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Host Lab: Hoekstra Lab
Dates of Visit: 5/27/11-8/5/11
Title of Protocol: Spectrometry Measurement and Analysis of Fur Brightness
Purpose of Protocol: In order to measure the "brightness" of coat color in deer mice, we used the Ocean Optics program and a field spectrometer to take measurements on the fur of mice. Although this protocol was specifically developed for deer mice fur, it can be used for almost any field organism with just a few modifications. The protocol below can also be used to quantitatively measure color and other variables as well, but brightness is specifically described here. I would recommend going to the descriptions of the various statistics associated with the CLR program (Step 1 of Analysis instructions) in order to find out where this protocol can be adapted for your needs.

SPECTROMETRY MEASUREMENT PROTOCOL (using the Ocean Optics program)

FIELD INSTRUCTIONS

To start:

1) Turn on computer and start Ocean Optics Program (field spectrometer should already be connected via USB to computer)
2) Check to see that spectrometer is connected (should appear in top left side of program)
3) Open up chosen file directory (whichever folder you wish to save data)
4) Create new folder named for given day of measurements(other organization patterns will also work, but this is the best for using the CLR program later on)
5) Set scans to average to 5
6) Set boxcar width to 5
7) Check spec box to turn on machine (should hear clicking sound)

To take recordings (repeat as needed):

8) Place spec on light blank to calibrate (click white lightbulb)
9) Place spec on black blank to calibrate (click black lightbulb)
10) Hit R button on top menu
11) Test standards again. If they differ by more than ~5% from original value, recalibrate (repeat steps 8-11)
12) **HIT THE PAUSE BUTTON!!!!!**
13) Go to File – Menu – Save Spectrum
14) Once window opens, find appropriate folder for files. Set number of padding digits to 2 (reduce from default of five), and name file RB### (include space at end)

15) Click Accept and place cursor over play/pause button
16) Click once to take each measurement (3 on belly, then 3 on flank, then 3 on back)
17) Check to see that measurements have saved
18) Go to File – Menu – Stop Save
19) Make sure that spectrum has stopped saving (stop button will turn grey), then hit the play button.

To shut down:

20) After hitting play, uncheck the spec box (clicking noise will stop)
21) Transfer files from the spec computer directory onto a flash drive
22) Shut down Ocean Optics program and close the computer

ANALYSIS INSTRUCTIONS

1) Download and install the CLR 1.05 program from
http://post.queensu.ca/~mont/color/analyze.html

2) After reading the "READ ME" file, open CLR.jar. The home page of the program should open with three possible programs to choose from.

3) Choose "CLRfiles". Your raw spec measurements should already be organized into folders by this point, and be in .txt format. Keep in mind that the program won't run if both folders and spec text files are in a single folder together – each folder should either contain other subdirectories (folders) or spec files, never both.

4) Go to EDIT then "Preferences" on the menu. Open a random text file of your spec data. The data marker you enter will tell the program where to start collecting your measurements in the text. Look at the first few digits on your file (i.e. 177) and enter them in (these digits should be the first ones on every spec text file in your directory).

5) Hit "Choose Project" on the CLR program and find your project directory. You can either have the program analyze all the files within a directory, or go through each subdirectory one by one. Depending on which approach you take, you will either choose a directory full of subdirectories only or a subdirectory filled with only raw spec data.

6) A screen will come up asking for your species code. You can enter in anything – initials, etc.

7) A screen will come up asking how your data is organized. Confirm whether you want to analyze an entire project directory or just do one subdirectory at a time by clicking on the appropriate button

8) A screen will come up asking for the lambda values and bin size. Leave bin size at 1. The default lambda values are 300 and 700; for mice, you should change them to 400 and 700.

9) A screen should pop up asking what you want the file saved as. The file will be saved in the folder or folders you chose to analyze as a .clr file. Check the file to make sure that there are no obvious errors (the program should have basically converted each spec data file into one large excel file). If there were more than 250 original files, you will have two output .clr files with an extra number attached to the end of their file name. If you have trouble opening the file with Excel, try opening up Excel first and then using it to open up the .clr file.

10) Hit Home on the CLR files screen. Choose "CLRvars."

11) A screen should pop up asking whether you want to analyze every .clr file in a folder, or just one particular .clr file. Choose whichever one you like. Remember that you might have had more than one output file for folder than had over 250 spec data files in them; in this case you will need to select the "choose a directory option." (I would recommend just using the "choose a directory" option on each of your subdirectories since it will work just as well if there is only 1 .clr file in a folder too and you are guaranteed not to miss any of your files by accident).

12) The .var file will be saved in the project directory with the same name as your .clr files had (if you choose to analyze all files within a directory, the word "ALL" will be attached to the end of the name). Open the file with Excel.

13) You can now start going through and looking for mistakes in the data. Depending on how many replicates of each measurement you took and what animal is used, you could want to use a variety of the stats displayed to clean up your data (all the stats are explained in the "READ ME" file in the CLR directories download).

[For example: With the Hoekstra Nebraska project, I found that looking at the B1 value was the best way to get rid of faulty data. With three measurements, I took the difference between the two closest values; if the third measurement was not within two times this difference from its closest other measurement, it was removed from the data set. A very general estimate for deer mice is that the belly measurements should have a B1 from about 70 to 150, the flank measurements should be between about 40 to 80, and the top (back) measurements should be about 20 – 50]