Generation of Correction Factors for Fluorolog Fluorimeter

Correction factors must be generated once a year

Guidelines: Slits: 1.5 nm (bandpass)

Personal Protection Equipment

When handling the lamp: Safety Glasses, Cloth Gloves

When turning the lamp on wear darkened safety glasses or sunglasses and do not look directly at the lamp.

Hazards

Aside from electric shock, the main risks here are associated with the lamp; it is under pressure, so it might explode – handle it carefully and wearing cloth gloves! Also, it is *really* bright, so don't look directly at it or your vision might be temporally impaired.

Equipment: Electrical Tape, Screwdriver, Cloth Gloves, Allen wrench set, Scattering Unit, Standard Lamp and Holder, Light Screen Blocker, Red Black and Brown Wires, Lamp Power Supply.

Setting Up

1. Use electrical tape to fasten the lamp-holder to side of the fluorimeter, such that it is centered over the sample chamber.
2. Remove the lid from the sample chamber.
3. Remove the cell holder from the sample chamber and install the scattering unit (no screws necessary). Orient the scattering unit such that the light coming from the lamp will reflect off the white surface, pass through the grate, and then proceed to the detector. See Figure 1.

Lamp (above, not pictured)

To detector

Figure 1

4. Place the screen/light blocker over the port and make sure it is centered. Use electrical tape to hold it in place.
5. Remove the screws on the lamp holder. ***While wearing cloth gloves,*** remove the standard lamp from its storage and ***gently*** place the lamp so the silver wires are underneath the screws on the holder. Tighten the screws onto the lamp. Your set-up should look like Figure 2.
6. Connect the black and red wires to the lamp but NOT TO THE POWER SUPPLY.
7. While the power supply is off, make sure no wires are plugged in and begin to zero the power supply by turning the voltage knob all the way counterclockwise and the current knob all the way clockwise (the knob will not rotate any further than zero).
8. Turn on the power supply and adjust the voltage to 30.7, then turn off the power supply.
9. Attach the short brown wire (short circuit) to both terminals on the back of the power supply, and then turn it on again. ***Without changing the voltage***, adjust the current to 6.50, and then turn off the power supply.
10. Wait five minutes to allow the current to dissipate, then remove the
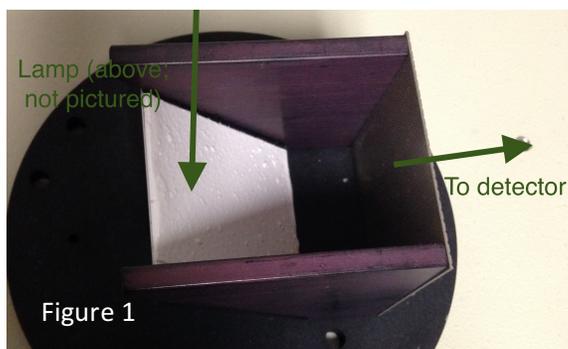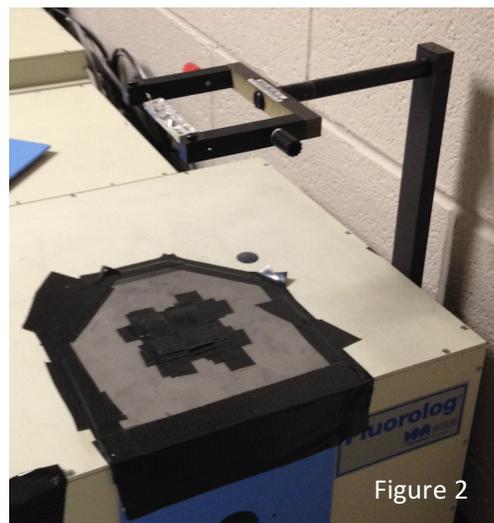
Figure 2

brown cord and then attach the wires which are already attached to the lamp to the power supply (red to red and black to black)

11. Turn on the lamp (avoid looking at the lamp, it is really bright) and let it stabilize for a few minutes. Adjust the voltage so that *current* is maintained at 6.50.

12. The set-up is complete and the fluorimeter is now prepared to take the necessary spectra.

Acquiring Spectra

1. On the fluorimeter computer, go to the "C:\UserData\EMCorrection" directory and create a new folder that indicates the date of the correction using the format DDmonYY.

2. Using the interface software, prepare to collect a full emission spectrum of the lamp from 350-900 nm with a 2 nm increment. Initially set the integration time to half a second. The slit widths for emission should be left open at 1.5 nm for both entrance and exit slits. The file should be named lampfull.spc and it should be recorded in your new folder.

3. While making sure the current is maintained at 6.50, initiate and observe the progress of the scan. The scan should ideally have a maximum signal of at least 500,000 but no greater than 800,000. Scans that do not fall in the above parameter should be aborted.

a. To lower the intensity: close the hole with electrical tape. To increase intensity: make the hole larger by removing electrical tape.

b. Make sure the signal intensity does not hit 1,000,000 counts per second. If it does you will fry the detector. If the signal goes over 800,000 cps, abort the scan.

4. If the scan was aborted because it did not meet the above requirements re-run and overwrite the previous file.

5. Once the signal is within the desired parameters, reset the integration time to 2.0 seconds, and retake the spectrum once again. This will take approximately 10 minutes. This final file should be "lampfull" (i.e., overwrite the one with 0.5 s integration time).

6. Turn off the standard lamp. Do not touch the lamp immediately because it is extremely hot.

7. Using the same settings and instrumental setup as were used for the final "lampfull.spc" spectra, take a dark spectral scan. This means that it will be the same as step 5, but with the lamp off. The file should be named "darkcnt.spc"

8. The spectra collection is now complete! Be sure the lamp has cooled for about an hour before touching and packaging it. Remember to wear cotton gloves while handling it.

Creating Correction Factors File

1. In the interface program, load the "lampfulla.spc" signal and then subtract the "darkcnta.spc" from "lampfulla.spc" (use the subtract function in the arithmetic menu). Save this result as "corr1.spc" NOTE: We are only interested in the raw signal files, those that end with 'a'.

2. Right-click on the newly created spectrum, choose copy data and paste this into notepad. Find the wavelength where the maximum signal is observed (usually around 550 nm). Record the wavelength and its corresponding signal.

3. Open the "corr1.spc" file in the interface program and normalize the signal by dividing the "corr1.spc" by the maximum observed signal. This is done by using the operation option in the arithmetic menu. Save this new signal as "corr2.spc"

4. In the computer in the "corrections" directory, there should be an excel-converted version of the standard lamp output file "M868intp.xls". Open this file and find the signal value for the lamp at the wavelength where the "corr1.spc" signal was the highest. Record the standard lamp signal intensity at this wavelength (For example: the M868intp signal at 550 nm is 1354).

5. Now open the "M868intp.spc" file in the interface program. Normalize the file at the maximum wavelength by dividing it by the intensity of the M868intp signal that was recorded in step 4. Record this result as a new file: "corr3.spc". ***Do NOT overwrite the M868intp.spc file.***

6. To generate the final correction file, open the "corr3.spc" file in the interface program and use the arithmetic features to divide the "corr3.spc" signal by the "corr2.spc" signal. This result should be saved as "corr-use.spc" in the working directory.
7. Congratulations! You have generated new corrections factors without breaking anything in the process. You are now allowed a brief break. Then go back to SCIENCE!

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