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Fluorometry Protocol

Getting Started

1. Open HP palmtop
2. Make sure all necessary cables are attached to PAM:
    a. Fiber Optics
    b. RS232 (to both PAM and Palmtop)
    c. Ext. DC
    d. Leaf clip
3. Turn on PAM-2000. A green light will flash regularly when on.
4. On Palmtop, hit On.
5. At the DOS prompt, type: DA-2000 <ENTER>
6. You are now in the program.
7. If you see graph axes rather than normal screen (filled with boxes), hit <N> to return to the normal screen. If this does not work, you are probably in the wrong mode: hit <ALT-I> to get the change mode dialog box, and then choose Sat. pulse Mode by either scrolling down and hitting <ENTER> or by hitting <S>.

Taking Measurements

The first measurement that should be done is the dark measurement (Run 2); this is also the preferred measurement. If there is only one leaf, the dark measurement (Run 2) must be done, not run 3! If there are no rosette leaves large enough to use, choose cauline leaves and make a note of this on in the comments column on the observation sheet. If there are no leaves anywhere on the plant large enough to use, write leaves too small in the comments column on the observation sheet. Leaves are only considered too small to use if no portion of them can be captured in the perimeter of the clip. A chosen leaf does not have to fill the clip. The same leaf may be used for both the dark and light portions of Run , however the leaf must sit for 5 minutes in the light if it is to be used for the light measurement and has already had the dark measurement done.

1. As plants come over from IRGA they should have a dark clip placed on one of their leaves so that they will be ready to go when you are ready for them. The dark clip must stay on the leaf for a minimum of five minutes for a proper measurement. The leaf should fill the entire area of clip, but if it does not, measurements will still be valid. Center the leaf to be measured in the leaf clip, gently capturing part of the stem if necessary. If the leaf does not fill the clip, make a note of it in the comment column on the fluorometer observation sheet.
2. Once the five minutes are up, FIRST insert the fiber optic cable into the dark clip and SECOND open the shutter on the dark clip
3. On the palmtop, you should be in the normal screen, the one with the boxes. At the Run Box select run 2 by either pressing the <-> or <+> buttons (To get to the Run box, use the arrow up or arrow down keys. The box the cursor is in will have a dashed line instead of a solid line). Once run 2 has been selected, hit <I> to initialize.
4. Then hit <CTRL-R> then <ENTER> if you are in the Run box.
5. If the palmtop beeps, the light intensity is too high. Go to box 1 and hit <-> to lower the light intensity. Hit CTRL-R again; do not initialize first.

6. Replace the light source in the leaf clip, the upper rim of the light source should be even with the second to last black line. If the leaf does not fill the clip, lower the light source in the source holder until it is as close as possible to the leaf. Select a second leaf and hit <Y>.

Once that is done, hit <CTRL-E> and write pot #### at the beginning of the data for that plant. Only write the pot number once, at the beginning of the data for that pot, do not write the pot number anywhere else. Otherwise the person cleaning up the data will get very angry.

8. If for any reason, you ever need to stop a run, press <ALT> and <MENU>, that should break into the run.

Saving at the End of the Day

1. When all the plants are done for the day, save the file:
    a. Exit the program using <ALT-X> and get to the root directory of the C drive by typing "C:" and hitting <ENTER> at the dos prompt.
    b. Type "dir" and <ENTER>. You should find a file named 'STANDARD.RPT' which will be fairly large. If you don't see it, you are probably in the wrong directory.
    c. At the DOS prompt for the C drive. Type "rename standard.rpt XXXX.rpt" where XXXX is a unique identifier of the plants sampled such as NLL213.
    d. Double check to make sure the file is there: Type "dir" and <ENTER>: you should see the file you renamed in the C root directory. Standard.rpt will now be missing but the program will make a new standard.rpt the next time it starts up.
2. At the DOS prompt, type "RPT2WKS" followed by a space and the filename you just saved the day's work under. This will convert the RPT file to a file more easily read by Excel.

Transferring Data from the Palmtop to the PC

1. Remove the HP 200 from its Velcro connection on the top of the PAM 2000 and unplug the RS232 cable from the HP 200.
2. Unplug the power supply cable from the outlet and take the HP 200 and the power cable to the PC.
3. Plug the HP 200 power supply to the electrical outlet and connect the cord from the PC to the HP's RS 232 slot.
4. Open the HP 200 and turn it on.
5. Type 200 at the c:/ prompt (or hit <ALT-X> to escape the program if still in the DA-2000 program).
6. Press the greenish-blue button that has the file folder on it. This starts the Filer application.
7. On the PC go to the Start Menu then All Programs, then Transfile Win 200, and finally, Transfile Win 200.
8. Once in the Transfile program there will be pull down menu that says HP Palmtop. From that menu select Connect to HP. The computer will now be linked to the Palmtop and the Palmtop will only acknowledge commands from the PC.

9. The left side of the Transfile program shows files on the PC and the right side shows files on the c: drive of the HP.
10. On the PC side, you should be in the E drive. If you are not already in the DEVSTA~2 <DIR> folder then double-click on it under the E drive. The destination location is therefore the Dev Stability folder in the E drive of the PC.
11. Select the file you wish to move from the C drive of the palmtop and go to the pull down menu that says “File” Under File is the command for move, select that. You do not want to copy the files, you want to move them completely off the Palmtop.
12. A dialog box will ask you if it is ok to delete the file from the source directory. Click Ok.
13. The file will be moved and you will be asked again if it is ok to delete the original file in the source directory. Click yes again.
14. The file should then appear on the left side of the Transfile application and shouldn’t be anywhere on the right side of the application.
15. Move as many files as necessary.
16. Once you are done moving files, select the pull down menu HP Palmtop and then select Disconnect from HP. The connection between the HP Palmtop and the PC will now be broken.
17. On the PC, close the Transfile application and open Excel. Open the files that you have moved in Excel and check that everything looks normal.
18. On the HP, exit Filer by using <ALT> then <F> or the E<X>it. Follow that by pressing the blue-green “&…” key, then <ALT> the <A>pplications, the <T>erminate All. Hit <ENTER> to accept the dialog box, and you should shortly see a dos prompt.
19. Turn off the HP 200 and plug it back on the PAM 2000.

THE FOLLOWING PROCEDURE WAS TERMINATED PART WAY THROUGH RUN 2 OF DEVELOPMENTAL STABILITY, DUE TO THE EXPENDITURE OF TIME AND DEARTH OF DATA WHICH RESULTS FROM THIS RUN.

9. Hit <ESC> and then <Y> to save.
10. Select another leaf that is big enough to fill the leaf clip and is relatively healthy looking. Place it in the leaf clip carefully and make sure that the leaf clip is properly balanced and will stay balanced for 5 minutes, without ripping the leaf off of the plant. If the leaf is ripped off of the plant, take the measurements anyway.
11. In the normal screen go to the Run box and hit <+> to increase the Run number from 2 to 3.
12. Hit <I> to initialize.
13. The light source should be LED instead of halogen. Box H in the middle of the normal screen is the box that lets you know which light is selected. Hit <H> to switch from halogen to LED if it is not already selected. This only has to be done once at the beginning, after that, unless you change it, the light type will not change.
14. After you have initialized hit <CTRL-S> to turn on the Far Red light. You will know that it is on when there is an asterisk in the Far Red box.
15. Hit <CTRL-R> and <ENTER> to start Run 3. This run will take 5 minutes and 20 seconds.
16. Once it is done hit <CTRL-E> to enter into the report screen. At the start of the data measured for Run 3 write pot ####, once again only at the start of the data.
17. Hit <ESC> and then <Y> to save changes.
18. You will end up back in the graph screen. To go from the graph screen to the normal screen, the one with the boxes, press <N>.
19. If for any reason, you ever need to stop a run, press <ALT> and <MENU>, that should break into the run.

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