

# **MEASURING LEAF ABSORPTIVITY WITH THE CI-780 MINIATURE LEAF SPECTROMETER**

PREPARED BY: TIMOTHY M. PEREZ

LASTED EDITED: SEPTEMBER 2<sup>ND</sup>, 2017

## **Setting up the Spectrometer:**

1. First, read the manual associated with the CI-710 spectrometer. It will make following these instructions much easier:  
[https://cidbio.sharepoint.com/sites/Support/\\_layouts/15/WopiFrame.aspx?docid=02aae723b4c324af1923f5870b24ef713&authkey=AYMixXdEC-u9-ywayQbzsJM&action=view](https://cidbio.sharepoint.com/sites/Support/_layouts/15/WopiFrame.aspx?docid=02aae723b4c324af1923f5870b24ef713&authkey=AYMixXdEC-u9-ywayQbzsJM&action=view)
2. Make sure that the CID spectrometer software is properly installed on the computer or device that you'll be using as the power source for the spec.
3. Assemble the spectrometer with care. The fiber optic cables are fragile and should be handled with care. Set up the spec. with the appropriate configuration to measure transmittance.
  - 3.1. The fiber optic cable should be running into the top of the spectrometer's leaf clip.
4. Connect the spectrometer to a computer, open the CID Spectrasnap spec. program, and turn on the switch on the spec. This will turn on the light source.
5. Let it warm up for ~15 minutes, because as the spec heats up, it will influence the readings. This makes a difference in readings.
6. After the spec. has warmed up, press the play button in Spectrasnap. A line should appear on the graph in the program.
7. Be sure to place caps on any fiber optic ports that aren't being used to prevent excess light from influencing any reading.
8. Check that all parts of the line can be seen by going to Set-up>Mode >Scope.
9. Next, go to Set-up>Select integration time>
  - 9.1. There are several options to choose from here, and other options may be best for different purposes, but I have found that an integration time of 350ms is required for both estimations of Transmittance and Reflectance. Select 350ms.
  - 9.2. Choose a boxcar width of 10 (or is it 3 - I'll have to check).

## **Transmittance readings:**

10. Set up the spec to read Transmittance by going to Setup>Mode> and select Transmittance
  - 10.1. Calibrate the Transmittance reading by going to Setup>Calibrate, and follow the instruction on the screen.
  - 10.2. After calibrating, the graph on the spectrometer should read that there is 100% transmittance
11. Before measuring, be sure to save each reading.
  - 11.1. To set up the file where you want to save readings, go to Setup>Advanced> and select the folder/flash drive etc. where you want your files saved.
  - 11.2. Then, go to Setup>Specimen ID to make sure that the program prompts you to name and save each file.
12. Once everything has been appropriately set up, place a leaf adaxial (top) surface facing up, into the leaf clip. Make sure the leaf is entirely covering the the hole on the base of the leaf clip.

- 45 12.1. To ensure accurate readings, make sure you are measuring a part of the leaf that  
46 appears to be representative of the entire leaf (i.e. avoid large veins, and discolored  
47 areas).
- 48 12.2. Wait a few seconds so that the spec. can integrate multiple readings.
- 49 13. Click the save button when ready to record each measurement. Each file name should have  
50 the following format: accession number.leaf number.reading type  
51 Example: If you are saving a transmittance reading from leaf 3 of a plant with the  
52 accession number 34534\*C, the file name should read: 34534\_C.3.trans
- 53 14. After saving the transmittance file, remove the leaf from the leaf clip, and place a new leaf  
54 into the leaf clip.
- 55 15. After all leaves of all plants have transmittance readings, attach the fiber optical cables to the  
56 base of the leaf clip for Reflectance readings.

57  
58 **Reflectance readings:**

- 59 16. Be sure to place caps on any fiber optic ports that aren't being used to prevent excess light  
60 from influencing any reading.
- 61 17. Before taking reflectance readings, go to Setup>Mode> and select Reflectance. The boxcar  
62 width and integration time should remain identical to the settings used for Transmittance.
- 63 18. To calibrate the Reflectance readings, use the reflectance standard and follow the instructions  
64 in the Spectrasnap program.  
65 Note: The white reflectance circle goes face-down. It's also important to not touch this white  
66 circle, and to keep it clean.
- 67 19. After calibrating, insert a leaf into the leaf clip.
- 68 19.1. To ensure accurate readings, make sure you are measuring a part of the leaf that  
69 appears to be representative of the entire leaf (i.e. avoid large veins, and discolored  
70 areas).
- 71 19.2. Make sure the leaf is upside down.
- 72 19.3. Place the reflection standard face-up on top of the leaf to prevent light-loss.
- 73 20. Click the save button when ready to record each measurement. Each file name should have  
74 the following format: accession number.leaf number.reading type  
75 Example: If you are saving a reflectance reading from leaf 3 of a plant with the  
76 accession number 34534\*C, the file name should read: 34534\_C.3.refl

77  
78 **Obtaining Absorptivity:**

- 79 21. Once one transmittance and reflectance have been measured for each leaf, make sure all files  
80 are compiled into one folder. Every time the spec. saves a reading, it saves a "calculations"  
81 file. These are empty and can be deleted.
- 82 22. After deleting all of the "calculations" files all that should remain are 2 files for each leaf (1  
83 reflectance and 1 transmittance). The remaining files should be .csv files with some  
84 information at the top, followed by 3 columns: wavelength, raw spectra, and the  
85 transmittance or reflectance values.
- 86 23. Below is the R script that I have written to process all of the transmittance and reflectance  
87 files within a given folder.
- 88 24. Some important things to consider. For some reason, for any of the "raw" data files are  
89 opened, some internal format of the file is altered and the code below will error. If you have  
90 to open a file for some reason, you have then also have to delete the blank rows in the file. If

91 you have to delete a row in one file, you have to delete empty rows in the corresponding  
92 transmittance/reflectance file for the same leaf. This is why careful attention must be given to  
93 naming and saving files

94 25. After clearing up any of the bugs, the code below will run and the object “zxc” will be a  
95 dataframe contain the absorptivities for each leaf.

96  
97

98 R-script:

```
99
100 setwd("/Users/timothyperrez/Dropbox/CH2/Spec.data/")
101
102 ufn = list.files("/Users/timothyperrez/Dropbox/CH2/Spec.data/6.15/", pattern="*.csv",
103 full.names=T)
104 rfl=trl=list()
105 #ufn
106 for(i in 1:length(ufn)){
107   x=ufn[i]
108   df=read.csv(paste(x), fill=T, header=F, sep=",", row.names = NULL)
109   # extract name of measurement, split name into parts, then recombine name so that each
110   name has 4 parts.
111   split.names=strsplit(as.character(df[2,3]),".", fixed=T)
112   name.df=data.frame(name=apply(data.frame(matrix(unlist(split.names), ncol=4, nrow=1,
113 byrow=T), stringsAsFactors = F),2,function(x)gsub("\\s+", "",x)))
114   ind.name=paste(name.df[1,1],name.df[2,1],name.df[4,1])
115   #extract data, remove "%" sign and divide by 100 to calculate appropriate spec. data. #
116   255:3383 are the rowsdata from 400nm to 1000nm
117   waves=as.numeric(as.character(df[255:3383,1]))
118   par.spec.data=as.numeric(gsub("%", "", df[255:3383,3]))/100
119   #combine names
120   d=data.frame(rep(ind.name, length(par.spec.data)), waves, par.spec.data)
121   names(d)[c(1:3)] = c("ID", paste(df[5,1]), paste(df[4,1]))
122
123   #sort "d" data.frames into lists if they are transmittance or reflectance data
124   if(as.character(factor(paste(df[4,1])))=="Transmittance"){
125     trl[[i]]=d
126   }else{(as.character(factor(paste(df[4,1])))=="Reflectance")
127     rfl[[i]]=d
128   }
129 }
130
131 #trl=Filter(function(x) dim(x)[1] > 0, rfl)
132 df.trl= do.call("rbind", trl)
133 str(trl)
134 #Filter(function(x) dim(x)[1] > 0, rfl)
```

```
135 #rfl[lapply(rfl, function(x) dim(x)[1]) > 0]
136 df.rfl= do.call("rbind", rfl)
137 str(rfl)
138
139 tID=unique(df.trl$ID)
140 rID=unique(df.rfl$ID)
141 #tID=unique(df.trl$Wavelength)
142 #rID=unique(df.rfl$Wavelength)
143
144 setdiff(tID, rID)
145 setdiff(rID,tID)
146 str(df.trl)
147 str(df.rfl)
148
149 abs=merge(df.trl, df.rfl, by=c("ID", "Wavelength"), all=T)
150 str(abs)
151 abs$abs=1-abs$Reflectance-abs$Transmittance
152 max(abs$abs)
153 zxc=aggregate(abs[,3:5], list("ID"=abs$ID), FUN=function(x)mean(x))
154
```