

Operating instructions for the portable low temperature spectrofluorometer AVANTES

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1 Contents of the package

1.1 Light source



1.2 Black Tower (pre-packed with 3 optical filters in the input port, 1 optical filter in the output port, Dewar bottle inside and sample holder with lid)



1.3 Spectrometer Avantes USB2000 (with optical fiber and USB cable BEWARE: The light guide (black) in the envelope is easy to break – do not bend it too much!)



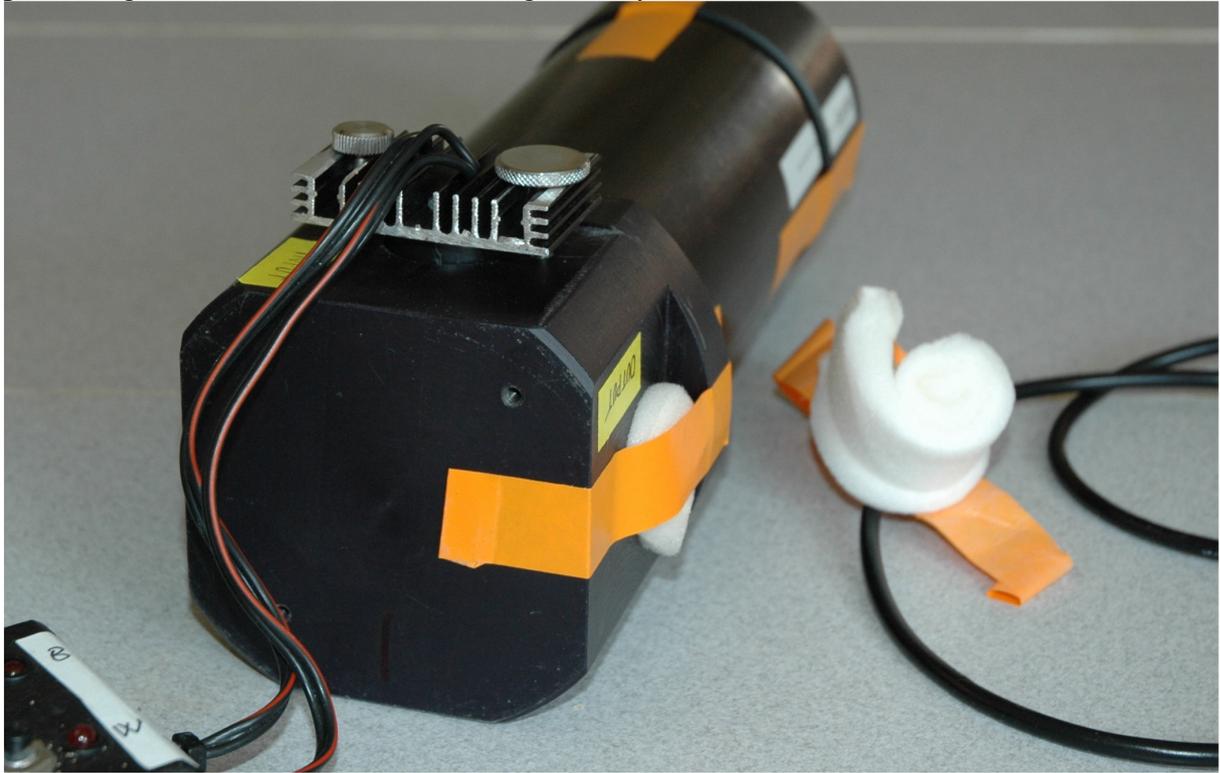
1.4 Cutting tool



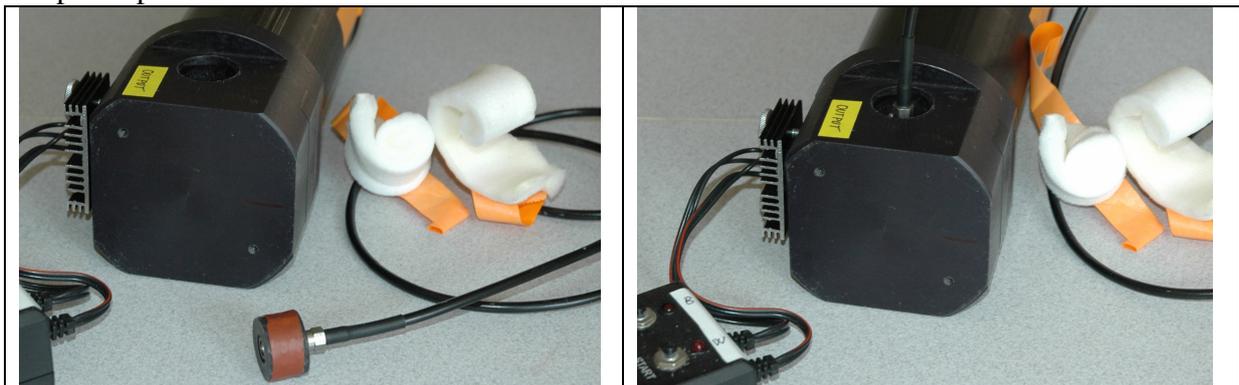
1.5 **Not packed, but necessary:**
Sharp tweezers
Hot air gun (or hair dryer)
Cutting plate (wooden or plastic)

2 Assembly of the instrument

After unpacking, lay down the Black Tower so that the input port is on the top. Remove the foamy filter holder and screw in the Light Source. The light source should be screwed in-place with power but carefully, because the LED diodes or the glassy light filters (which are pressed against each-other) can be damaged easily.



Turn the Black Tower CCW so that the Output port is on the top. Remove the foamy filter holder and plug the thick end of optical cable to the port and screw the other end to spectrophotometer.



- c) Stand-up the Black Tower, open the top lid, remove the foam rubber and napkin spacers from within, plug the light source socket plug to 230V/50Hz, connect the Spectrophotometer to USB port and the instrument is “ready 2 go”.



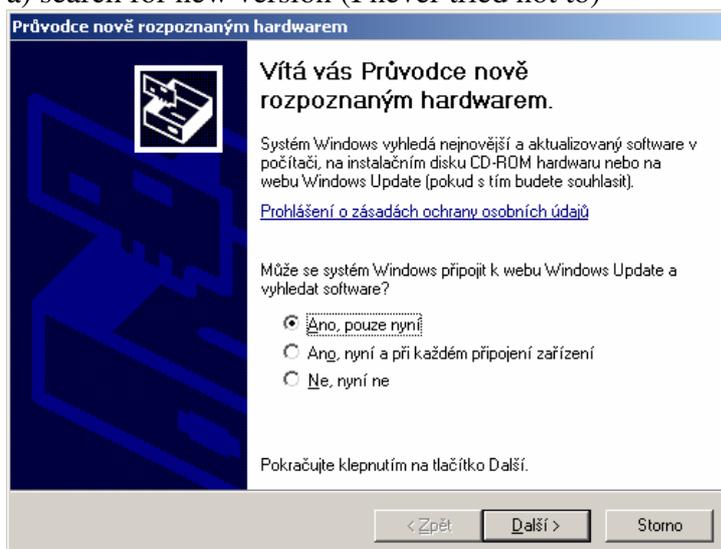
3 - HW and SW installation to PC

We have tested this only on Windows XP prof. (SP2)

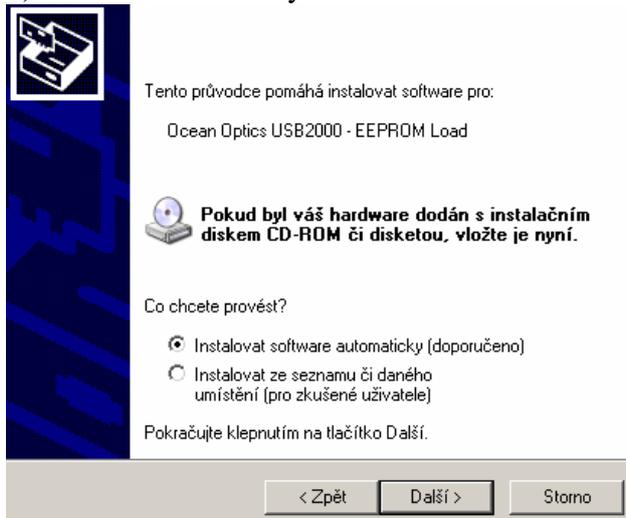
- 3.1 First install the SW. Run Setup.exe from AvaSoft_5 directory, then click Next, Next, OK, Next, Next, Next, OK and Finish. Once you have the SW installed, you can attach the Spectrophotometer.

- 3.2 Plug-in the USB cable and pray. While praying, go through the “New Hardware found” dialog as follows:

a) search for new version (I never tried not to)



b) install automatically



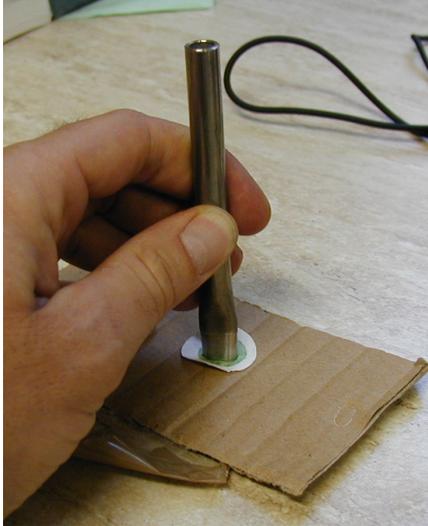
c) it does not have XP logo certificate, install anyway



d) and finally click Finish. If some steps are missing/different, just follow your common installation routine.

4 Sample preparation

Prepare the sample: filter enough seawater so that your glass fiber filter is slightly green.
Use the supplied cutter to cut out piece of filter of the required size.



Using any long object (e.g. wooden skewer), remove the cut filter and using the forceps place it into the copper filter holder. You can slightly wet the surface of the holder for better adhesion.

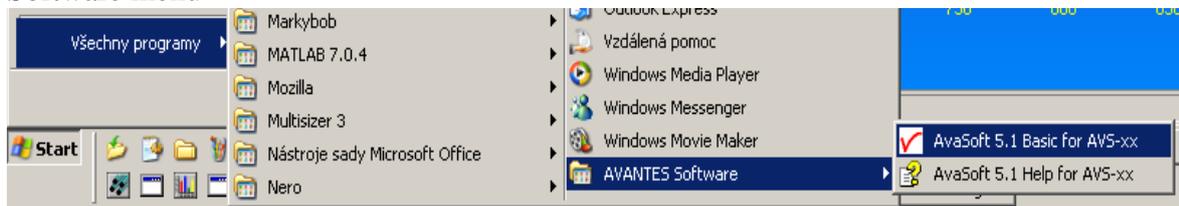


Fill the Dewar with liquid nitrogen. Insert the Dewar into the black plastic holder so that it fits into the cutting.

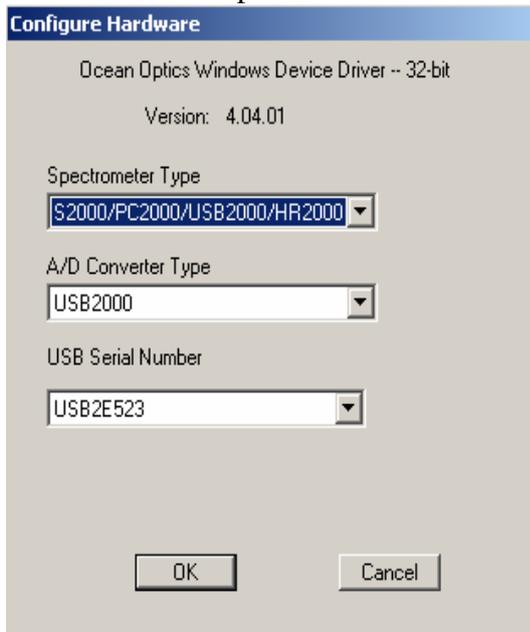
Insert the holder into the Dewar with liquid nitrogen - let it cool gradually. Make sure the sample is attached to the holder. Position the mark "SAM" on the upper holder against the similar mark (black line on white sticker) on the lower part of the cylinder - this will assure the proper orientation of the sample against the excitation and the detector.

5 - Setup and Dark measurement

5.1 Start the spectrometer software by clicking on the AvaSoft5.1 icon in the AVANTES Software menu

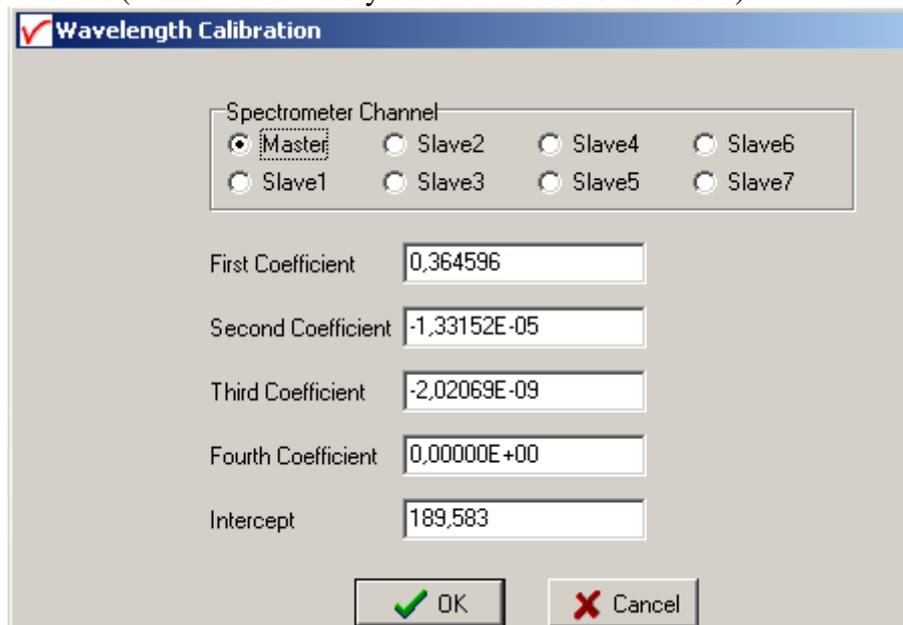


5.2 From the Setup menu choose Hardware and set the following 3 parameters:



5.3 Set-up the calibration coefficients

In the menu Setup/Wavelength Calibration Coefficients input the coefficients obtained from Avantes (we do not have any data for Fourth Coefficient)



5.4 Select the option “Change the graph scale” from the View menu and set the desired wavelength range (measured range is 354-922nm).

5.5 Make sure that the Black Tower is properly assembled (Led diodes plug and 3 filters in the import port, 1 filter and light harvesting plug in the output port, dewar bottle with sample holder in place and the Top lid is closed).

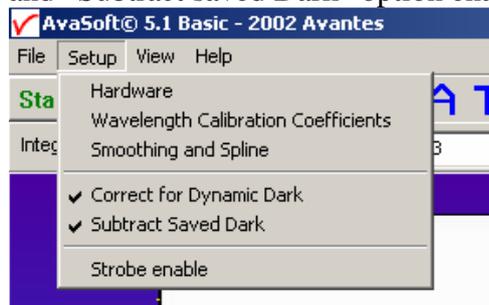
5.6 Adjust the sensitivity of the detector. Integration time is directly proportional to the signal intensity and Average sets the number of averaged measurements. In oligotrophic areas, with weak signal you can use up to 5000 ms (5sec) integration time and averaging (for example 3). This means that one experiment will take 15sec!!! During this time you will not see any changes on the screen. Zoom the graph to the desired region (see previous step or with mouse) and set the integration time to make the signal high enough, but not too high to overflow the detector (~3700 counts).



THIS STEP DECIDES ON THE QUALITY OF SPECTRA! SO TRY TO USE AS MUCH OF THE DYNAMIC RANGE OF THE DETECTOR (0-3700 COUNTS) AS POSSIBLE- EXPERIMENT WITH THE INTEGRATION TIME TO GET THE BEST SIGNAL. TOO SHORT TIME MEANS NOISY SIGNAL, TOO LONG CAN OVERSATURATE THE DYNAMIC RANGE. (Of course, you need to have the excitation LEDs on and sample in place when adjusting the sensitivity = see the next paragraph).

5.7 Now switch on the LED source, both W(white) and B(blue) buttons and see how the signal looks like. You should get peaks in the spectrum in the range 680-720 nm, if phycobilins then peak between 600-660nm. The peak around 560 nm is an artifact coming from the excitation source. **BE CAREFUL – DO NOT LEAVE THE LED ON LONGER THAN NECESSARY FOR MEASUREMENTS – THE POWER RESISTOR GETS VERY HOT AFTER PROLONGED USE AND MIGHT BE DANGEROUS OR BREAK DOWN. FORTUNATELY THERE IS THE AUTOMATIC FUSE THAT SWITCHES LEDS OFF AFTER ~5MINUTES.**

5.8 Always before sample measurement (or at least once or two times a day or whenever you change the integration time or average) perform Dark measurement. Measure with Lights off and save the measurement by clicking on the black square (or in the menu File/Save/Dark). When measuring the samples, always have the “Correct for dynamic dark” and “Subtract saved Dark” option enabled.



Last, but not least, the software has very good Help.

6 Routine measurements

6.1 Once all parameters are tuned-up (selection of the integration time, number of averages, dark signal has been saved for reference), start and save new experiment (from the File menu). At this time, you can provide short name for the experiment (e.g. CTD001) – the software will then number the measurements automatically (CTD0010001.ROH etc.). Each graph is stored in separate file with comments - these are important to know later what is what.

6.2 You can run the spectrometer in continuous mode (press Start only once for each experiment). If the spectra looks OK for you, you can save them just by clicking on the save icon on the menu bar.

6.3 **BLANK SAMPLE** - from time to time (once or two times a day or whenever you change the integration time) it is necessary to record the background signal. This is done by taking a blank sample - wet GF filter (with no sample filtered) and place it into the holder and then record and STORE the spectra in the same way (the same integration time etc.) exactly like the real sample. This signal will then be subtracted from the real samples – **SO IT IS IMPORTANT TO MEASURE AND STORE THE BLANKS!!**.

6.4 Once you finish the measurement, then you can take out the sample holder from the liquid nitrogen and either leave it to warm up in the room temperature or (if you want to do next measurements right away) you can use the hair dryer and warm the tip of the copper sample holder. If you do not plan another measurements, pour out the liquid nitrogen from the Dewar and let it dry out completely. It is important to **start with the dry Dewar** - the ice or humidity deteriorates the measurements significantly. Clean the dust or other debris from inside the Dewar tip by rinsing with ethanol and using cotton swabs. The cleaning should be done only on dry Dewar at room temperature, then allow the ethanol to evaporate fully.