

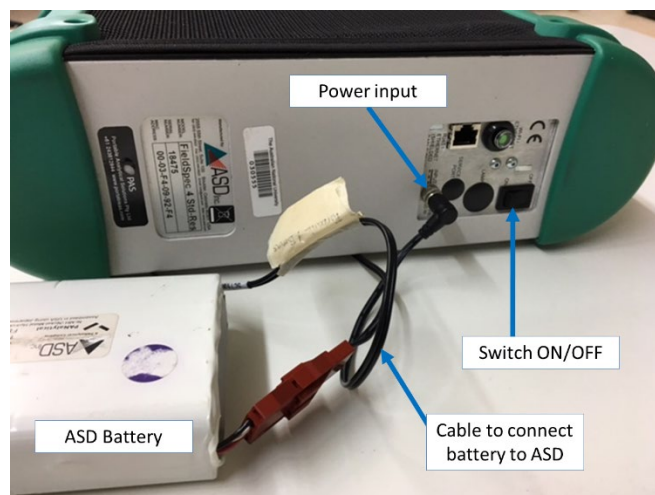
Protocol to measure wheat leaves using a hyperspectral radiometer

ASD Fieldspec3 + Leaf-clip to predict leaf nitrogen, leaf thickness,

SPAD, Rubisco activity and electron transport rate.

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1. Make sure to plug the ASD Field Spec (I call it ASD) to a power source: connected to an electric power point or a battery (be sure that ASD batteries and laptop batteries are charged).

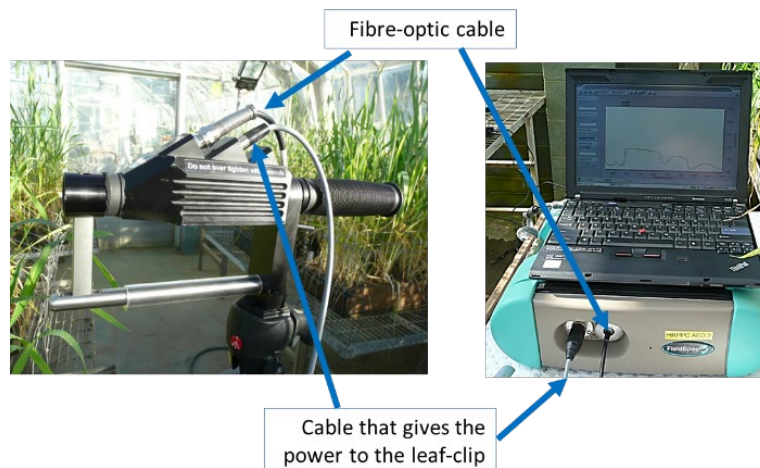


2. Turn ON the ASD and let warm up for 10 min, during this time you can continue the next step.
3. Put the mask in the leaf-clip of the ASD FieldSpec.
 - 3.1. Remove the default O-ring from the leaf-clip and keep it in a safe place. Place the mask in the leaf-clip, the oval should be looking down (see picture below, ①).
 - 3.2. The screw of the mask should match a screw that is on the side where the O-ring was removed before (see picture below, ②).
 - 3.3. Tight the screw with an Allen (hex) key to avoid the mask moves during the measurements (see picture below, ③).



Ask Viri if you are unsure about placing correctly the mask, since it is critical to obtain good predictions from the spectra.

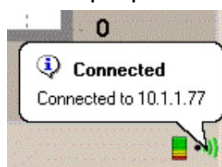
4. Connect the cable that gives the power to the leaf-clip to the ASD box. Then, connect the fibre-optic cable in the leaf-clip, tight well the fibre-optic cable to avoid it moves during measurements but not too tight since the fibre-optic cable is fragile and very expensive.



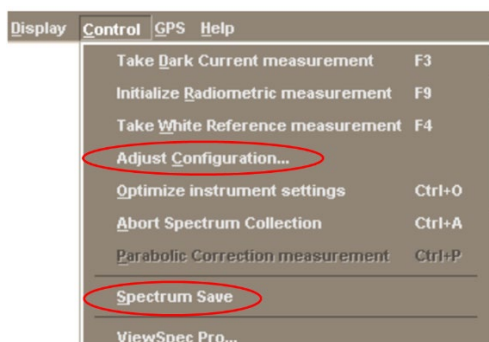
5. Turn on the laptop, give time to the computer to start.
6. Create a folder using Windows explorer to save the spectra. For example: C:/ASD/Viri/ create a sub folder (YYMMDD_ your name)
7. Open the program.



8. Wait the laptop connect with the ASD Box.



9. Select the parameters to use, for that go to "Control":

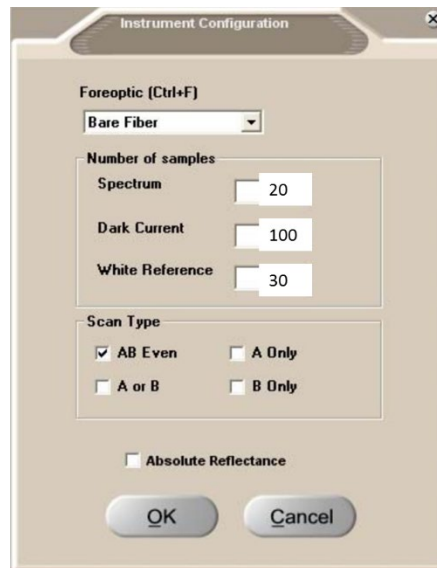


- 9.1. Adjust Configuration
 - 9.1.1. Spectrum (set 20)
 - 9.1.2. Dark Current (Default, 100)
 - 9.1.3. White reference (set 30)

9.1.4.AB Even

9.1.5.OK

You can decide the resolution/time of the measurements, if you set 'Spectrum' to 20, it will last 2 seconds to record the measurement, if you set to 100 it will last 10 seconds, etc.



9.2. Spectrum save have these options:

9.2.1.Path name: Path of the folder where the data will be saved.

9.2.2.Base name: If you want to include a base name in the files. In the example was written 'spectrum'

9.2.3.Starting Spectrum Number: You can modify the number when the first measurement is taken. Usually I start in 0

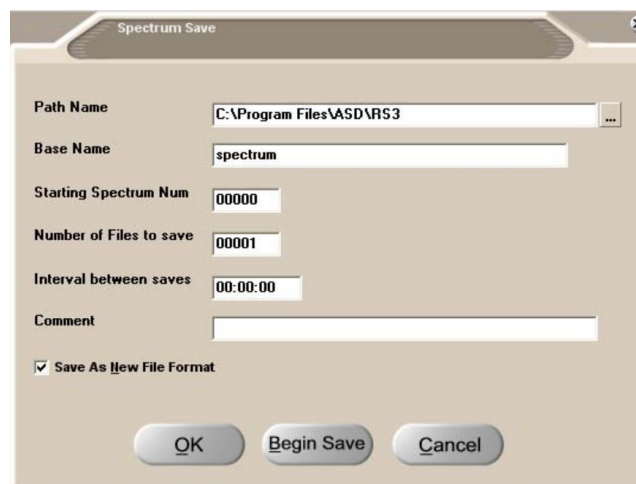
9.2.4.Number of files to save: Usually I use 1

9.2.5.Interval between saves: You can control autosavings by time. In this case 0

9.2.6.Comment: To include some comments like trial name

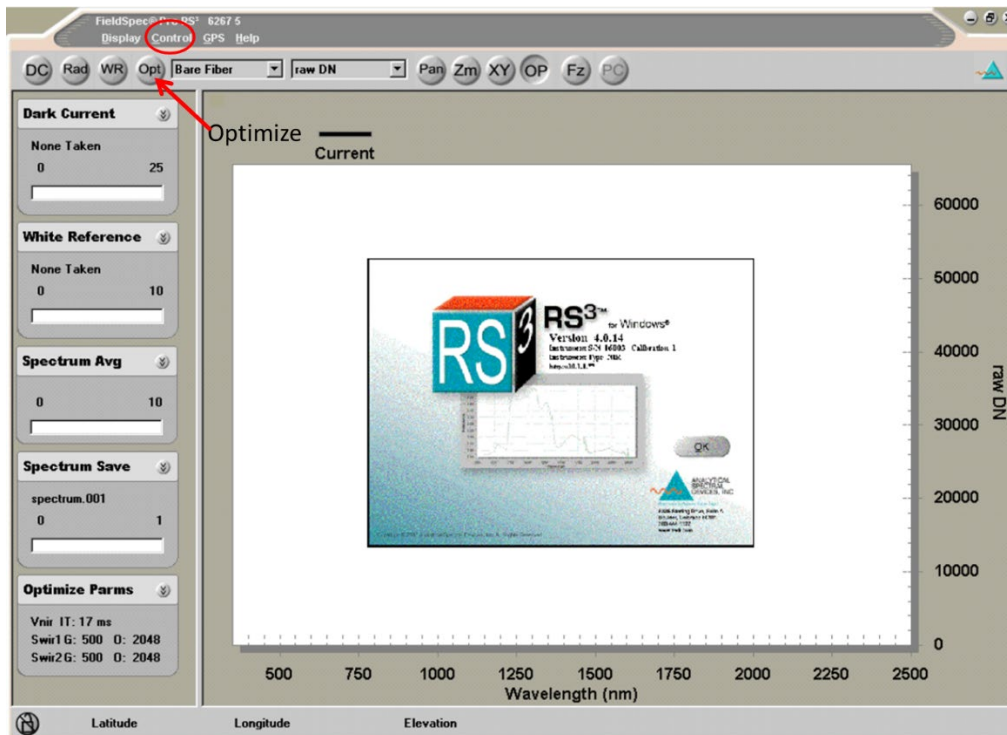
9.2.7.Save as new file format (tick)

9.2.8.OK



10. Close the leaf-clip with the white background looking the internal lamp, and turn ON the leaf-clip. A loud noise might be heard after each reading, to turn the sound off, to silence, it is necessary to optimize the internal light using the virtual button on the

screen “Opt” or Ctrl+O with the keyboard. Save the plot shown in the optimization pushing the space bar of the laptop.



11. Still using the white background, calibrate the white reference selecting the virtual button on the screen “WR” or use F4 from the keyboard. After that, it will start to run the calibration for Dark Current, White Reference and it will start to detect the reflectance, this can be seen in the Spectrum line. It will plot reflectance in y scale =1 with a horizontal line that will indicate that the calibration is done. Save the plot shown in the calibration pushing the space bar of the laptop.
12. Just before start measuring, put the leaf-clip in de direction that will be used during the measurements (like in the picture) and close the leaf-clip (no leaf inside) and repeat the white calibration (step 11). It is important to calibrate constantly, mainly when moving from position. In the field, calibration is good each 8-10 plots. To check it, close the leaf clip with the white background (no leaf inside), the line displayed should be flat (y scale=1), if not you need to calibrate again pressing F4.

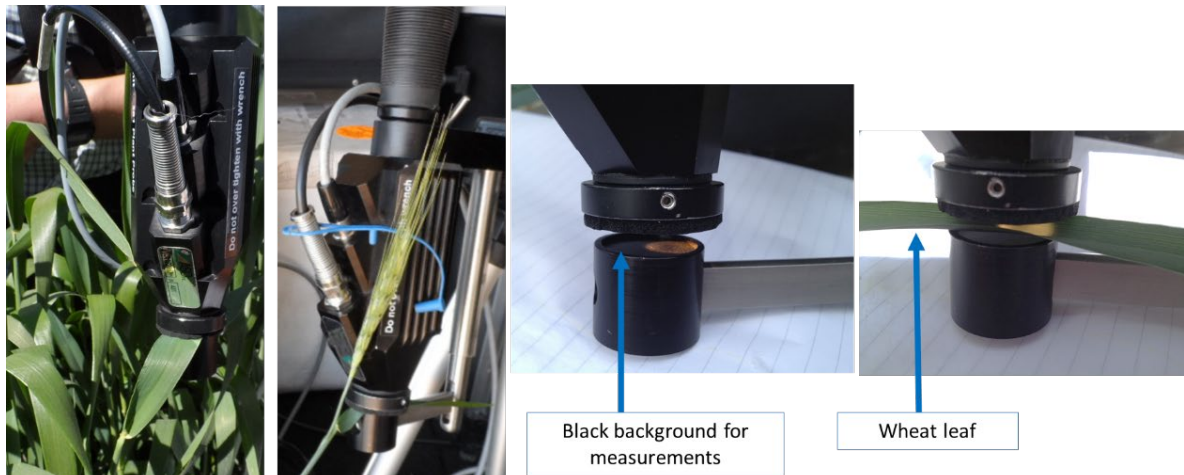


Position to measure wheat leaves

13. I recommend to save the white reference each time that is done, it can be each 10 plots or at the end of the row in the field. The current number, in this case: spectrum.001 is the number that will be recorded. Press the space bar of the lap-top to save the spectrum 001. You can move the leaf-clip after listen the sound of the laptop.



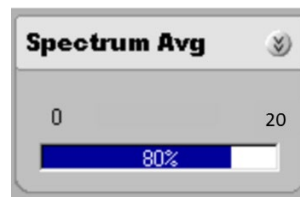
14. To measure reflectance, use the black background looking the internal lamp (if the white is used, the measurement is wrong!!), clip the leaf and measure as shown in the picture. The wheat leaf should be aligned with the longest part of the mask oval.



Position to measure wheat leaves

15. Save two completed bars of average spectrum (100%). The bar is moving constantly, if you clip the leaf when (1) the bar was at 80% as shown in the picture, wait to be finished, (2) wait for the next bar to be 100%, (3) when the next bar start running press the space bar of the laptop to save the spectrum. The spectrum will be saved after the (3) is completed (100%) and you will listen a sound. Don't move the leaf-clip from the leaf during this process (this process will take max 6 seconds). You can remove the leaf-

clip after listen the sound of the laptop. The number that need to be written down with



the plant/pot/plot number (ID) will be 002.

- 16.** The number of spectrum (spectrum.003) will change after save the one before (002). Therefore now, it will be measuring the next spectrum (003), so you can change of leaf and repeat point 15 and 16 for next measurement.
- 17.** Close the program RS3 when finish.