

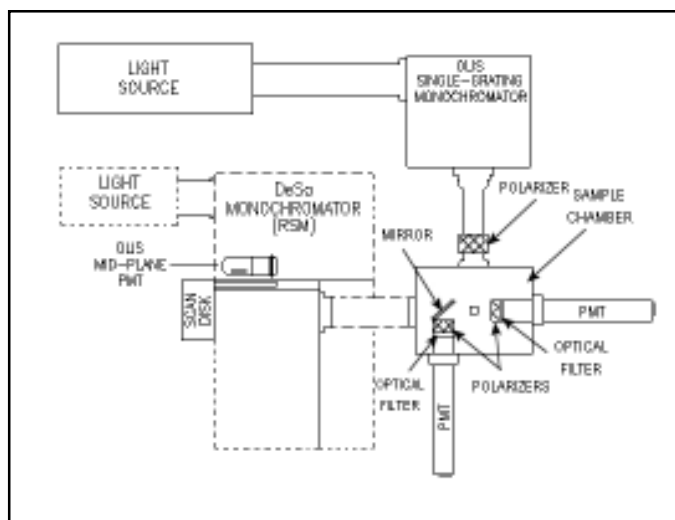
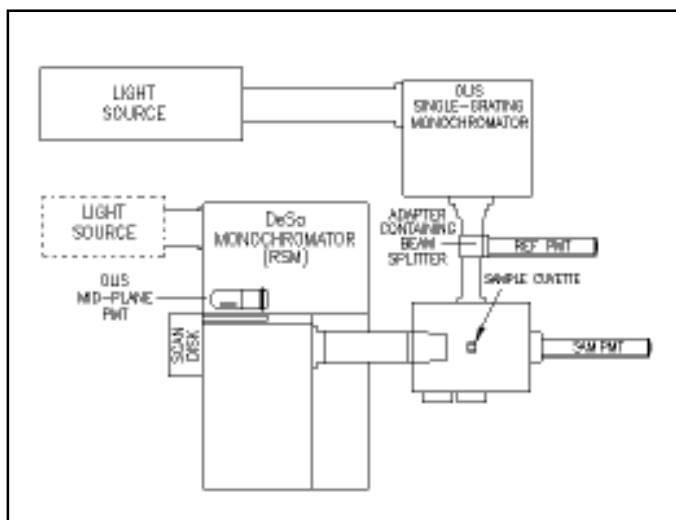
CONVERTING THE RSM 1000 FROM FLUORESCENCE MODE TO POLARIZATION OF FLUORESCENCE



OVERVIEW: You will be converting your RSM 1000

From this fluorescence configuration with no polarization,

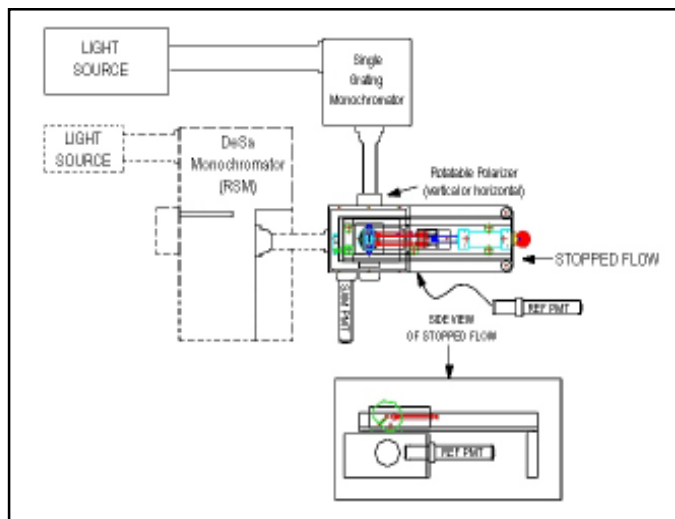
to polarization of fluorescence and anisotropy using the single grating monochromator



OR to polarization of fluorescence with stopped flow

This conversion involves five steps:

1. Removing the cuvette holder from the sample chamber.
2. Placing the excitation, horizontal, and vertical polarizers into the sample chamber.
3. Placing the PMTs on the sample chamber with appropriate optical filters.
4. Placing the stopped flow unit on the sample chamber.
5. Changing the appropriate software parameters.



If at any time during the following procedures you have any questions feel free to contact Olis at 706-353-6547 or email your questions to techsupport@olisweb.com.

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PROCEDURES

The following procedures will aid in your transition from fluorescence to stopped-flow polarization of fluorescence. An asterisk (*) indicates the step may be skipped if the stopped flow is not used. Photos below show sample chamber that is not attached to the RSM. Your chamber should remain attached to complete these procedures.

1.* Note that the cuvette holder should not be removed if the stopped flow attachment is not to be used. Skip to step 5 if this is the case. Disconnect any tubing going into the cuvette holder from underneath the sample chamber.

2.* Open the sample chamber and remove the two thumb nuts holding the cuvette holder in place. One is located on the far left of the cuvette holder assembly while the other is somewhat enclosed on the right side.



3.* Remove the cuvette holder by lifting straight up.

4.* Cover the hole in the bottom of the chamber with the metal plate and tighten the thumb nuts to hold it down.



- Slide out the light pipe from inside the excitation port. A black plastic cylinder is in the excitation port fitting; slide this forward into the chamber and set aside.



- Take the excitation polarizer (shown in picture) and slide the shorter nose cone over the end containing the polarizer.



- Insert the end without the nose cone into the excitation port so that the screw hole shows through the slot labeled "HORIZ. VERT."



8. Screw the polarizer lever with knob into the screw hole and tighten. The polarizer should easily be movable from horizontal to vertical position.



9. Remove the red labeled PMT from its holder.

10. Insert the horizontal polarizer (shown in picture) over the exit port, making sure that the plastic set screw is on top and the screw heads on the port fit into the cutouts on the polarizer mount.



11. Tighten the setscrew making sure that the assembly is flush with the chamber wall.



12. Slide the long nose cone over the horizontal polarizer; its function is to insure that only light from the sample reaches the PMT.



13. Insert the vertical polarizer into the left front exit port of the sample chamber. Make sure that the white setscrew is on top and that the polarizer assembly is flush with the chamber wall.



14. Tighten the setscrew holding the polarizer assembly in place.

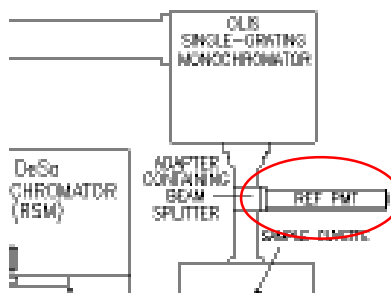
15. Insert the beam mirror (looks like a beam splitter) into the fitting at the left end of the sample chamber (same position as the beam splitter) in front of the vertical polarizer. Be sure to insert the mirror assembly such that the notch engages the alignment pin.



16. Tighten the white thumb screw holding the beam mirror in place.



17. Slide the blue ratio PMT (circled in the drawing) out of the beam splitter assembly (ratio position).



18. Insert the appropriate optical filter into the collar in the nose of the PMT by:



Loosen the set screw.



Remove collar.

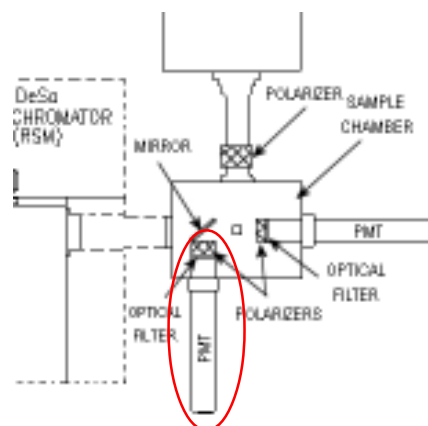


Remove aperture.



Insert filter. Replace collar. Tighten screw.

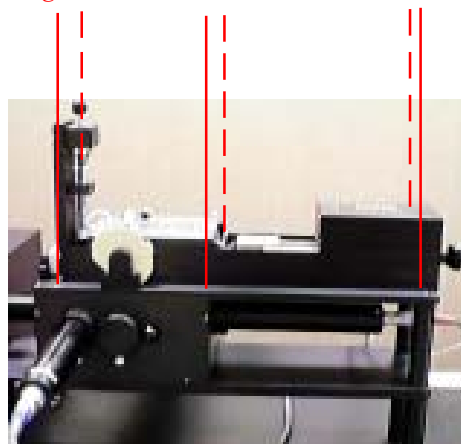
19. Slide the blue reference PMT into the left front exit port until it stops, then back 1/8".



20. Replace the collar with an appropriate optical filter and slide the red PMT back into the right exit port.
- 21.* Carefully remove the stopped flow unit from its stand and place it on the sample chamber. Note, skip to step 25 if stopped flow is not to be used.

- 22.* Secure the stopped flow unit with the six screws (Two 5/32" allen screws at the "trigger" end and four 3/32" screws at the syringe end). Turn each screw enough such that the screw engages, but do not tighten until all six screws are engaged, and you are sure that the stopped-flow unit is seated correctly.

secure using 3/32 Allen wrench *secure using 5/32 T-bar*



- 23.* Connect the gas tubing from the stopped flow unit to the gas box.
- 24.* Connect the gray cable from the stopped flow unit to the "SF Sensor" connections on the back of the electronics control box.
25. Ignite the lamp.
26. Turn on the control box and the computer.
- 27.* Enter the RSM-POF program and press "C" until fixed wavelength appears, "D" to highlight dual beam mode, and "F" to enable stopped flow.