO4 250511 #139:Scan #2:CD Sig □ = AC225 10mMPO4 250511 #139:Scan #3:CD Sig ■ 10 mMPO4 250511 #138:Scan #2:CD Signal □ = 10 mMPO4 250511 #138:Scan #2:CD Signal	r Right Axis Traces			
Resulting Data Set Experiment : Data Set :				
Clear Average Trace	Save Average Trace		Return]

Click "Average Selected Traces":

Data Average	- 🗆 X
Left Axis Traces	5
Resulting Data Set	Data Review Average X
Average Selected Traces	Average Complete
Save Average Trace	ОК

Dismiss the notification pop-up by clicking "OK". Click "Save Average Trace". The software will ask if you want to create a new experiment with the same name, but missing the # suffix. Click "Yes":

Data Average	- • ×
Left Avis Traces	Right Axis Traces 13.00 10.60 10.60 5.800 5.800
Resulting Data Set Experiment : AC225_10mMPO4_250511 Data Set : Ave Results Average Selected Traces Clear Average Trace Save	Create Experiment X WARNING- The following experiment does NOT exist : AC22S_10mMP04_250511 Do you want to create this experiment ??
eg C -12.000	Yes No

And dismiss the confirmation window that follows ("OK").

Now, as necessary, deselect the first set of scans and select the next for averaging:

Data Average		-		×
Left Axis Traces	□ Right Axis Traces			
Resulting Data Set Experiment : AC225_10mMPO4_250511 Data Set : Ave Results				
Average Selected Traces Clear Average Trace S	ave Average Trace		Return	

Repeat the averaging and saving as necessary until you have average scans saved for all your experiments.

3) OPTIONAL: If you wish to check these look OK, you can display them in the main window. First, remove the current, non-averaged, scans from the display:

Nota Review						-	×
File Configure Experime	nt Displays	Math Operations	Control Panels	Axis Definitions	Help		
Biomedical, Inc.	Data Review D	isplay : Wavelength		Left Axis Defi Left- Multi-D	nition ata Set		
Experiment Type Wavelength	14.00	A		Right Axis De Right- Multi-	finition Data Set		
CD - PMT	11.40 -			Clear Left Axi	s Definition		
Signal : 193.18 m deg	8.800 -	f		Clear Right A	xis Definitio	n	
Dynode : 407.24 v	6.200	<u> </u>		Data Review	Average		
Fluorescence PMT	3.600 -			Data Collectio Data Collectio	on Display S on Average	election	
Signal : N/A Dynode : N/A	1.000 -			Save Data Op	tions		
	-1.600	restration	Ana				-11
Monochromator Wavelength : 574 10 nm	-4.200 -						
Bandwidth : 1.00 nm	-6.800 -						
Sitwidth : CLOSED	-9.400 -		where the	1			
25.00 deg C	-12.000	5.00 200.00	215.00	230.00	245.00	260.00	
RUN EXPERIMENT							
Experiment is IDLE	Ready					Stirring: OFF	

Then "Select ALL":

Multi-Experiment Selector	\times					
Select Left Axis Traces to Clear						
 AC22S 10mMPO4 250511 #139:Scan #1:CD Signal AC22S 10mMPO4 250511 #139:Scan #2:CD Signal 						
AC22S 10mMPO4 250511 #139:Scan #3:CD Signal						
Image: Second # 1985.5call # 1985.						
⊠ = 10 mMPO4 250511 #138:Scan #3:CD Signal						
Select ALL UN-Select ALL						
Clear Selected Traces Return						
	-					

And "Clear Selected Traces" (this doesn't delete them, just removes them from the display). Return to "Axis Definitions" -> "Left Multi Data Set"...

—			
File Configure Experiment Displays Math Operations Control Panels Axis Definition	ns Help		
Multi-Data Seview Dienlay · Wavelength			
Experim Wave Available Data Sets		Г	
10mMP04 250511 #138 CD Scan #1 CD Signal Scan #1 Eror			
Signal:1 □ Scan #1 CD Dynode Dynode:4 □ Scan #2 CD Signal			
Boon #2 CD Dynode Fluoresce Scan #3 CD Signal Scan #3 CD Signal			
Signal : I ☐ Scan #3 CD Dynode Dynode : I ☐ Data Review Average Ave Results AC225 10m/RPO4 250511			
I Ave Results Monoci 10 mHPO4_250511 I Ave Results			
Vavelength Bandwidth OK Cancel Sittwidth			
Sample			
25.00 deg C	ì		
RUN EXPERIMENT			

Scroll to the bottom of the list to find the new averaged data sets you created:

And check all looks well with the data:



NB: A bug in the software generates an extra white trace that can be safely ignored, or deleted from view by double-clicking on it directly.



4) Now we have to subtract the buffer signal. Navigate as follows:

Click "Select Data Set A" and find your averaged sample scan:

Math Operations	×	Data Browser	×
Data Set A		Multi-Experiment Selection Export Data Set	
Experiment Name: Data Set Name:	Select Data Set A	Wavelength Experiment AC22S_10mMPO4_250511 : Ave Results Options	7
Operation or Constant		Select Luta Set	
Operation Constant		Review Data Set Image: Control 2:00:11 #158 Delete Image: Control 2:00:11 Delete Image: Control 2:00:11	
Data Set B		Rename	
Experiment Name: Data Set Name:	Select Data Set B	Read Data Set <- Disk	
Results			
Experiment Name:	Calculate		
Data Set Name:	Return	Return File Name :	
Sample 11 000	-	Default Dataset Path: \\Mac\Home\Desktop\Biophysics\CD\data\KS Br	rowse

Click "Select Data Set". Find "Subtract Data Sets" in the "Operation" drop-down menu:



"Select Data Set B" (buffer) using the same procedure as for A:

Math Operations	×	Data Browser		×
Data Set A		Multi-Experiment Selection	Export Data Set	
Experiment Name: AC22S_10mMPO4_250511 Data Set Name: Ave Results	Select Data Set A	Wavelength Experiment	10 mMPO4_250511 : Ave Results	1
Operation or Constant		Select Data Set	AC22S 10mMPO4 250511 #139	
Operation Constant Subtract Data Sets		Review Data Set		
		Delete	Ave Results	
Data Set B		Rename		
Experiment Name: Data Set Name:	Select Data Set B	Read Data Set <- Disk		
Results		Save Data Set -> Disk		
Experiment Name: AC22S_10mMPO4_250511	Calculate			
Data Set Name:	Return	Return	File Name :	
Sample 11 000	~	Default Dataset Path: \\Mac	Home \Desktop \Biophysics \CD \data \KS	rowse

"Experiment Name" is automatically taken from A. The "Data Set Name" will be the column heading within the file. Name it something explicit like "minus buffer", and click "Calculate".

Math Operations	×	elp	
Data Set A Experiment Name: AC22S_10mMPO4_250511 Data Set Name: Ave Results	Select Data Set A	Г	arch cds
Operation or Constant Operation Constant Subtract Data Sets Data Set 8 Experiment Name: 10 mMP04_250511 Data Set Name: Ave Results	Select Data Set B	Data Set Operations	AMC File Text Doct Applicatio GID File Help file PDE File X
Results Experiment Name: AC22S_10mMPO4_250511 Data Set Name: minus buffer	Calculate	Calculation Com Data Set saved	plete

The data are saved automatically (dismiss the notification pop-up). Repeat as necessary, changing Data Set A as appropriate for all your samples. When you have subtracted the buffer from all your sample scans, you can dismiss the Math Operations window by clicking "Return", or continue straight to 5).

If you want to check these scans look OK, you can repeat step 3, this time selecting the "minus buffer" scans for display.

5) Almost all published CD wavelength data has been subjected to a

smoothing operation (usually a sliding polynomial) and therefore we recommend you do the same. It doesn't improve the data quality *per se* but does remove visually-distracting high-frequency noise components.

Return to "Math Operations" -> "Wavelength", and select your "minus buffer" data for A:



This time select "Smoothing" from the drop-down menu:

Math Operations	×
│ Data Set A - Experiment Name: AC22S_10mMPO4_250511 Data Set Name: minus buffer	Select Data Set A
Operation or Constant Operation Constant	
Smoothing NO Operation Add Constant Data Add Data Sets Subtract Constant Exp Subtract Data Sets In Multiply Constant Dt Multiply Constant Dt Wute by Constant Dt Work by Constant	Select Data Set B
Divide Data Sets Result Convect by Molar Elipticity Convect to Molar Elipticity Exep Convect to Deta Epsilon Convect Intensity to Abs. De Granothing Derivative	Calculate

For the Data Set Name, choose something explicit such as "minus buffer smooth". I also like to rename the Experiment to create a new one with the suffix "final" so that I end up with a single column of *y* values, which is easier for e.g. importing into Dichroweb or Excel:

Math Operations	~
Math Operations	^
Data Set A]
Experiment Name: AC22S_10mMPO4_250511	Select Data Set A
Data Set Name: minus buffer	
Operation or Constant]
Operation Constant	
Smoothing	
Data Set B]
Experiment Name: 10 mMPO4_250511	
Data Set Name: Ave Results	Select Data Set B
Results]
Experiment Name: AC22S_10mMPO4_250511 final	Calculate
Data Set Name: minus buffer smooth	
,	Return

Clicking "Calculate" will then ask if you wish to create a new experiment. Click "Yes", and a new window describing the smoothing operation will be displayed:

Data Set A Experiment Name: AC22S_10mN	IPO4_250511	Select	: Data Set A
Data Set Name: minus buffer	Smoothing	×	
Operation or Constant Operation Smoothing Data Set B Experiment Name: 10 mMP04_2 Data Set Name: Ave Results	Smoothing Option Manual Smoothing C Automatic Smoothing Degree : 2 Window Width : 11 (must be ODD) Number of data points in Data Set : 76 Save Residuals as a Data Set	othing	: Data Set B
Results Experiment Name: AC22S_10m1 Data Set Name: minus buffer s	Smooth Data Set Cance	4	alculate Retum

The options displayed are the default, and work well in the majority of cases so there is no need to change them. Click "Smooth Data Set" and dismiss the next two pop-ups that have the D_2/S_2 value[§], and the saved notification.

 D_2/S_2 is a measure of the randomness of the residuals and should be close to 2; use "Automatic Smoothing" to optimise the smoothing parameters using this criterion.

Repeat step 3 if you wish to view your final, averaged, buffer-subtracted and smoothed data.

6) Your processed data is NOT SAVED AUTOMATICALLY, but it is easy to do on exiting the software. Go to File -> Terminate CDS Program, and click "OK". A list of your unsaved processed data will appear. Click "Select ALL" and make sure everything you wish to save is highlighted in blue before clicking "Save Selected Experiments":

Multi-Experiment Selector	×
The following experiments contain data NOT saved to the hard drive. Terminating will cause the data to be lost. Select Cancel to prevent terminating.	
10 mMPO4_250511 AC22S_10mMPO4_250511 AC22S_10mMPO4_250511 final	
Data Set Path \\Mac\Home\Desktop\Biophysics\CD\data\KS Brows	se
Select ALL UN-Select ALL Cancel	
Save Selected Experiments Terminate	

Click "OK" when the various notifications appear. All your processed data will now be saved in the same directory as your original experiments (the difference being the raw data has a # suffix). The software will terminate automatically a few seconds after this process has completed.

Further points to note:

- "Math Operations" also has functionality for converting your acquired data in millidegrees (the unit produced by Aviv) to molar ellipticity, and for taking the derivative of thermal melt curves, which is a quick method to estimate T_m.
- To compare CD spectra of different proteins, they are usually normalized to the number of peptide bonds present in each sample (mean residue ellipticity, Θ):

 $\Theta_{mre}(\lambda)$ (usually expressed in deg . cm² . dmol⁻¹) = millidegrees / {(AAs - 1) * concentration (M) * cell pathlength (cm) * 10}

Further fitting and post-processing (including the conversion above) can be carried out using Dichroweb, for which you need to apply for a personal user account:

http://dichroweb.cryst.bbk.ac.uk/html/home.shtml

Katherine Stott 17th October 2017