# 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:

Data Review					- 0
ile Configure Experimen	t Displays	Math Operations	Control Panels	Axis Definitions	Help
Biomedical, Inc.	Data Review Di	splay : Wavelength		Left Axis Def	
				Left- Multi-I	
Experiment Type	7			Right Axis D	
Wavelength				Right- Multi	-Data Set
CD - PMT				Clear Left Av	is Definition
Signal : 196.91 m deg	-			Clear Right A	Axis Definition
Dynode : 402.25 v				Data Review	Average
PMT DC : 1.045 v				Data Collect	ion Display Selection
Fluorescence PMT	÷			Data Collect	
Signal : N/A	_			Save Data O	ations
Dynode : N/A	1				Configuration
	-				
Monochromator	_				
Wavelength : 574.10 nm					
Bandwidth : 1.00 nm	7				teğitetetetetetetet
Slitwidth : CLOSED	4				
Sample					
25.00 deg C	7				1 1
RUN EXPERIMENT					
periment is IDLE	Ready				Stirring: OFF

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

Data Review File Config	r gure Experiment Displays	Math Operations	Control Panels	Axis Definitions Help	×
	Multi-Data Set Selection	Disnlay - Wasslanoth	×		
Experim Wavel	Available Data Sets				Γ
CD -	AC22S 10mMP04 250511 Scan #1 CD Signal Scan #1 Error	#139	<b>^</b>		
Signal : 1 Dynode : 4 PMT DC : 1	Scan #1 CD Dynode Scan #2 CD Signal				
Fluoresce Signal : 1 Dynode : 1	Scan #2 CD Dynode Scan #3 CD Signal Scan #3 Error Scan #3 Error Data Review Average Aver	Rends			·····
Monoci	10 mMPO4 250511 #138 Scan #1 CD Signal Scan #1 Error Scan #1 CD Dynode		- _		
Bandwidth	ок	Cancel			
Sampl	le				
25.00	deg C	1 1	1		
RUN EXPE	RIMENT				
xperiment is ID	LE Ready				Stirring: 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:

Pata Average		-		×
Left Axis Traces	r Right Axis Traces			
Resulting Data Set				
Average Selected Traces	ave Average Trace		Return	

Click "Average Selected Traces":

Data Average		- 0 X
Left Avis Traces	Sigi Sigi	
Resulting Data Set Experiment : AC22S_10mMPO4_250511		Data Review Average
Data Set : Ave Results Average Selected Traces		Average Complete
Clear Average Trace	Save Average Tra	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":

Right Axis Traces
10.60 
Create Experiment
WARNING- The following experiment does NOT exist : AC225_10mMPO4_250511
Do you want to create this experiment ??

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

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

ita Average			-	>
Left Axis Traces	R	ight Axis Traces —		
AC225 10mMPO4 250511 ≠1     AC225 10mMPO4 250511 ≠1     AC225 10mMPO4 250511 ≠1     S = 10 mMPO4 250511 ≠138:5cc     = 10 mMPO4 250511 ≠138:5cc     = 10 mMPO4 250511 ≠138:5cc     = 10 mMPO4 250511 ≠138:5cc	39:Scan #2:CD Sigi 39:Scan #3:CD Sigi an #1:CD Signal an #2:CD Signal			
Resulting Data Set	04.259511			
Experiment : AC22S_10mMP	04_250511			
	04_250511			
Experiment : AC22S_10mMP	04_250511			

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:



### Then "Select ALL":

- circi	Experiment Sele	ctor	2
Selec	ct Left Axis Traces	s 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
⊠-	10 mMPO4 25051	1 #138:Scan #1:CD Signal	
⊠ -	10 mMPO4 25051	1 #138:Scan #2:CD Signal	
⊠-	10 mMPO4 25051	1 #138:Scan #3:CD Signal	
Γ	Select ALL	UN-Select ALL	

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

	Data Review Display - Waveley Multi-Data Set Selection	ath X		
177551 121	Multi-Data Set Selection	~		
Experim	Available Data Sets		a a a	Г
CD - Signal : 1	10 mMPO4 250511 #138 Scan #1 CD Signal Scan #1 Error Scan #1 CD Dynode	^		······
Dynode :4 PMT DC : 1	☐ Scan #2 CD Signal ☐ Scan #2 Error ☐ Scan #2 CD Dynode ☐ Scan #3 CD Signal			
Signal : I Dynode : I	□ Scan #3 Error     □ Scan #3 CD Oynode     □ Data Review Average Ave Results     AC225_10=MPO4_250511	-		
Monoch Vavelength	Ave Results           10 mMP04_250511           Ave Results	-,		
Bandwidth Slitwidth	OKCancel		]	
Samp	le			
	deg C		1 1 1	

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.





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

fath Operations Data Set A	×	Data Browser Multi-Experiment Selection Export Data Set	~
Eperment Name. Data Set Name.	Select Data Set A		_
Operation or Constant Operation Constant Data Set 8 Experiment Name: Data Set Name:		Select Data Set         Image: Control of the select s	
Results Experiment Name Data Set Name	Calorie	Save Data Set -> Dak	
uese per name.	Return	Return File Name :	
Sample 11 000	~	Default Dataset Path: ()Mac (none (Desktop)(Biophysics)(CD)(data)(/S	Browse

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:

fath Operations	×	Compared and the second s	>
Data Set A Experiment Name: AC225_1GmMP04_280511 Data Set Name: Ave Results	Select Data Set A	Multi-Experiment Selection Export Data Set Wavelength Experiment Options	_
Operation or Constant Operation Constant Subtract Data Seta 💌		Select Data Set         III - Ø AC225 10m/MP04 230511 #138           Review Data Set         III - mMP04 230511 #138           Delete         III - mMP04 230511	¢
Data Set II Experiment Name: Data Set Name:	Select Data Set B	Remane Road Data Set <- Disk	
Results Epsement Name: AC225_10mMP04_250511 Data Se Name:	Deserv	Save Data Set -> Dak	
iample	Return	Return Pile Name : Default Dataset Path: \\Mac\#ane\Desktop\Bophysics\CD\data\#S	Brown

"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".

Data Set A	-		
Experiment Name: AC22S_10mMPO4_250511 Data Set Name: Ave Results	Select Data Set A	Г	sarch cds
			Туре
Operation or Constant Operation Constant		_	AMC File Text Doc
Subtract Data Sets			Applicatio
Data Set B		······	GID File Help file
Experiment Name: 10 mMPO4_250511 Data Set Name: Ave Results	Select Data Set B	Data Set Operations	X
Results	_	Calculation Co	
Experiment Name: AC22S_10mMPO4_250511	Calculate	+>	·
Data Set Name: minus buffer	- Return	P	ок

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:

ath Operations		
Data Set A Experiment Name: AC22S_10 Data Set Name: minus buffe		Select Data Set A
Operation or Constant Operation	Constant	
Smoothing	•	6
NO Operation Add Constant Data Add Data Sets Subtract Data Sets Multiply Constant Divide by Constant Divide by Constant Divide by Constant Divide by Constant	511	Select Data Set B
Result Convert Dynode to ABS Convert to Molar Elipticity Exp Convert to Delta Epsilon Convert Intensity to Abs.	04_250511	Calculate
Da Smoothing Derivative		Return

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:

lath Operations	×
Data Set A Experiment Name: AC22S_10mMPO4_2 Data Set Name: minus buffer	50511 Select Data Set A
Operation or Constant Operation Smoothing	Constant
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	Betum

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:

moothing	
ineeuning	×
Smoothing Option       Image: Smoothing in the second	ning
Window Width : 11 (must be ODD) Number of data points in Data Set : 76	. Data Set B
Smooth Data Set Cancel	alculate
	<ul> <li>Manual Smoothing</li> <li>C Automatic Smooth</li> <li>Degree : 2</li> <li>Window Width : 11</li> <li>(must be ODD)</li> <li>Number of data points in Data Set : 76</li> <li>I Save Residuals as a Data Set</li> </ul>

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<sup>§</sup>, 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":

iuit	i-Experiment Selec	tor	×
ſ	The following expe	eriments contain data NO	T saved to the
	hard drive. Terr	ninating will cause the dat	ta to be lost.
	Select C	ancel to prevent terminal	ting.
	10 mMPO4_250511		
	AC22S_10mMPO4_ AC22S_10mMPO4_		
	AC225_10MMPO4_	250511 mnai	
	1		
Da	ta Cet Path		
Da	ta Set Path		
_		Biophysics \CD \data \KS	Browse
_		Biophysics \CD \data \KS	Browse
_		Biophysics \CD \data \KS	Browse
_		Biophysics \CD \data \KS	Browse
_	Mac\Home\Desktop\		Browse
_	Mac\Home\Desktop\g		

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<sub>m</sub>.
- 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 . cm<sup>2</sup> . dmol<sup>-1</sup>) = 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

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