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MAKING BRILLIANT BREAKTHROUGHS HAPPEN. SINCE 1980

HARDWARE HANDBOOK

for the

RSM 1000, the RSM 1000F, and the RSM CD

Revised Oct-96

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PREFACE

The purpose of this handbook is to gave a name to each component in your new rapid-scanning spectrophotometer, to identify where it "belongs" and what secondary functions it might also fulfill, and to show you how to repair and/or replace it.

A basic understanding of optics is expected, along with a reasonable level of competence with the notion of light, intensity, slits, etc.

Perhaps, after reading this booklet, you will have not only a richer appreciation for the potential of the tool at hand, but also some mild dissatisfaction with the immutability of lesser workstations. If all spectrometers were this modular, we would need far fewer of them lining our benches!

For the literal novice, a lamp is a source of photons, a monochromator is a means of selecting colors, a photomultiplier tube is an eyeball, and a sample is a thing which absorbs or glows.

Light in, colors are selected, the light is divided into halves—one of which interrogates the sample, one of which is a reference—and two "eyes" capture (1) the light from the lamp effected by the sample, and (2) the light from the lamp. By imposing other influences in the light besides color, e.g., polarization, we can increase our Q & A potential.

Reasonable comparison of our CD and absorbance model, and/or our CD and anisotropy model, for instance, confirms that the qualitative differences are minimal. Ultimately, what you do not see—the software—is the most powerful determinant of what results are returned.

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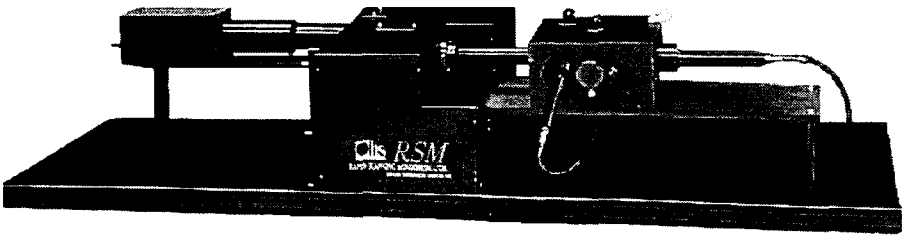
RSM CONFIGURATIONS

Each RSM can be used for a variety of measurements by combining appropriate components. Several popular configurations are described in the following pages. Optimization can be performed by the user. In addition, *OLIS* can assist RSM owners in optimizing their systems for specific applications by building special components and accessories. Please call if we can help with your particular experimental situation.

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OLIS RSM 1000

For absorbance and fluorescence



Basic hardware for the *OLIS* RSM 1000 includes the patented DeSa rapid-scanning monochromator, a ScanDisk, two 50-mm gratings, a sample chamber, a 75- or 150-watt xenon arc lamp, two PMT detectors, a P5 133 computer with twin 2MHz 12-bit A/D converters, and the *OLIS* software.

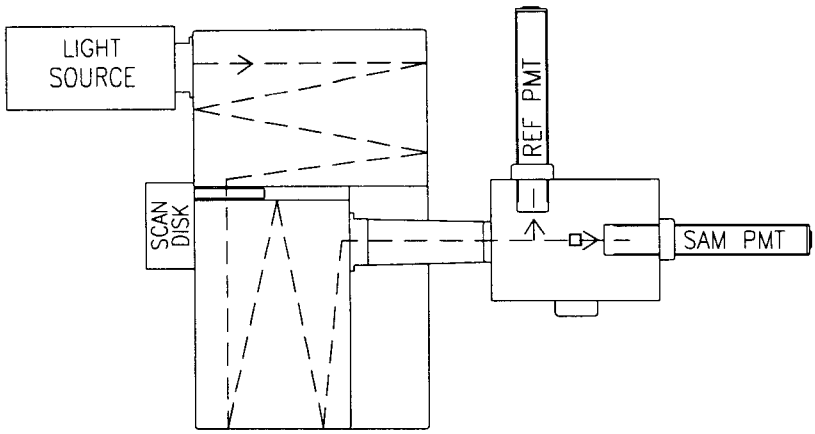
Optional hardware is available for purchase along with your basic RSM system or later as the need arises. "Extras" include a color printer, red-sensitive PMTs for scanning in the 700-1100 nm range, additional gratings and ScanDisks, an optical cable, a means of inducing a flash, a stopped-flow mixing unit, etc. These optional items can also be used with the RSM 1000F and the RSM CD.

Absorbance and fluorescence measurements can be made using the RSM 1000. The optical paths for both configurations follow; details of each configuration are given on page 41.

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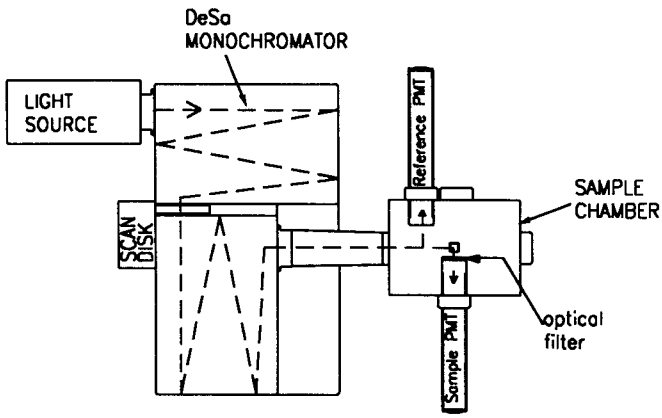
Single-Wavelength or Rapid-Scanning Absorbance

The optical path of the RSM 1000 (the absorbance configuration) is shown in the diagram at right. See page 41 for details of the absorbance configuration.



Single-Wavelength or Rapid-Scanning Fluorescence

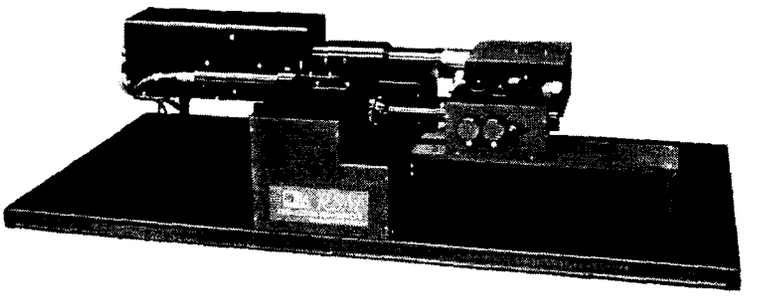
The basic RSM 1000 can be used for fluorescence measurements by moving the sample PMT to the 90° port and adding a filter to select the emission wavelength; the optical path for fluorescence measurements using the RSM 1000 is shown at right. See page 41 for details of this configuration.



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OLIS RSM 1000F

For fluorescence and absorbance



The RSM 1000F includes the same components as does the RSM 1000 plus a 150-watt, 450-watt, or 1000-watt xenon arc lamp for an exciting source, a single-grating monochromator for sample excitation, a middle-plane PMT for increased sensitivity, a beam-cooling accessory (necessary when using a 450- or 1000-watt lamp), and either the *OLIS* closed-cycle cooling system or the *OLIS* fail-safe sink box.

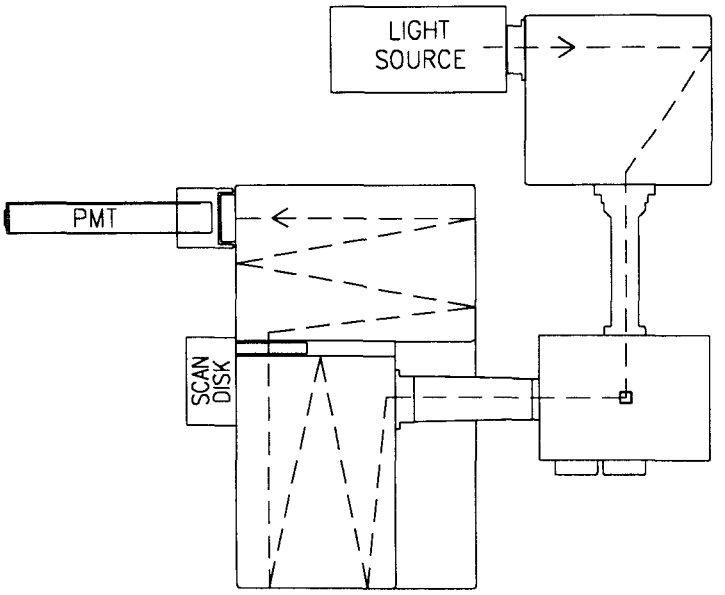
Optional hardware for the RSM 1000F includes a shutter that can be mounted inside the single-grating monochromator to protect photolabile samples from the excitation beam between measurements and to provide a pulsing excitation beam; and an external shutter box (containing the same shutter as would be mounted inside the single-grating monochromator and serving the same purpose), which can be mounted between the single-grating monochromator and the sample chamber or between the sample chamber and the DeSa monochromator. The external shutter box can also be used with the RSM 1000 and the RSM CD.

Three fluorescence configurations can be produced using the RSM 1000F in combination with the *OLIS* single-grating monochromator. The optical paths for these configurations follow; details of each configuration are given on pages 42-43.

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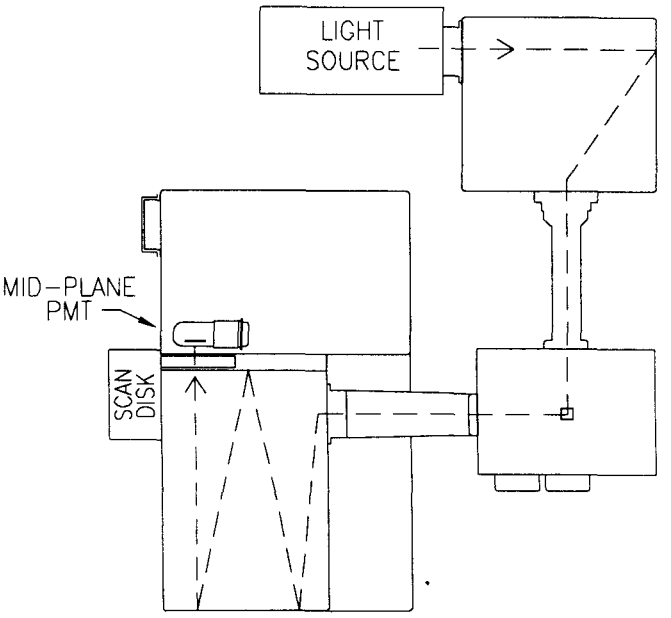
Single-Wavelength Excitation and Single-Wavelength or Rapid-Scanning Emission Detection

At right is the optical path for the RSM configuration in which the *OLIS* single-grating monochromator is used to excite the sample while the DeSa monochromator collects fluorescence emissions. If the rapid-scanning ScanDisk is used in the DeSa monochromator, millisecond spectral scans are recorded; if a fixed-wavelength ScanDisk is used, microsecond single-wavelength data points are collected. See page 42 for details of this configuration.



Single-Wavelength Excitation and Maximized Single-Wavelength or Rapid-Scanning Emission Detection

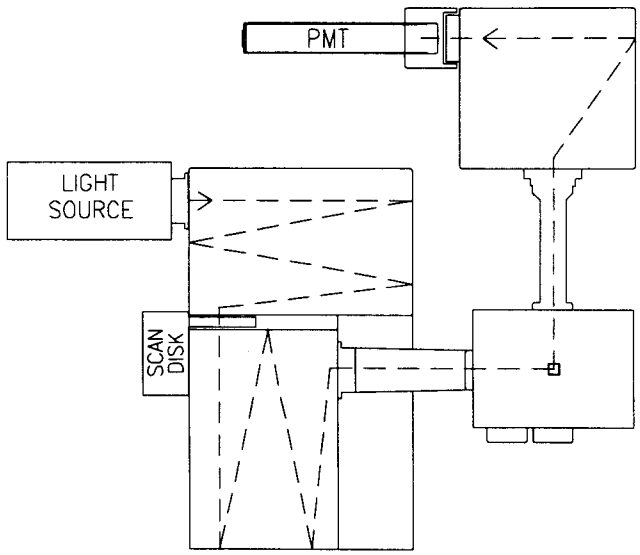
At right is the optical path for the RSM configuration recommended for samples having weak fluorescence emissions. A mid-plane PMT is positioned in the optical plane just beyond the ScanDisk module, eliminating two mirrors and a grating from the path of the emission beam. The *OLIS* single-grating monochromator is used to excite the sample while half of the DeSa monochromator is used to collect fluorescence emissions. See page 42 for details of this configuration.



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Rapid-Scanning Excitation and Single-Wavelength or Slow-Scanning Emission Detection

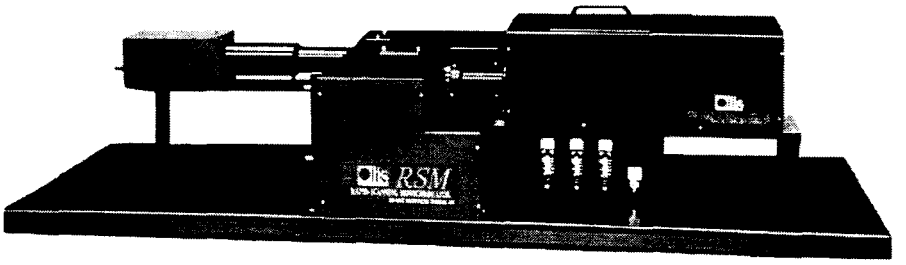
The diagram at right shows the optical path for the RSM configuration in which the DeSa monochromator is used to excite the sample while the *OLIS* single-grating monochromator collects fluorescence emissions. If the rapid-scanning ScanDisk is used in the DeSa monochromator, the excitation beam comprises a selected span of wavelengths and a single wavelength of the emission beam is measured by the PMT; if a fixed-wavelength ScanDisk is used in the DeSa monochromator, the sample is excited by a single, appropriate wavelength of light while the PMT makes measurements at the fluorophore's emission wavelength. See page 43 for details of this configuration.



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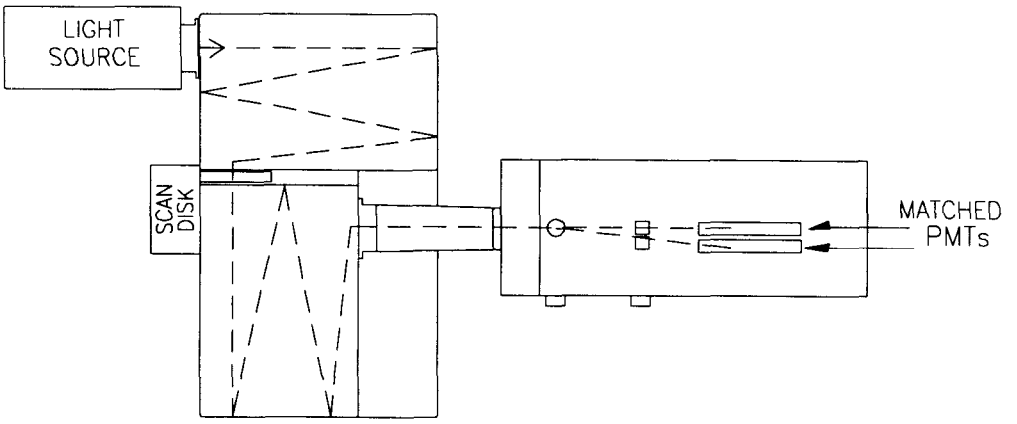
OLIS RSM CD

For circular dichroism and absorbance



The RSM CD system includes the DeSa monochromator, a lamp of your choice, plus a piezo-electric modulator (PEM), a polarizer, and the OLIS CD sample chamber. The RSM CD uses two matched PMTs in a unique dual-beam system.

The diagram below shows the optical path of the *OLIS* RSM CD instrument. See page 43 for more about the CD configuration.



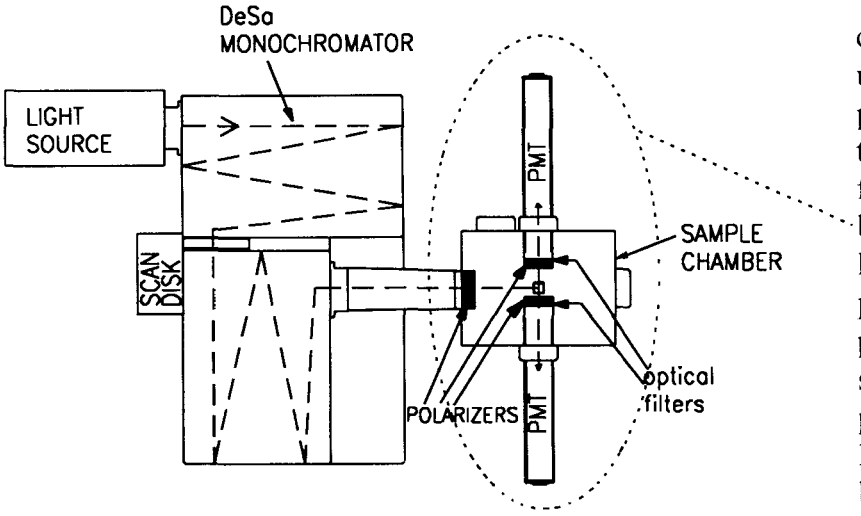
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POLARIZATION OF FLUORESCENCE & ANISOTROPY USING THE RSM

When excited by polarized light, fluorescent samples emit polarized light. This polarization is caused by the photoselection of fluorophores oriented in the same direction as the polarized excitation. The emission can be depolarized by a number of phenomena, including rotation diffusion of the fluorophores, which in turn depends on the viscosity of the solvent and the size and shape of the diffusing species. Fluorescence polarization measurements are used to determine the average angular displacement of the fluorophore occurring between the time that a photon is absorbed and subsequently emitted.[1]

Four RSM configurations are available supporting polarization of fluorescence and anisotropy measurements. One utilizes the RSM 1000, two utilize the RSM 1000F, and the other utilizes the *OLIS* single-grating monochromator. In all four configurations, the software returns the answer as (1) polarization of fluorescence, (2) anisotropy, or (3) total fluorescence.

Fluorescence Polarization and Anisotropy Using the RSM 1000



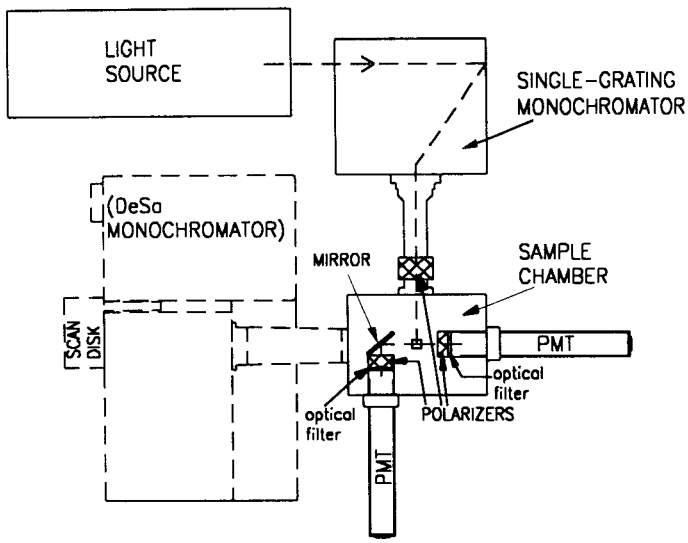
The diagram at left shows the optical path of the configuration used to measure fluorescence polarization and anisotropy using the RSM 1000. The classic T-format is used (shown enclosed by the dotted oval). The exciting light from the RSM is vertically polarized by the excitation polarizer upon entering the sample chamber. Emission polarizers positioned before each PMT polarize the light collected by the PMTs such that one collects light oriented parallel to the exciting light and the other collects light oriented perpendicular to the exciting light. Optical filters at the input end of each PMT select the wavelength at which emissions are collected. The ScanDisk determines the data-collection mode (rapid-scanning, fixed-wavelength, or conventional slow-scanning).

[1]Lakowicz, JR. (1983) *Principles of Fluorescence Spectroscopy*. New York: Plenum Press.

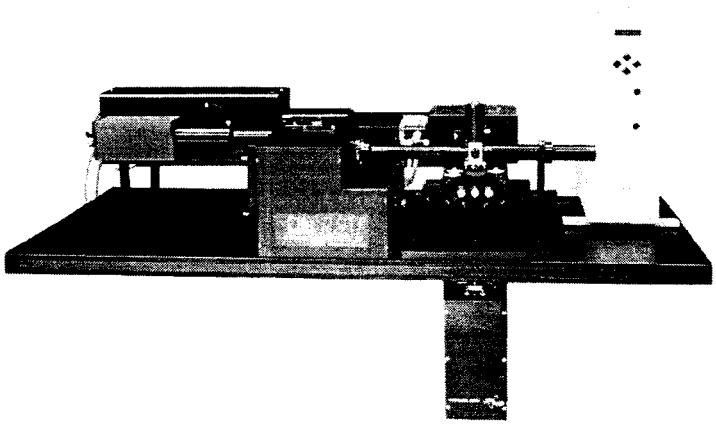
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Fluorescence Polarization and Anisotropy Using the OLIS Single-Grating Monochromator

The diagram at right illustrates the configuration used to measure fluorescence polarization and anisotropy when greater sensitivity is needed than can be accomplished by rapid-scanning. In this configuration, the DeSa monochromator is not used; instead, the *OLIS* single-grating monochromator provides the exciting light. An excitation polarizer is positioned just before the sample chamber and emission polarizers are placed just before each PMT. The exciting light is vertically polarized; the emission polarizers are positioned such that one PMT collects light oriented parallel to the exciting light and the other collects light oriented perpendicular to the exciting light.



Pictured below is an RSM 1000F custom-fitted with a bioLogic SFM-3 three-syringe stopped-flow mixing unit. The fluorescence polarization configuration shown above was developed to allow this RSM owner to make polarization measurements. Modifications to the RSM in this case included cutting a hole in the base plate to accommodate the vertically oriented bioLogic stopped-flow unit. This highly customized instrument graphically illustrates OLIS' willingness and ability to customize RSM systems to meet the experimental needs of the user.

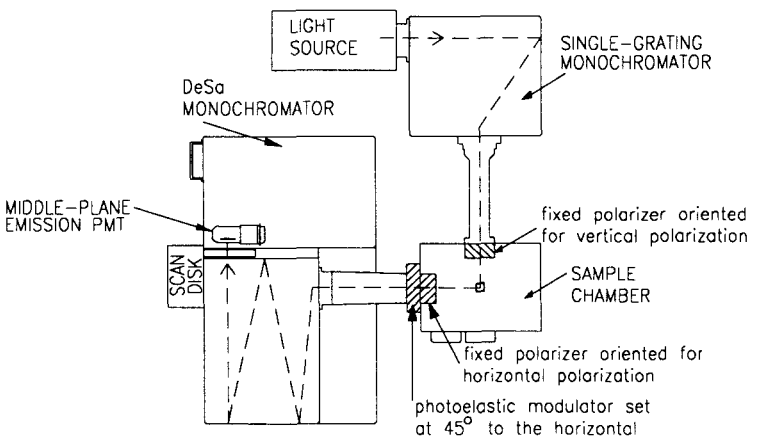


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SPECIAL POLARIZATION OF FLUORESCENCE APPLICATIONS

A PEM-based polarization of fluorescence format was developed to allow two special applications of the RSM 1000F. The optical layout is similar to that described by Wampler & DeSa[2] in 1974. A photoelastic modulator positioned just before the sample chamber is set at 45° to the horizontal. The sample is "bracketed" by fixed polarizers; i.e., both the exciting and the emitted light pass through these fixed polarizers, making the polarization characteristics of the excitation source and the detector system irrelevant. The emitted light, as viewed by the detector, changes only in intensity.

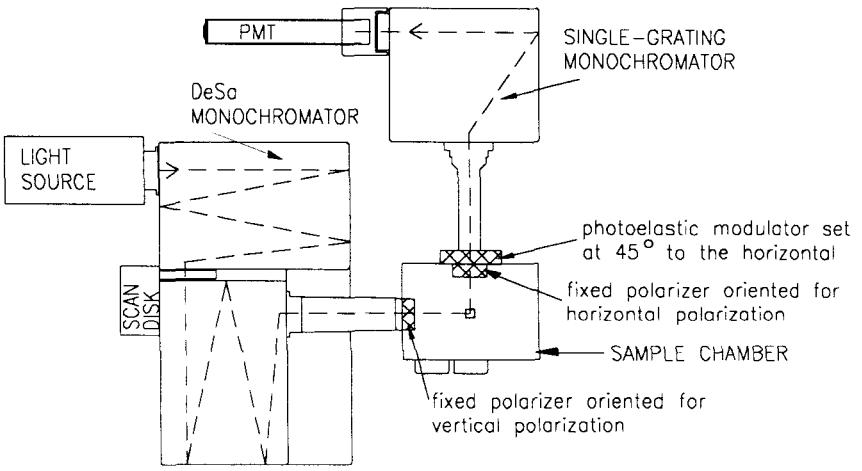
Rapid-Scanning Polarization of Fluorescence Emission Spectra



The special configuration diagrammed at left allows RSM 1000F owners to collect polarization of fluorescence emission spectra.

Rapid-Scanning Polarization of Fluorescence Excitation Spectra

The special configuration diagrammed at right allows RSM 1000F owners to collect polarization of fluorescence excitation spectra.



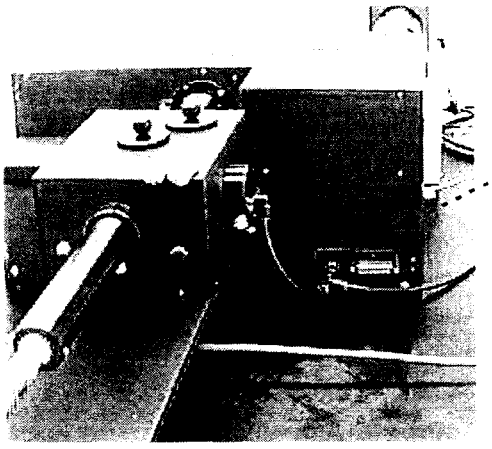
[2]Wampler, JE and DeSa, RJ (1974). "Recording Polarization of Fluorescence Spectrometer—A Unique Application of Piezoelectric Birefringence Modulation." *Analytical Chemistry*, 46:563-567.

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ROUTINE MAINTENANCE

Nitrogen Purging

To ensure the longest life, the monochromator should be purged with nitrogen gas during use. The nitrogen line attaches to the port on the right side of the DeSa monochromator (see illustrations). To keep dust out of the monochromator and ensure optimal performance, purge with nitrogen during the changing of optical components—gratings, mirrors, slits, and filters—as well as ScanDisks and sample compartments.

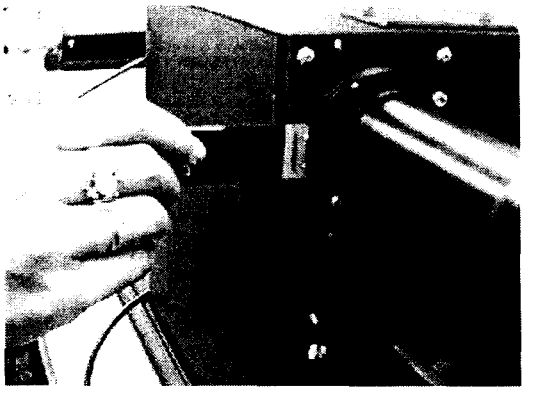




Nitrogen purging is essential for optimizing detection in the deep-UV and is encouraged. With this said, many labs never purge their mono-chromators. **Oxygen effects the coating on the mirrors, and degradation will occur more quickly without nitrogen purging.**

Slit Manipulation

Another recommended practice is to protect the RSM's optics from light when the instrument is on but measurement is not being made. Both the entrance and exit slits can be pulled out ½" from their seated position, just enough to move the slits out of the optical path (see illustration). This blocks the light beam from passing through the slit (entrance slit during absorbance work and exit slit during fluorescence work), preventing sample photo-degradation between measurements and extending the life of the system's optical components.



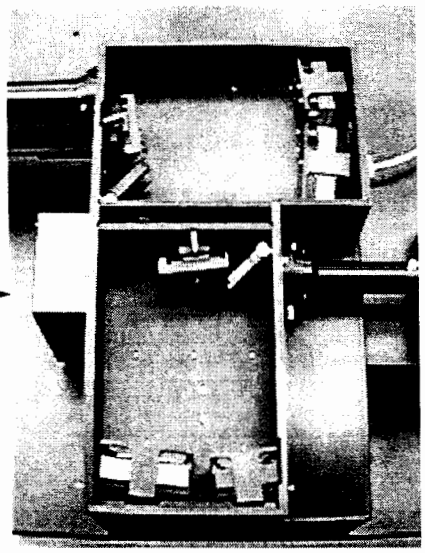
During experiments, of course, both slits will be fully inserted to allow the light beam to travel the optical path.

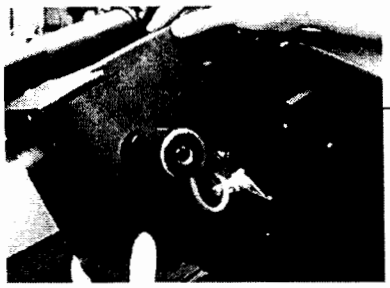
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MONOCHROMATORS & FILTERS

THE DeSa MONOCHROMATOR

The DeSa monochromator is the heart of all RSM systems and the key to rapid scanning. The photo at right—an aerial view with the lid removed—shows the optics of the DeSa monochromator; the photo below shows the ScanDisk, which has been pulled out for viewing.





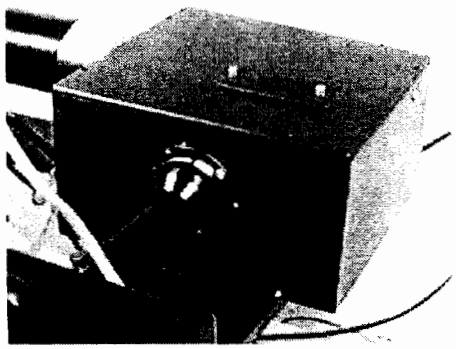
Instructions for removing and replacing ScanDisks are given on page 20.

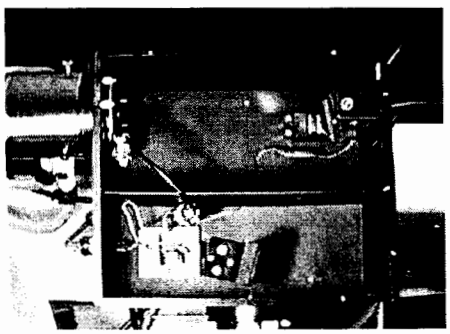
The DeSa monochromator is a subtractive double-grating model. The two halves of the DeSa monochromator are positioned as mirror images of each other, so that the first half diffracts the incoming light beam and the second half recombines the diffracted beam coming from the first half to produce a spectrally homogeneous output beam. The DeSa model can be used as a rapid-scanning or fixed-wavelength excitation monochromator and as a rapid-scanning or fixed-wavelength emission monochromator.

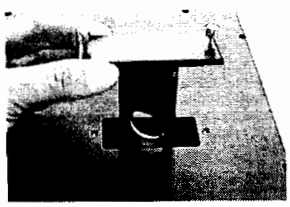
The DeSa "rapid-scanning monochromator with moving intermediate slit" was awarded a U.S. patent (no. 5,285,254) on February 8, 1994. *R & D Magazine* recognized the RSM as one of the 100 most technologically significant new products and processes to emerge in 1993.

THE OLIS SINGLE-GRATING MONOCHROMATOR

The *OLIS* single-grating monochromator can serve as either the excitation or the emission monochromator in fluorescence work. It has a 40x45 mm concave holographic grating driven by a small servo motor, traditional entrance and exit slits, and a place for a second-order filter (attached to underside of lid). A computer-controlled shutter, used both to protect photolabile samples between measurements and to effect pulsing of the excitation beam, is optional.







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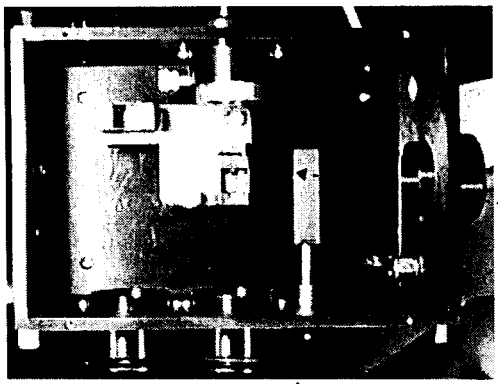
FILTERS

Filters may be used to calibrate the monochromator, to select a specific wavelength of interest, and to minimize the effects of higher-order radiation.

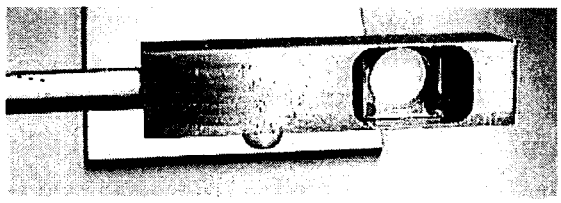
Using a Holmium Oxide Filter to Calibrate the Monochromator

Because holmium oxide exhibits sharp peaks throughout the UV/ Vis/ NIR spectrum, a holmium oxide filter is the standard used to calibrate the DeSa monochromator. When you purchase an RSM, it will arrive calibrated for the gratings you have selected. Each time you change gratings, you will need to calibrate your RSM.

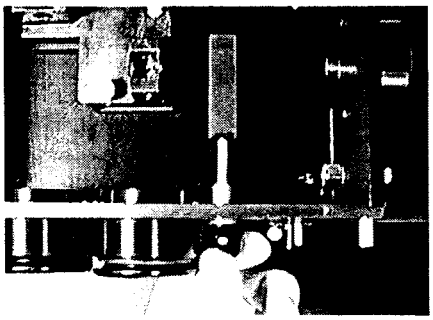
Calibration is also advisable any time you suspect that the wavelength(s) you have selected are not being transmitted, although you should first ensure that the gratings are seated correctly (you should hear a distinctive "click" when you have correctly seated the gratings—see page 27).



The holmium oxide filter holder is permanently mounted to a retractable rod inside the sample chamber of the RSM. The rod extends through the front wall of the sample chamber and has a black knob on its outside end.

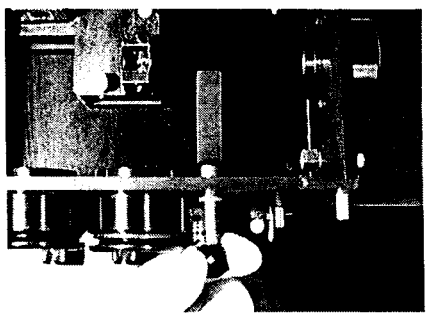


Because the rod can rotate freely 360°, a white dot on the knob is used to indicate the correct rotational position for the rod during wavelength calibration.



To use the holmium oxide filter, move the filter into the optical path by pushing in the black knob and rotating the rod so that the white dot is on top of the knob, as shown in the photo at left.

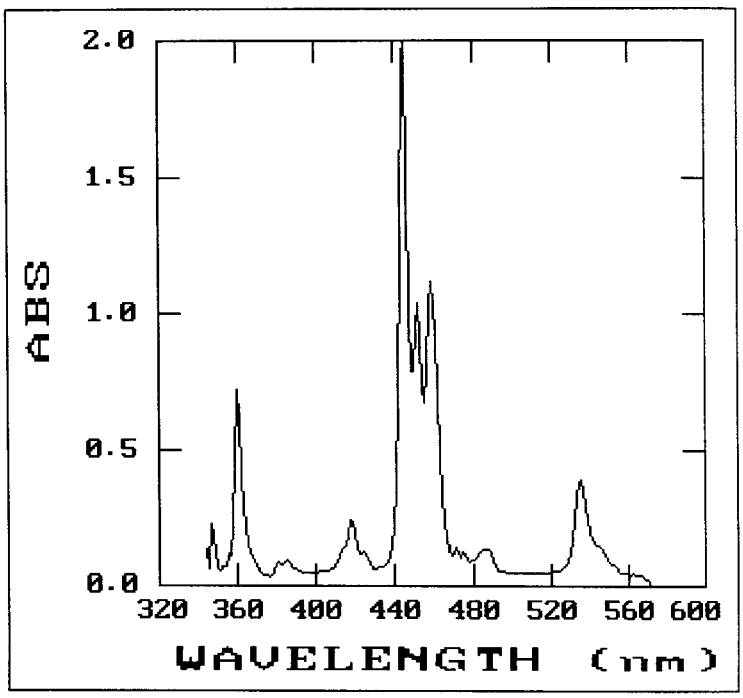
When not in use, remove the filter from the optical path by pulling out on the black knob.



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Following are the procedures for calibrating RSM systems for accurate measurements in the visible spectral range:

1. In Command Mode, press "V" to Enter Live Display.
2. Press "M" to Move RSM Wavelength, then select 450 nm as the center wavelength. If your RSM has a shutter, ensure that it is open.
3. Adjust the High Voltage on the PMTs using the "A—Auto HV" option.
4. Insert the holmium oxide filter in the light beam path; the live display should look like the picture at right.
5. Set the Collection time (option "T") to 0.2 sec.
6. Collect data. Do not collect Baseline since the absolute spectrum is of no interest during calibration.
7. Go to Global Fitting (option "G"). For number of species, enter "1," then choose a Model-Free Fit by entering "2" when prompted to select a kinetic fit.
8. Press the spacebar, then press "Q" to go to a full-screen display of the resulting spectrum. Press "C" to enter Cursor Mode; using the arrow keys, move the cursor to each peak to verify that the wavelength of every peak corresponds to those shown in the standard holmium oxide spectrum at right.
9. If the spectrum you collected does not correspond exactly to the standard, go to the Parameter Setup menu and enter a new value for the calibration wavelength.
10. Repeat steps 1-8.



Using a Filter to Select Specific Wavelengths

Optical filters can be used to select a particular wavelength as your experimental needs require. For example, RSM 1000 owners can make simple fluorescence measurements by moving the sample PMT from the straight-on position to the 90° port and adding a 1" circular filter to the input end of the PMT to select the emission wavelength. This configuration is described in detail on page 4.

Using a Filter to Eliminate Higher-Order Radiation

The *OLIS* single-grating monochromator accommodates a second-order filter to remove unwanted wavelengths that may be transmitted when a very bright light source is used. Filter holders are also mounted at the input end of all PMTs purchased from *OLIS*. And, of course, should the need arise, you can position a filter temporarily anywhere in the RSM system.

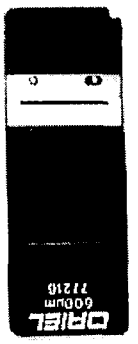
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SLITS/ SCANDISKS

Like all double-grating monochromators, the DeSa model has an entrance slit, an intermediate slit, and an exit slit, each of which effects the amount of light passing from the illumination source (lamp or emitting sample) to the detectors. Entrance and exit slits are interchangeable; the intermediate slit is either a simple slit or multiple slits radially dispersed on the slit wheel (i.e., a ScanDisk).

CONVENTIONAL SLITS

The typical absorbance configuration includes a 0.6-mm entrance slit and an exit slit ranging from 0.6 mm to 0.12 mm. The RSM uses Oriel slits in addition to slits made by *OLIS*. Many sizes are available, including 1.24 mm, 3.12 mm, and 6.32 mm, and sizes not available elsewhere can be made to order. If the slit width you need is not available, call us. The *OLIS* Fixed-Slit ScanDisk also utilizes a conventional slit—the slit width is selectable by the user and, again, can be commercial or custom.



When installing a slit in the RSM, insert the notched edge first with the notch facing down. ----------►

When a slit is not in use, store it in a safe place, such as in the *OLIS* Grating & Slit Holder.



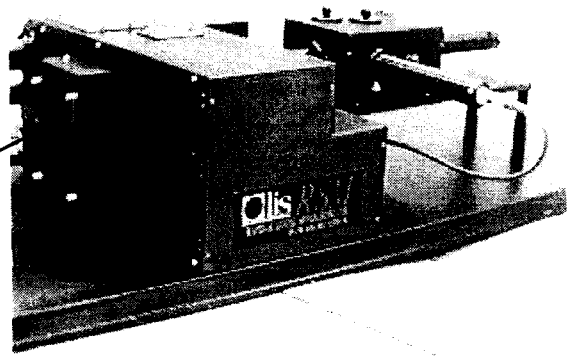
As explained on page 12, pulling out the entrance slit slightly (1/2" or so) moves the slit aperture out of the optical path, protecting photolabile samples and the RSM's optical components from potential light damage between experiments.

The final optical resolution of the RSM is determined by the size of the two narrowest slits. Thus, sometimes it is desirable to remove the entrance and exit slits, using only the gratings and intermediate slit to determine resolution. A stopped-flow experiment is one situation in which you may want to remove the entrance slit during measurement; doing so minimizes any ringing that may occur when the arc lamp vibrates due to the shock caused by the stopping syringe hitting its stop.

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OLIS ScanDisks

The ScanDisk module is what makes *OLIS* RSM systems unique. Serving as the intermediate slit, the ScanDisk effects scanning; changing ScanDisks therefore enables the user to change data-collection modes quickly and easily.



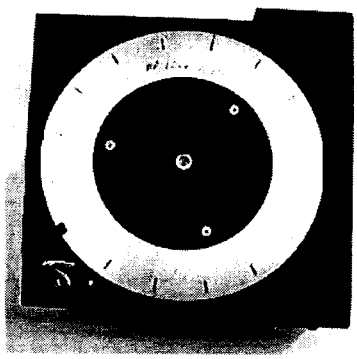
The ScanDisk module is located on the left side of the DeSa monochromator. Instructions for changing ScanDisks are given on page 20.

Three basic types of ScanDisks enable several data-collection modes.

Rapid-Scanning ScanDisk

For collecting absorbance, fluorescence, and CD spectra

To scan multiple wavelengths at 62.5 Hz (collecting 1,000 spectral scans/ sec), a ScanDisk containing 16 slits is used; the ScanDisk is caused to spin by a synchronous motor. This view of the spinning disk inside the ScanDisk module shows the 16 "intermediate slits" that enable RSM systems to collect 1,000 spectral scans per second



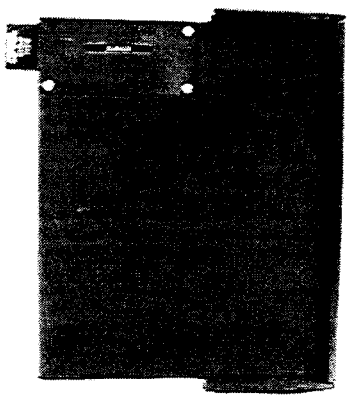
You may choose a ScanDisk with 0.2-mm, 0.5-mm, or 1.0-mm slits. Combine various pairs of gratings with either slit-width ScanDisk to achieve the desired resolution (see chart on page 19).

When rapid scanning, the data are written directly to the computer, which has 16 Mbytes of RAM; 1,000 scans per second can be stored in the computer for as long as RAM lasts. A maximum of 14,000 scans can be stored.

Fixed-Wavelength ScanDisk

To collect fixed-wavelength absorbance, fluorescence, and CD data at rates to 2 MHz

For single-wavelength measurements, a ScanDisk containing a slit holder is used. Oriel (or *OLIS*) slits of any width may be used—your choice depends on the desired resolution.

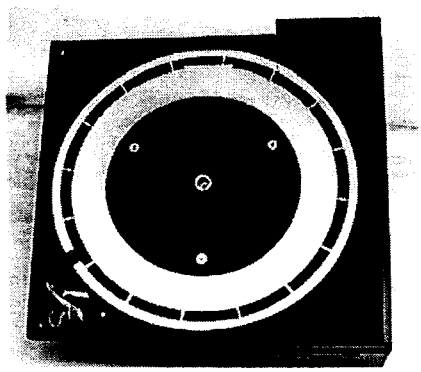


Fixed-wavelength data are collected at rates to 2 MHz, resulting in up to 2,000,000 readings per second or a reading every 500 nsec. Because the data are collected at such a rapid rate, they cannot be written directly to the computer and are stored in the RSM's A/D card. With 1 Kb of RAM on the RSM's A/D card, a maximum of 1,000 data points can be stored.

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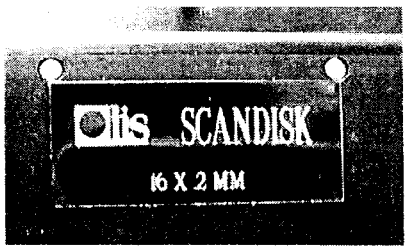
Spoke ScanDisk

For collecting fluorescence and luminescence spectra and studying the kinetics of emission

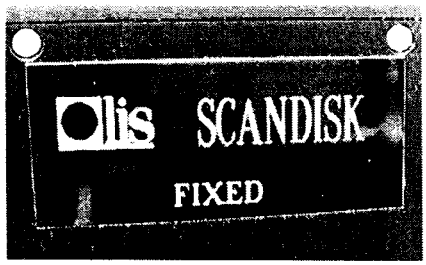


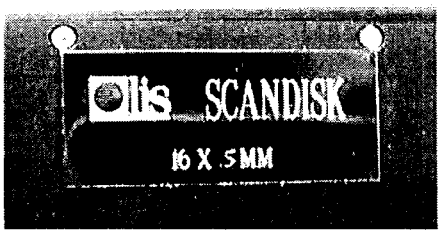
When collecting a weak signal, such as in fluorescence work, using the *OLIS* "Spoke" ScanDisk maximizes the light throughput by providing an intermediate-slit configuration that is essentially the opposite (or the "negative") of that provided by the rapid-scanning absorbance ScanDisk. As the picture shows, the spinning disk inside the Spoke ScanDisk has 16 1-mm "spokes" instead of 16 1-mm slits. The spokes serve as wavelength markers, enabling the user to collect maximum-intensity, time-resolved, multiple-wavelength spectral information about a changing sample with a weak signal.

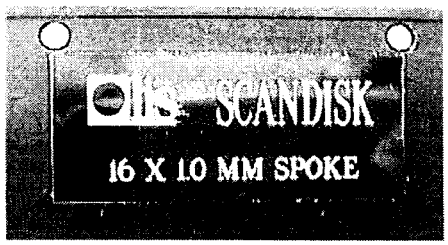
As with the rapid-scanning ScanDisk, data collection using the Spoke ScanDisk occurs at 62.5 Hz, which produces 1,000 scans/ sec. The data are written directly to the computer for the duration of RAM memory. Up to 14,000 scans can be stored.



Each ScanDisk module is clearly labeled.







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RESOLUTION

The spectral resolution of a DeSa monochromator is determined by the width of the intermediate (ScanDisk) slit and the groove density of the gratings. For example, using 400-line gratings and a 0.2-mm-slit ScanDisk results in a spectral resolution of 1.5 nm. The same resolution results when you use 1200-line gratings and a ScanDisk with either a 0.5-mm or a 1-mm slit. The Spectral Resolution Guide below shows the resolution provided by various combinations of gratings and a 0.2-mm intermediate slit.

Spectral Resolution Guide for the OLIS RSM

BLAZE WAVELENGTH	GROOVE DENSITY (NO. OF LINES)	SPAN (nm)	RESOLUTION (bandwidth in nm)	WAVELENGTH REGION (Primary*)
870	80	>500	7.5	580-1100
800	150	500	4	533-1100
500	300	300	2.5	334-750
200	400	230	1	200-300
550	400	230	1	366-825
200	600	155	0.7	200-300
300	600	155	0.7	200-450
400	600	155	0.7	267-600
500	600	155	0.7	334-750
750	600	155	0.7	500-1125
1000	600	155	0.7	667-1500
200	1200	76	0.4	200-300
250	1200	76	0.4	200-375
280	1200	76	0.4	200-420
500	1200	76	0.4	334-750
750	1200	76	0.4	500-1125
270	2400	38	0.4	200-405

* Although the primary wavelength range of a given grating is calculated as [range = 2/3 blaze wavelength to 3/2 blaze wavelength], the actual wavelength region may slightly exceed the listed primary range, especially at the upper end; e.g., the grating optimized for 334-750 nm is actually useable from approximately 300 to 1000 nm. The primary region is defined as the spectral region in which the grating efficiency is greater than approximately 20%; the useable region is defined as the spectral region in which the grating efficiency is greater than approximately 10%.

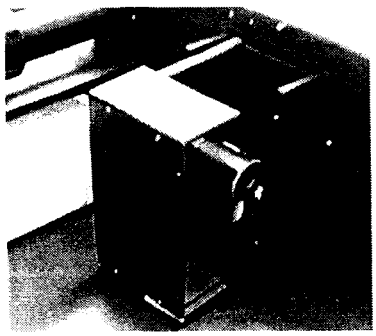
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CHANGING SCANDISKS

Changing ScanDisk modules is easy, quick, and requires no tools. Always store ScanDisk in a safe place when not in use. We provide a velcro-sealable bag for ScanDisk storage and recommend that you use it.

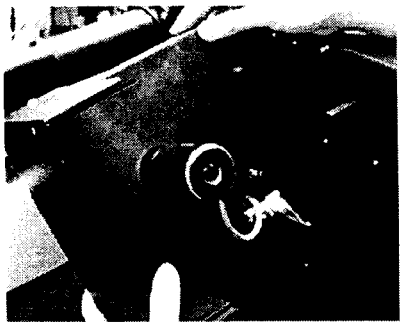


Step 1

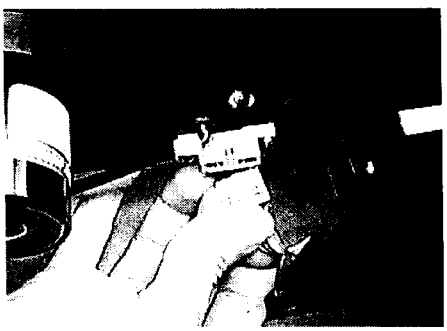


Step 2

1. Remove the four thumbscrews from the front of the ScanDisk module.
2. Gently slide the ScanDisk out of the DeSa monochromator.



Step 3



Step 4

3. Locate the ribbon (cable) at the bottom inside corner of the ScanDisk module.
4. Gently disengage the ribbon plug from the ribbon header.

5. Reverse steps 4, 3, 2, and 1 to install the new ScanDisk module.

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SCANDISK PURCHASE/ MAINTENANCE RECORD

Additional notes:

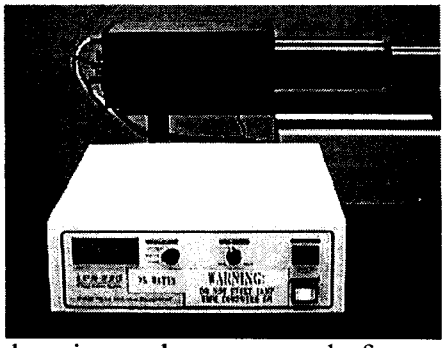
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LIGHT SOURCES

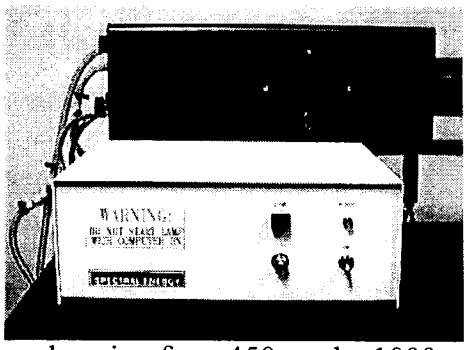
We use xenon arc lamps because (1) they are very bright light sources (2) they have a wide spectral range (UV/Vis/NIR) and (3) they are easy to use. Their principal drawback—instability—is more or less erased by the fact that a reference is used. Your choice of a lamp will be based on (1) the number of photons you need (2) the spectral range in which you are working (3) budgetary considerations. Options include: deuterium, halogen, lasers, flash lamps, pulse lamps, etc.

CHOOSING A LAMP

Most RSM system include xenon arc lamps because they produce bright light throughput in the 180–2400 nm spectral range. The wattage you choose for your particular experimental situation is a compromise between intensity (typically, the more photons, the faster the effective time resolution) and cost.



Lamp housing and power supply for a 75-watt air-cooled xenon arc lamp and a 150-watt water-cooled xenon arc lamp



Lamp housing for a 450- and a 1000-watt water-cooled xenon arc lamp; power supply for a 450-watt water-cooled xenon arc lamp (power supply for a 1000-watt water-cooled xenon arc lamp is available but not pictured)

According to the manufacturers, the typical lifespan of these lamps is 1000 hours. As lamps age, the electrodes inside the quartz envelope corrode, making the lamp harder to start; lamp instability also increases with age. Therefore, when to change a lamp is a somewhat subjective decision. You must evaluate the noise level as your lamp ages—when it becomes unacceptable, change your lamp.

A laser may also be used as the light source for an RSM system.

STARTING THE LAMP

***Never* start the lamp when the computer or any system-related electronic devices are on.** Start the lamp first. Then, turn on the computer and associated electronic devices. The label on the front of the power supply is a strong reminder of this important procedure!

WARNING:
DO NOT START LAMP
WITH COMPUTER ON

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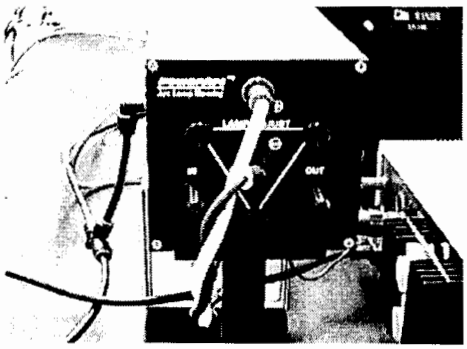
COOLING THE LAMP

If you are using the *OLIS* Closed-Cycle Cooling System or the *OLIS* Fail-Safe Sink Box with your RSM system, remember that the built-in safety feature prevents the lamp from being started unless the cooling system and the water are turned on. Therefore, if your lamp fails to start, ensure that the cooling system and the water are turned on. For more on lamp cooling, see page 51.

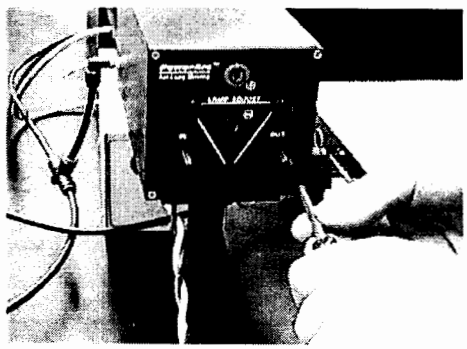
REPLACING THE LAMP

The process of replacing and aligning a lamp is tricky and involves some danger; if you prefer, *OLIS* will perform the procedure for you.

Always wear gloves and goggles when you handle a lamp; there is always danger of the lamp exploding, and the natural oil on your hands can damage the lamp.



Step 1

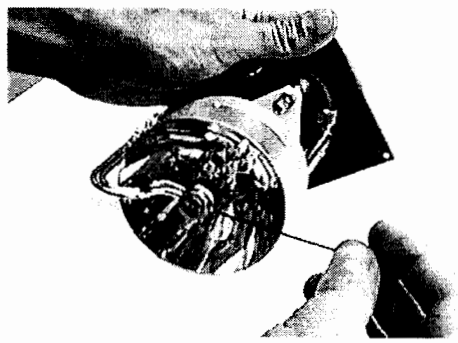


Step 2

1. Remove the wires (1 white, 1 black) from the lamp housing. (Note that the wires are twisted and fastened with a twist-tie; ensure that the wires remain twisted and fastened together.)
2. Remove the four screws on the rear on the lamp housing.



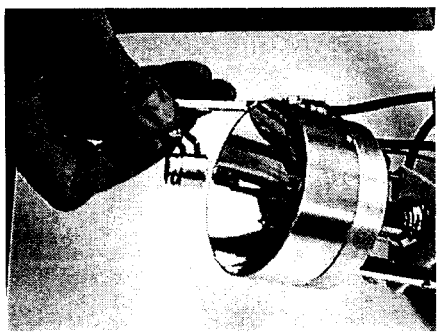
Step 3



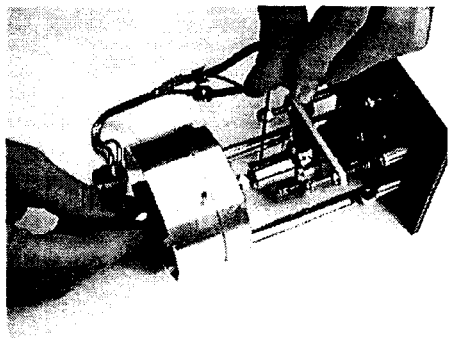
Step 4

3. Carefully remove the lamp/ reflector assembly.
4. Loosen the (Allen) socket head set screw on the anode.

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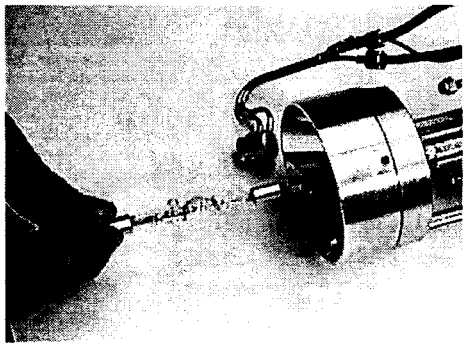


Step5

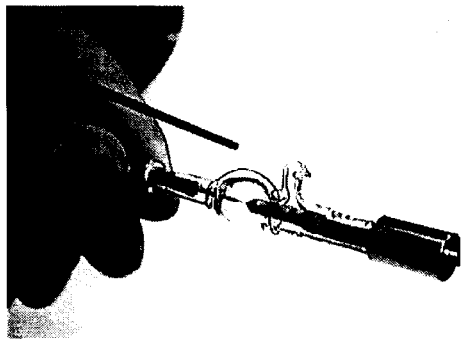


Step 6

5. Carefully slide anode off the lamp.
6. Loosen the (Allen) socket head set screw on the cathode.

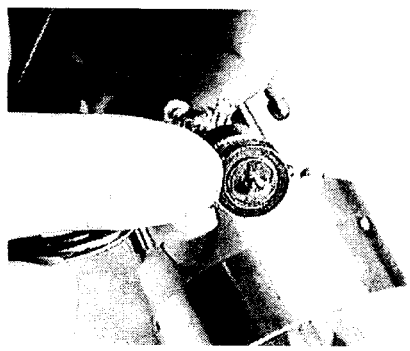


Step 7



Step 8

7. Carefully remove lamp from center of reflector.
8. Carefully replace the lamp. Position lamp with nipple facing up. Seat lamp fully in receptacle. If you touch the quartz envelope, wash it with methanol and wipe it gently with a Kimwipe® or a lens tissue.
9. Carefully replace anode, leaving it flush with the end of the lamp.
10. Tighten both set screws. Before reinserting the lamp in the lamp housing, familiarize yourself with the action of the adjustment screws by turning them while observing the direction in which the lamp rotates as you do. Understanding the effect of each adjustment screw on the position of the lamp within the housing will make it easier for you to align the lamp, which is required after lamp replacement.
11. Reverse steps 3, 2, and 1.



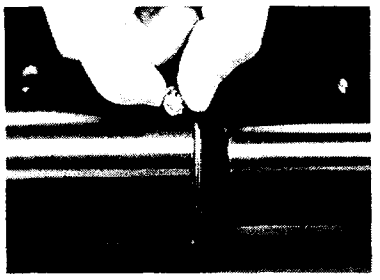
Step 9

If you have replaced an old lamp with a new lamp of the same wattage, you will need to adjust the lamp until maximum voltage level is attained. To do so, perform steps 5–10 of the lamp alignment procedure described on pages 25-27. If you have replaced an old lamp with a new lamp of different wattage, perform all of the steps of the lamp alignment procedure.

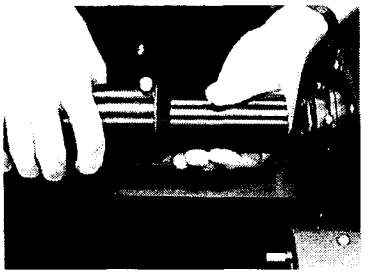
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ALIGNING THE LAMP

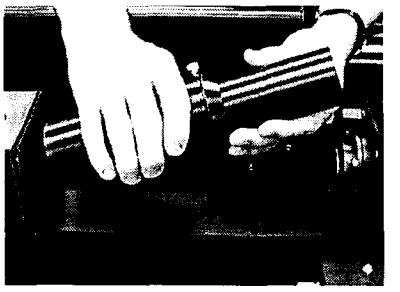
If you have replaced an old lamp with a new lamp of different wattage (i.e., of different physical size), you must align and adjust the voltage of the new lamp after installing it. Because the lamp is on during alignment, **you should always wear heavy goggles (such as welder's goggles) while aligning your lamp. Failure to wear goggles during alignment can cause blindness**. *OLIS* can perform lamp alignment for you if you prefer.



Step 1

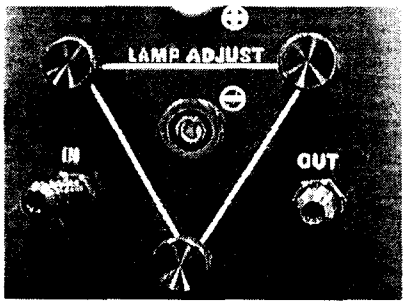


Step 2a



Step 2b

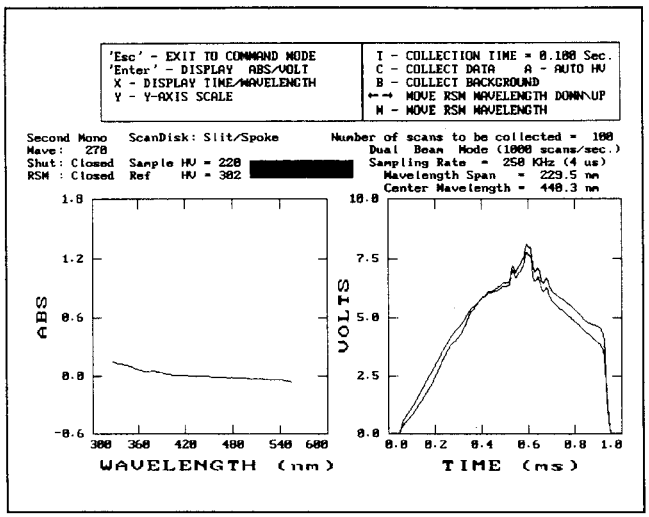
1. Loosen the thumbscrew on the telescoping tube between the lamp housing and the RSM.
2. Grasp one end of the telescoping tube with one hand and the other end of the tube with the other hand. Push the two sections of the tube together, causing the end with the smaller circumference to slide approximately 1" into the end with the larger circumference as shown in photo 2a and allowing the tube to be slipped out of its port and removed as shown in photo 2b.



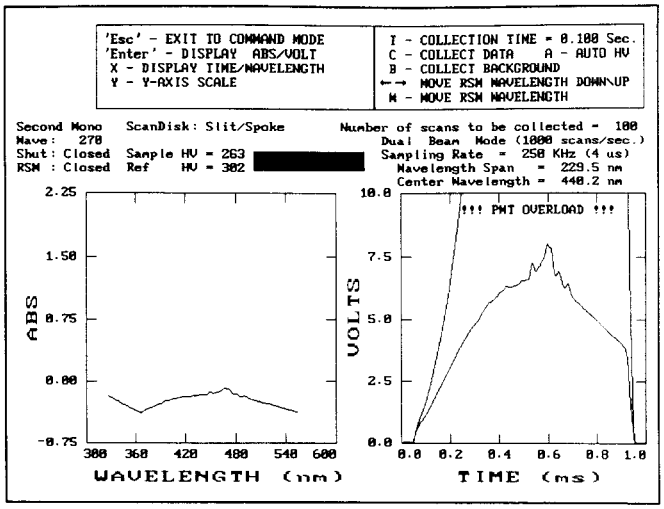
Step 3

3. The object of alignment is to maximize the level of light passing through the observation cell or cuvette; maximum light throughput will occur only when the lamp is accurately positioned in the lamp housing (relative to the mirror). Turn each of the three adjustment screws at the end of the lamp housing until the light beam is focused on the entrance slit. Continue to turn the screws until the light is a 'hot spot,' i.e., an intense spot of light, approximately the size of a dime (15–20 mm) projected at the middle of the entrance slit.
4. Reverse steps 2 and 1 to replace and secure the telescoping tube.
5. Insert 400-line gratings, a 0.6-mm entrance slit, a 0.12-mm exit slit, and a 0.2-mm ScanDisk in the RSM. Go to the Parameter Setup Menu in the RSM software. Under Data Collection Parameters, set Collect to Rapid-Scan Data, set Beam Mode to Dual Beam, and set Mode to No Average Mode. Under Monochromator Parameters, set Lines of the Grating to 400 and set Blaze Wavelength to 550 nm.

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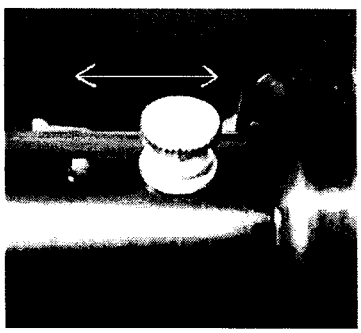


Step 6

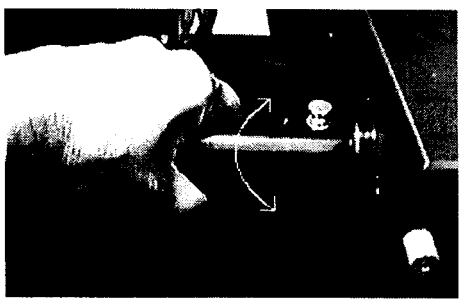


Step 7

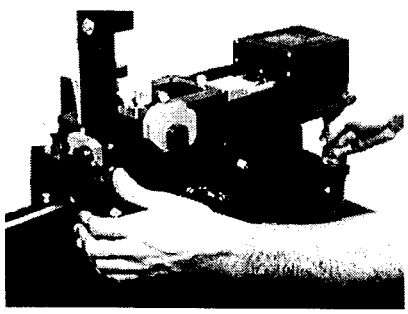
6. Go to Live Display; the <u>shapes</u> of the sample and reference beams should be as close to identical as possible and the two beams should lie one on top of the other. The photo for step 6 illustrates what you should be striving to attain; if you are using gratings other than the 400-line gratings, the shape of the beams will be slightly different than shown in the photo, but the beams should nevertheless be the same shape, should be superimposed one atop the other, and the signal should be maximized. If the shapes are not identical, you will need to fine-tune the alignment by turning the three adjustment screws on the lamp housing again; this time, your adjustments should be slight, since you made gross adjustments in step 3. While observing the voltage signal on the Live Display, gently adjust one screw to maximize the signal, then go to another screw and adjust slightly to maximize the signal, then repeat with the third screw. Continue adjusting each screw in turn until any adjustment causes a decrease in voltage.
7. The PMTs will very likely become overloaded during the alignment procedure, as illustrated in the photo for step 7. This screen is accompanied by a loud beeping sound. Each time this happens, press "A" for Auto HV to automatically adjust the voltage. The Sample HV and the Ref HV will decrease as the light throughput is maximized.



Step 8a



Step 8b



Step 8c

8. If the <u>shapes</u> of the two beams cannot be adjusted such that they are very similar, adjust the focal length by moving the thumbscrew on the lens tube as shown in photo 8a until the two beams are better aligned. If the beams are still not shaped almost identically, rotate the lens tube gently as shown in photo 8b to improve alignment. If you are using the *OLIS* stopped-flow, you may need to adjust its position very slightly, as shown in photo 8c, to see if the beam shapes improve.

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9. If the alignment is still imperfect, inspect the optical path to reaffirm that nothing is blocking the light. Be sure that the gratings are properly seated and that the entrance and exit slits are fully engaged. Purge the monochromator with nitrogen to clean the optical path. If you are using a stopped-flow, you should clean it to ensure that residue or air bubbles are decreasing light throughput. Also, double-check the Parameter Setup Menu to ensure that the settings reflect the hardware you are using.
10. If you cannot achieve proper alignment, or if you are unsure about your results, call *OLIS*.

LAMP PURCHASE/ MAINTENANCE RECORD

Additional notes:

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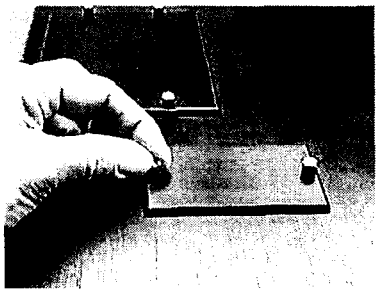
GRATINGS

Grating access doors should be removed **only** when you are changing gratings.

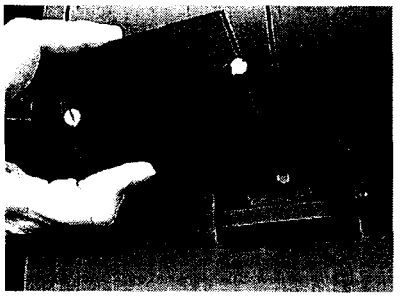
If the RSM is not producing spectra in the region of interest, make sure that you are using an appropriate pair of gratings. The number of lines per millimeter and the blaze wavelength are printed on the back of every grating. The number of lines determines the dispersive power, wavelength span and resolution of your instrument; the blaze wavelength is the wavelength for which the grating is optimized.

CHANGING GRATINGS

Before changing gratings, turn on the nitrogen gas to ensure positive pressure during the process and to prevent dust from entering the monochromator.



Step 1

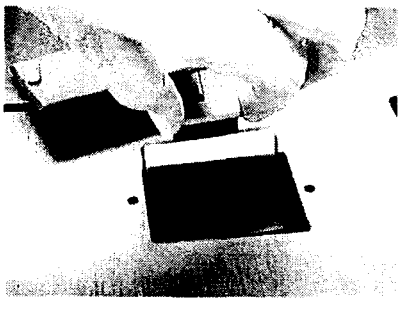


Step 2

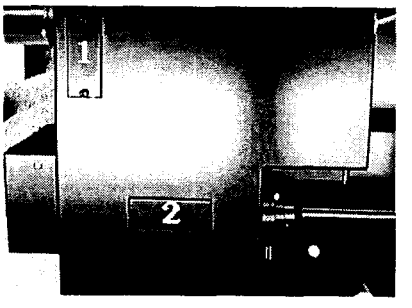


Step 3

1. Loosen the thumbscrews on the top of the grating access door.
2. Remove the grating access door.
3. Remove tension from the spring-locked clip that hold the gratings in place by pushing the handle at the top of the grating 90° in either direction.



Step 4



Step 5

4. Remove the grating by grasping the sheet-metal "ears" on the top of the grating and lifting the grating straight up and out of the monochromator.
5. Insert the new grating, ensuring that it is facing the correct direction. When you are standing in front of the RSM, the grating in the first position (labeled "1" in the photo) should be inserted with its mirrored surface facing the right and the grating in the second position (labeled "2" in the photo) should be inserted with its mirrored surface facing you (i.e., the front of the monochromator).

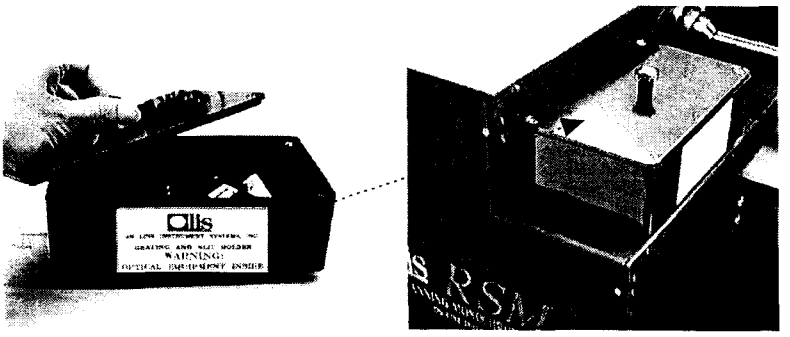
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6. Return the springclip to its original position; you will hear a distinctive click when the springs are set properly in place—if you do not hear a click, gently adjust the position of the springclip and/ or the grating until you hear the click.
7. Repeat steps 1–6 for second grating.
8. Change the monochromator parameters in the software to reflect the new gratings (the menu is found under "Parameter Setup" in Command Mode).

CLEANING & STORING GRATINGS

Do not touch or attempt to clean the surface of the gratings. Dust may be removed using a gentle stream of dry air.

When not in use, gratings should be stored in a safe place, such as in the *OLIS* Grating and Slit Box. The box fits neatly on top of the DeSa monochromator, as shown.



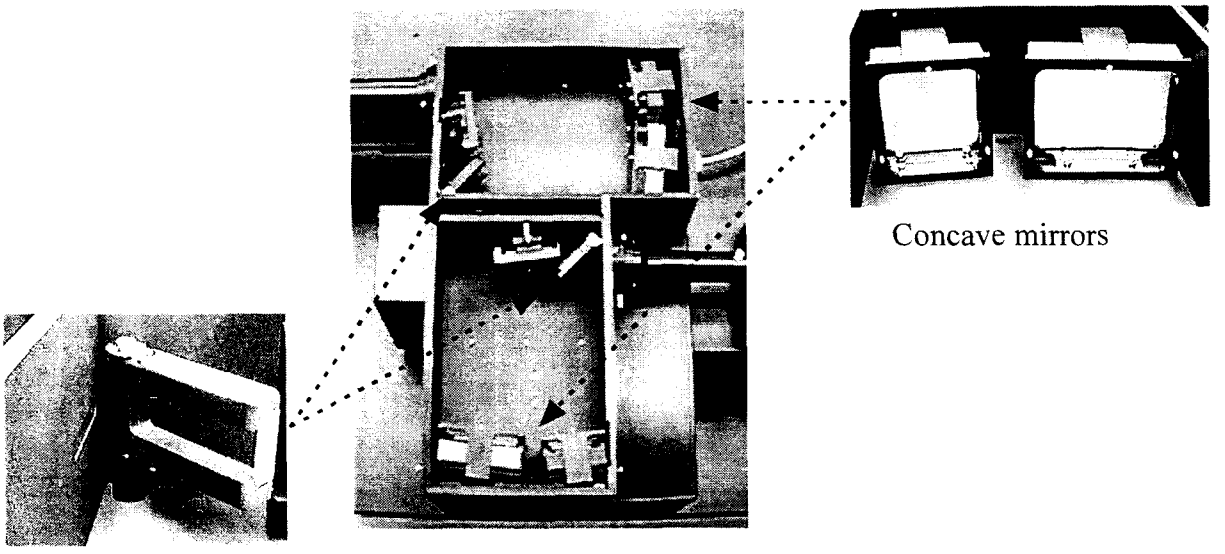
GRATING PURCHASE/ MAINTENANCE RECORD

Additional notes:

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MIRRORS

There are six mirrors inside the DeSa monochromator, four concave mirrors to reflect and collimate the light beam and two plane mirrors to turn the spectrally dispersed beam 90°. The photo below of the RSM optical path identifies the two types of mirrors used and their positions.



Concave mirrors

Plane mirror

These mirrors should last indefinitely, but can be damaged. Infrequently, the xenon light beam will damage the aluminum oxide coating on the mirrors, producing a white aluminum oxide film. To ameliorate this potential problem, we recommend that you block the light beam from reaching the optical components whenever the RSM is on but not making measurements. Three methods are available. *OLIS* offers computer-controlled shutters for this purpose; we also offer a "blank slit" that can be quickly substituted for the entrance slit between measurements. The "free" method, which was described on page 2, is to pull out the entrance slit approximately ½" between measurements; this moves the slit opening out of the optical path, preventing the light from entering the monochromator.

The magnesium fluoride coating on the mirror has a very low solubility in water. When the mirrors are exposed to humidity, they can begin to disintegrate. We recommend that you purge the mirrors with a steady stream of nitrogen to protect them from any damage caused by humidity (see page 12).

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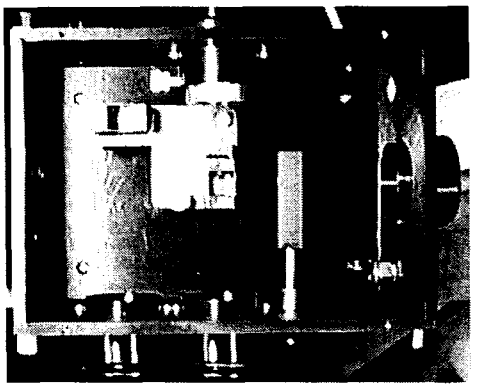
SAMPLE CHAMBERS & CUVETTES

Many different sample chambers are available from *OLIS*; we can also accommodate special experimental needs by making a sample chamber to order.

TYPES OF SAMPLE CHAMBERS

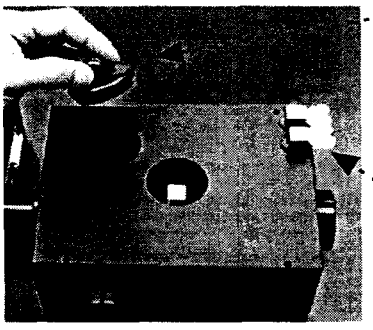
Standard Absorbance/ Fluorescence Sample Chamber

The typical sample chamber for absorbance and fluorescence contains holders for rectangular cuvettes; cuvette holders may be standard or jacketed. The cuvette holders are mounted on the floor of the sample chamber, as shown at right.

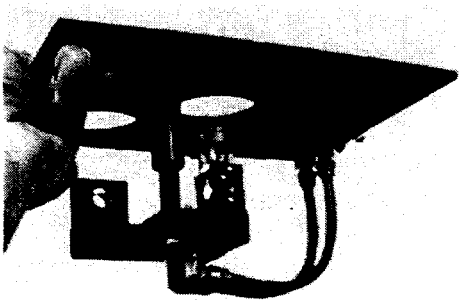


Hanging Cuvette Holder

The *OLIS* hanging cuvette holder offers two different cuvette positions and features built-in water lines for sample temperature control.

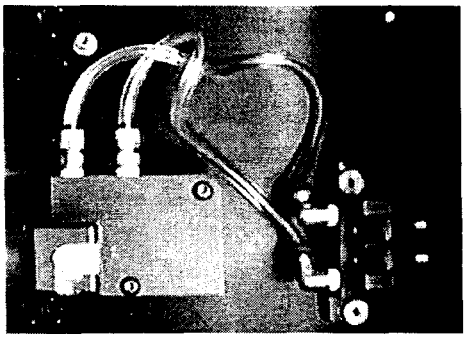


- Cuvette holders are easily accessible using the lift-off lids positioned over each cuvette position.
- Water line hook-ups are external.



CD Sample Chamber

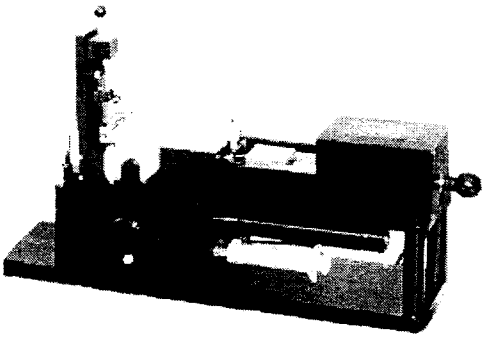
The CD sample chamber accommodates one cylindrical cuvette and two rectangular cuvettes. The rectangular cuvette holders are parallel to each other. Water lines are built in and hook-ups are external to the chamber.



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Stopped-Flow

To study reactions caused by mixing two solutions very quickly, a rapid-mixing device may be used. The *OLIS* U.S.A.-Stopped-Flow is one of the available "stopped-flows." The *OLIS* stopped-flow can be used with most spectrophotometers; it can be mounted to an existing instrument or connected by means of optical cabling to a stand-alone stopped-flow. Standard pathlength is 20 or 10 mm; deadtime is ≤ 2–3 milliseconds; reagent volume requirement is ≤ 70–150 microliters of each reagent per shot.

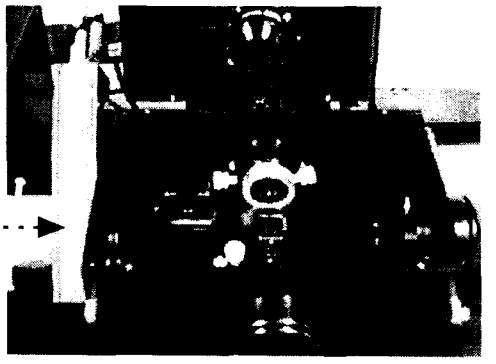


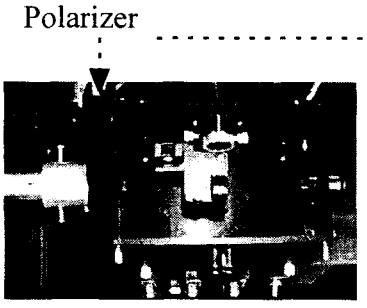
The *OLIS* USA–SF features a safety interlock system that does not allow the stopped-flow to be fired unless all connections are correct and secure. The USA-SF is computer controlled, but the safety interlock system works with or without computer control.

Illustrated instructions for changing stopped-flow syringes are provided on page 36.

Polarization Module

The *OLIS* PEM-based polarization module enables the user to introduce polarization to absorbance and fluorescence measurement (see page 11 for details). A T-format polarization module, diagrammed on page 9, is also available.





CUVETTES

Cuvette screws are adjusted in-house to accommodate typical cuvette size. Because cuvette size varies, two small adjustment screws are provided in each cuvette holder. Tighten or loosen the adjustment screws a half turn if necessary to produce a snug fit for the cuvette. Note: When tightening a cuvette holder, tighten it with the cuvette removed to avoid breaking the cuvette.

Cuvettes used with the RSM CD must be large enough to allow both light beams to pass through the sample, at least 10 mm wide. When using the RSM CD, any stress will induce bifringence. Be sure to use a high-quality cuvette made of fused silica.

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STOPPED-FLOW MAINTENANCE

Most maintenance procedures required to keep the *OLIS* U.S.A.-Stopped-Flow in good working order can be performed by the user. Routine maintenance practices are described below. Procedures for changing stopped-flow syringes are given on page 36.

Routine Maintenance

The importance of keeping your stopped-flow unit clean cannot be overemphasized. Treat your stopped-flow just as you would any sophisticated piece of laboratory equipment: keep it in good working order by cleaning it frequently.

1. Use denatured alcohol, followed by distilled water, to flush valves and syringes. Refer to the flow-path diagram on page 34. When flushing, push the plunger manually rather than firing the stopped-flow automatically through the software. Flush several times with the fill/flow handle and the exhaust handle in the "flow" position to clean out the drive syringes and the stop syringe. Then flush again with fill/flow handle on "flow" and the exhaust handle on "flush" to bypass the stop syringe and clean out the sample cell and viewing window.
2. If your *OLIS* stopped-flow is an anaerobic model, flush valves and syringes with de-gassed distilled water after flushing with distilled water to dispel any oxygen.
3. Use a toothbrush, cotton swab, test tube brush, or clean cloth as necessary to remove any dust, corrosion, or other type of contamination from all parts of the stopped-flow unit.
4. Gently wipe the stopped-flow unit using a clean cloth dampened with soapy water.
5. After the unit has been completely cleaned, finish by spraying furniture polish on a clean cloth and rubbing the cloth over the unit. Furniture polish protects the unit's anodized surfaces from chemicals and moisture.
6. Maintain clean water bath fluid at all times (see section on "water bath fluid mixture"). The water bath should be emptied and cleaned occasionally using a cotton swab or clean cloth. If the stopped-flow is to be left idle for some time (such as during a holiday), drain and clean water bath chamber and leave it empty during the period in which the stopped-flow will be left idle.

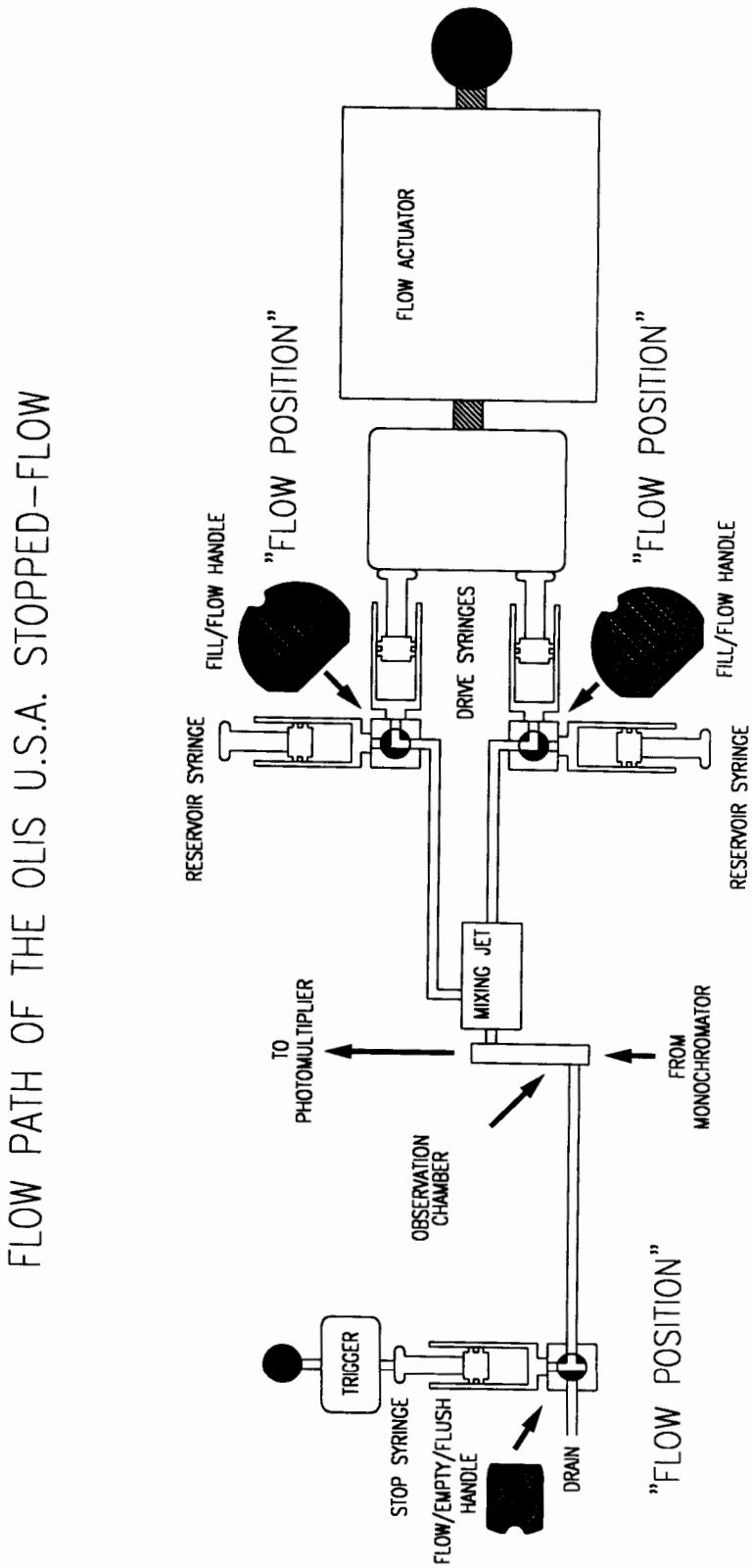
The *OLIS* U.S.A.-Stopped-Flow mixer will provide many years of service if properly maintained. **Failure to maintain your *OLIS* stopped-flow may void your warranty.**

Water Bath Fluid Mixture

Distilled water should be used as the stopped-flow water bath fluid. If you are using the stopped-flow at temperatures below freezing, add Methanol or antifreeze as needed to keep the water bath from freezing. As a general rule, approximately one part alcohol or antifreeze to ten parts distilled water is sufficient to prevent the fluid from freezing when the stopped-flow is used at temperatures to approximately –2°C. The temperature range is determined by the type of seals. *OLIS* has an ambient-range model (10–80°C) and a low-temperature model which has been tested to –10°C and likely can be used to –30°C or lower. Be sure to identify the type you have—operating a stopped-flow at temperatures outside of its range will damage it!

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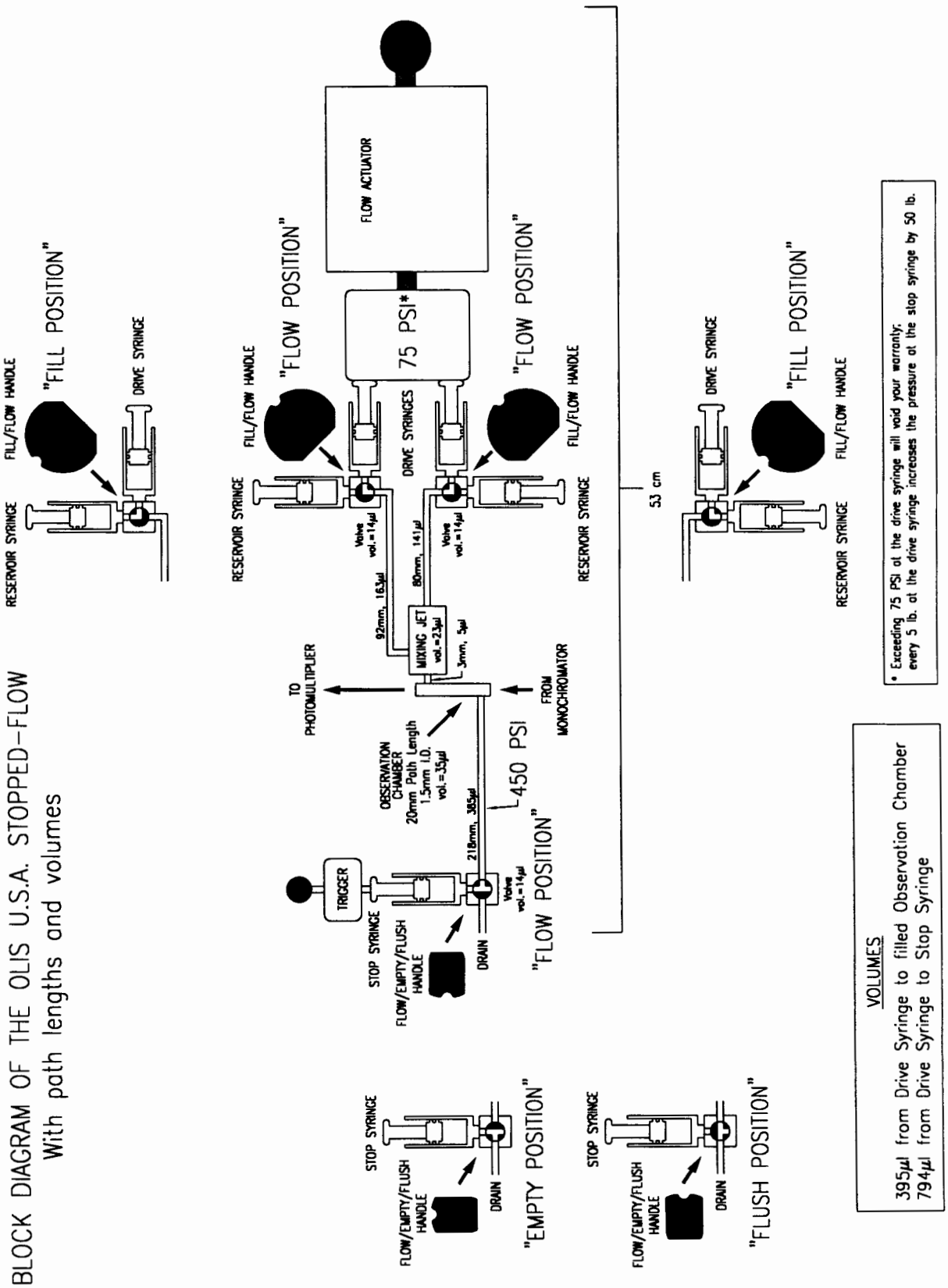
Flow Path of the OLIS U.S.A.-Stopped-Flow



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Block Diagram of the OLIS U.S.A.-Stopped-Flow

with path lengths and flow volumes



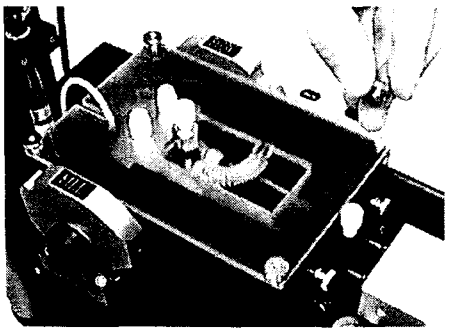
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Changing Syringes

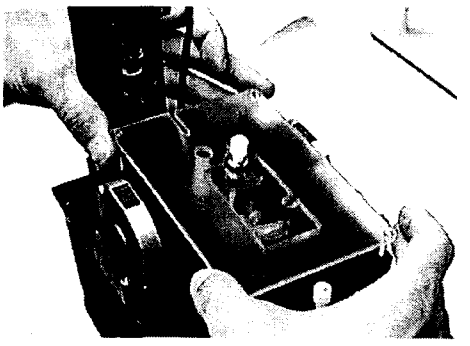
Changing the stopped-flow syringes is required when a syringe breaks or when you want to switch to variable-ratio mixing. Changing syringes is not difficult, but does involve the risk of damaging your stopped-flow. The following instructions take you step-by-step through the process and provide illustrative photos for most steps. **Before changing the syringes, read all the instructions to ensure that you understand the process and to assess your level of comfort in performing the task.**

Please note that overtightening a syringe during installation may severely damage the syringe and associated valve, rendering the stopped-flow inoperable and requiring expert repair by *OLIS* personnel. If you prefer not to change the syringes yourself, or if syringe replacement is not permitted by your institution, *OLIS* will be happy to replace syringes for you at no cost except the price of new parts and shipping and handling fees both ways. This warning is given not to discourage your from attempting to change the syringes yourself, but to ensure that you are aware of the risk of damage involved and to offer you the alternative of having *OLIS* take the responsibility.

The tools needed to change stopped-flow syringes are included in tool kit provided with purchase.

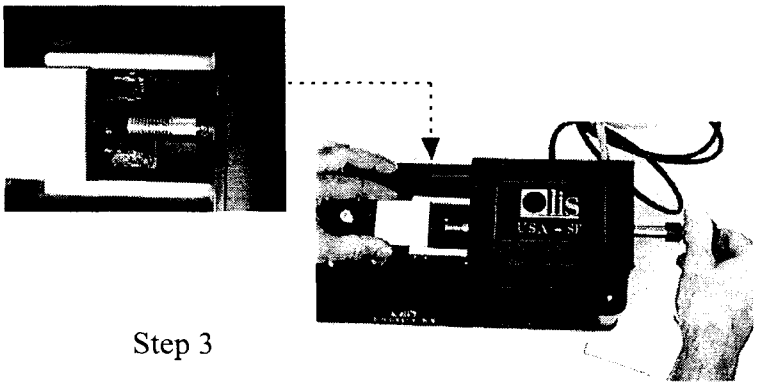


Step 1



Step 2

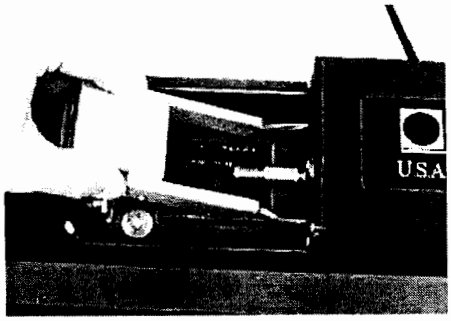
1. Remove the four thumbnuts from the cover of the water-bath chamber.
2. Gently remove water-bath cover, easing it carefully over syringe extenders.



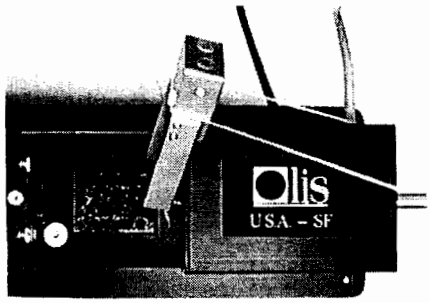
Step 3

3. In your right hand, grasp the black knob at the end of the cylinder shaft; in your left hand, hold the plunger block steady. Unscrew the cylinder shaft from the plunger block by turning the knob counterclockwise.

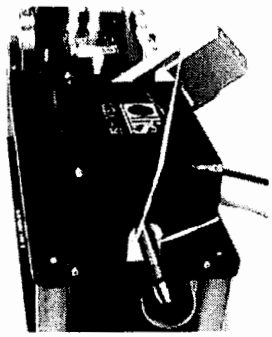
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Step 4a

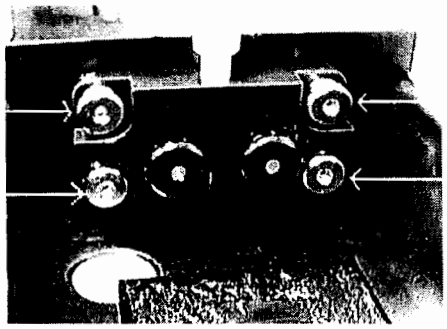


Step 4b

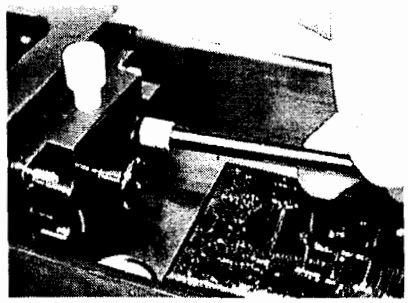


Step 4c

4. Lift the plunger block assembly as shown in photo 4a and move it out of the way; do not disconnect the wires, but allow the plunger block assembly to rest against the side of the air cylinder cover. To keep the plunger block assembly out of the way, you may secure it using a rubber band, as shown in photos 4b and 4c.

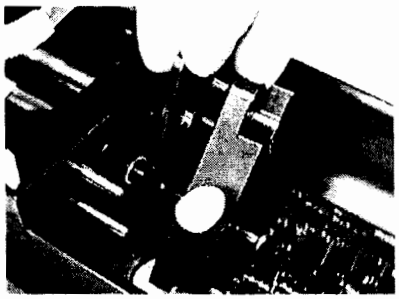


Step 5

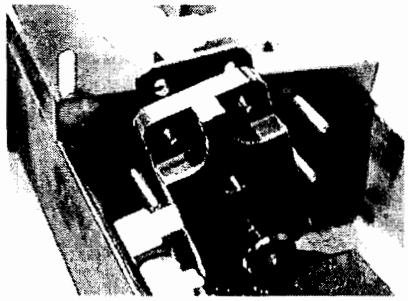


Step 6

5. Remove the four thumbnuts from the purge cap.
6. Remove the plunger from the body of the syringe by gently pulling it straight out.



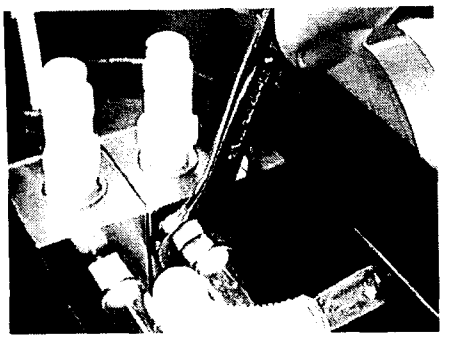
Step 7



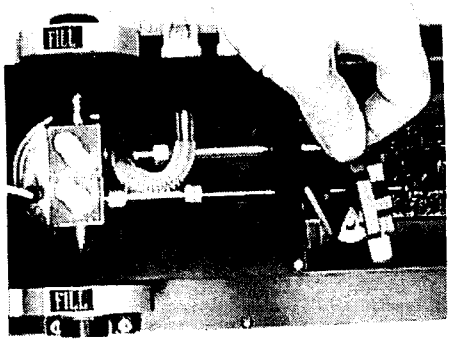
Step 8

7. Pull out the second syringe plunger slightly to free the purge cap; you will notice that the syringe clamp is directly behind the purge cap, as shown in photo 7. If the syringe clamp does not slide out along with the purge cap, use the tip of the Allen wrench to free it.
8. Rotate both the purge cap and the syringe clamp slightly and allow them to rest against the side of the stopped-flow in order to provide access to the syringe.

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Step 9

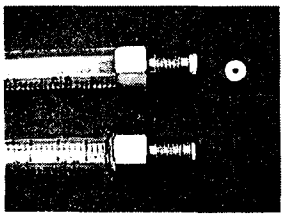


Step 10

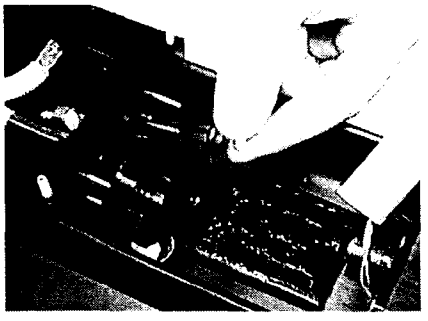
9. Using the 7/16" end of the wrench provided, loosen the stainless steel end of the syringe by turning the wrench counterclockwise.
10. Grasp the other end of the syringe and finish unscrewing it, then gently remove the syringe as shown in photo 10. Inspect the new syringe to ensure that a new black rubber O-ring is included (we provide a new O-ring with each new syringe); also, if you have the *OLIS* low-temperature stopped-flow with ceramic valves, ensure that a white safety washer is attached to the threaded end of the new syringe. The photo at right labeled "safety washer" shows a syringe <u>with</u> the safety washer attached (the syringe at the top of the photo) and a syringe <u>without</u> a safety washer (the syringe at the bottom of the photo); an unattached safety washer is lying to the right of the top syringe to show you what a safety washer looks like. As you can see, the safety washer looks very much like the white plastic tip of the syringe; with a safety washer attached, however, syringes appear to have a "double tip." Also, safety washers are slightly larger in diameter than the white plastic syringe tips. If you have the *OLIS* low-temperature stopped-flow and the new syringe does <u>not</u> include a safety washer or if the safety washer falls off during installation, do not use it; call *OLIS*. If you do not have the *OLIS* low-temperature stopped-flow, your new syringe should <u>not</u> have a white safety washer.



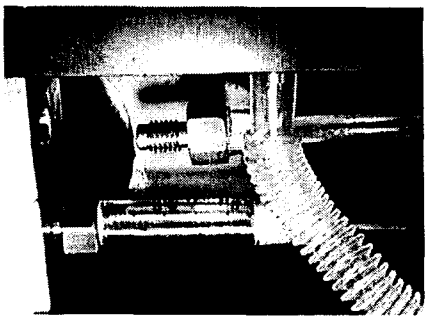
O-ring



safety washer



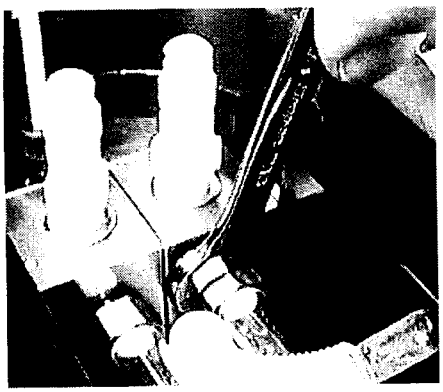
Step 11a



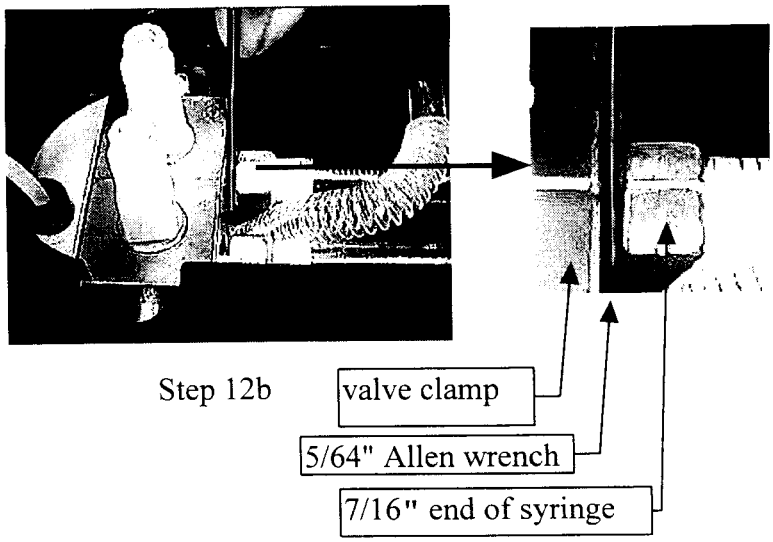
Step 11b

11. Carefully install the replacement syringe as shown in photos 11a and 11b. Secure the syringe in place by gently turning it clockwise; <u>hand tighten</u>.

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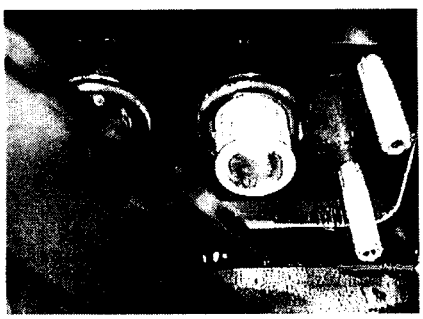
Step 12a



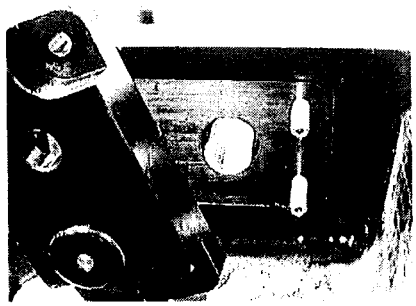
Step 12b

12. Using the 7/16" end of the wrench provided, complete syringe installation by turning the 7/16" end of the syringe approximately 45° clockwise. **This is a critical step because overtightening will cause permanent damage to the syringe and valves. It is better to undertighten than to overtighten; you can always further tighten the syringe if you find that the stopped-flow leaks. You will not feel a firm stop or hear a click when the syringe is sufficiently tight, and you should never attempt to tighten the syringe until it touches the valve clamp; when the syringe is correctly seated, a 5/64" Allen wrench will approximate the width of the space that should be left between the valve clamp and the 7/16" end of the syringe, as shown in the photos for step 12b.**

    Please note that the damage resulting from overtightening the syringe will cause the stopped-flow to leak just as undertightening will; however, leaking caused by under-tightening can be corrected by the user—leaking caused by overtightening causes damage (i.e., ruins the syringe and/ or valve) that requires expert repair by *OLIS* personnel, and parts are costly. Avoid damage by using the guideline described in the preceding paragraph to verify the width of the gap between the 7/16" end of the syringe and the valve clamp.



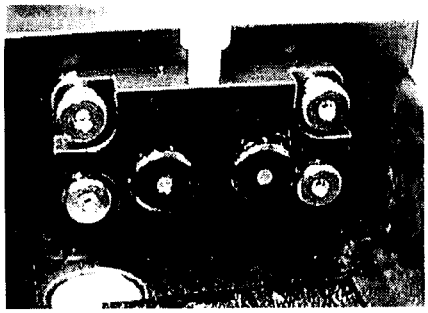
Step 13



Step 14

13. Gently push the O-ring until it is flush with the water bath chamber. The O-ring is a water seal for the bath chamber.
14. Slip the syringe clamp into place in the recession on the side of the water bath chamber.

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Step 15

15. Replace the purge cap and fasten it with the four thumbnuts. Dip the tip of the plunger in water to lubricate it for easy insertion, then install plunger in replacement syringe.* Gently push both plungers until they are flush with the purge cap as shown in the photo.

    **The syringe manufacturer suggests that placing the syringe plunger in boiling water for a few minutes before inserting the plunger in the syringe will cause the teflon tip of the plunger to swell, which may improve performance of the syringe.*

16. Replace the plunger block assembly (i.e., reverse step 4).
17. While holding the plunger block assembly in your left hand and the cylinder shaft knob in your right hand, screw the cylinder shaft into the plunger block assembly (i.e., reverse step 3).
18. Reverse steps 2 and 1 to replace and secure the cover of the water-bath chamber.

As stated in the introduction to this section, if you do not feel comfortable replacing stopped-flow syringes, or if equipment maintenance is not permitted by your institution, *OLIS* will gladly replace syringes for you. Call *OLIS* first to let us know that you are sending your stopped-flow for syringe replacement; your only expenses will be the cost of replacement parts and shipping/ handling fees to and from *OLIS*.

STOPPED-FLOW PURCHASE/ MAINTENANCE RECORD

Additional notes:

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PHOTOMULTIPLIER TUBES

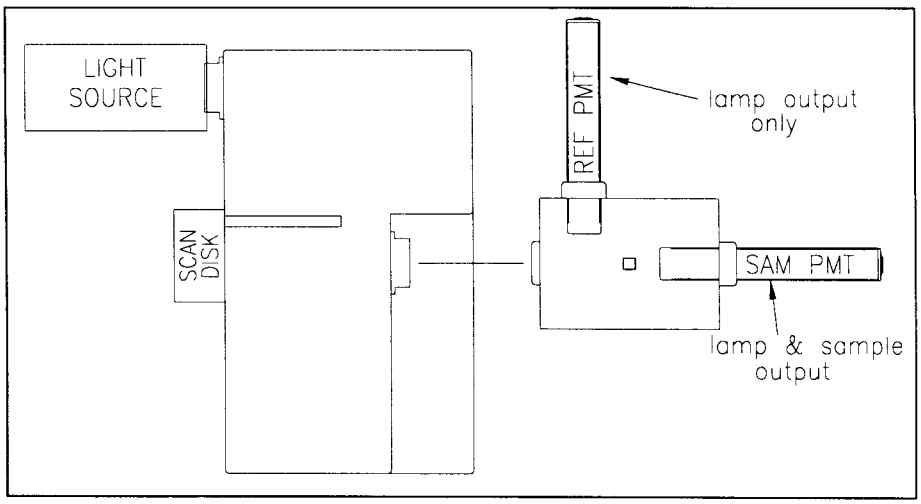
Because we use two PMTs and take the ratio of their signals, any difference between the PMTs is minimized to near insignificance. Nevertheless, we encourage you to heed any significant differences that appear over time, alerting you to the need to replace aging tubes.

APPROPRIATE PLACEMENT FOR SPECIFIC APPLICATIONS

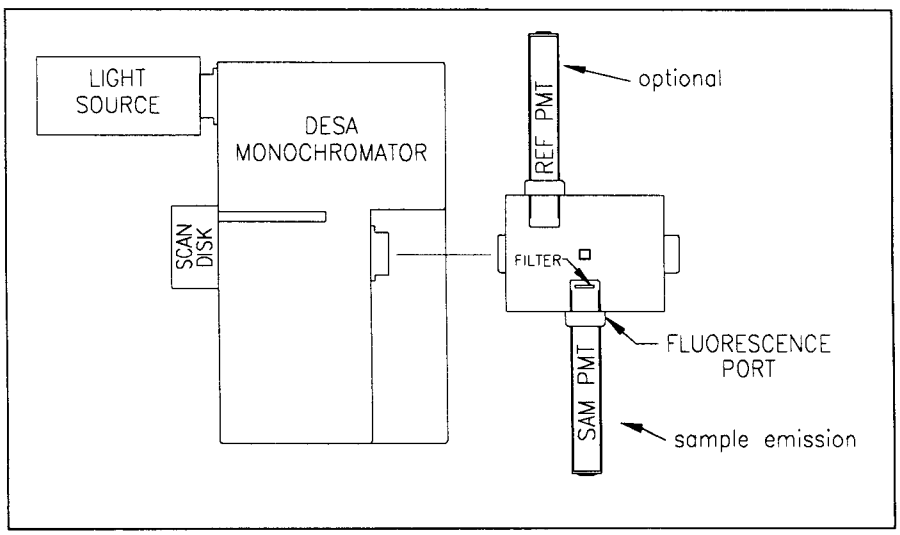
Because of the modularity of the RSM, one instrument can easily be optimized for multiple experimental situations. Some experimental needs can be met by merely changing the position and/ or type of PMT. The optical paths for each configuration are shown on pages 4-11.

Dual-Beam Absorbance

For dual-beam absorbance, the RSM 1000 configuration includes two PMTs positioned at right angles to each other.



Single- or Dual-Beam Fluorescence



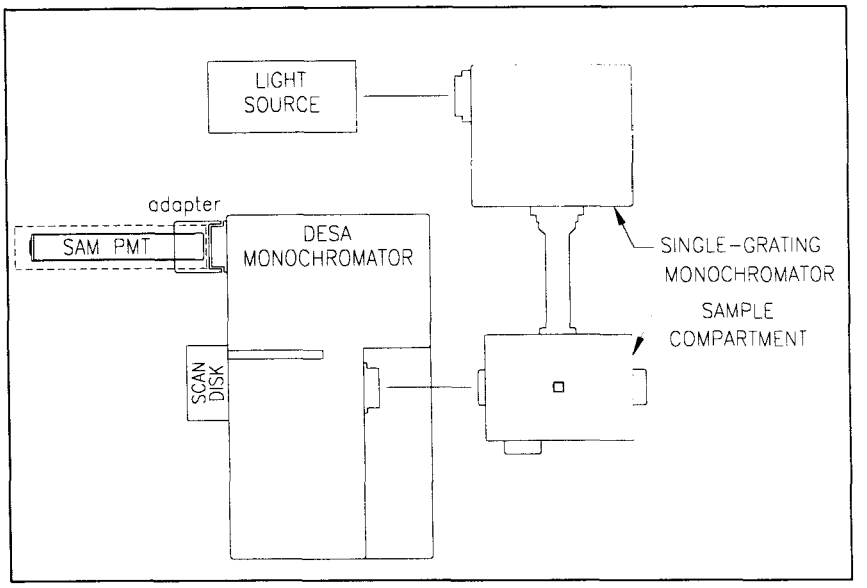
Fluorescence measurements can be made using the standard RSM 1000 simply by moving the sample PMT to the fluorescence port (at a 90° angle to the sample) and using a filter to select the emission wavelength. The reference beam is used or not, depending on whether the beam splitter is left in place.

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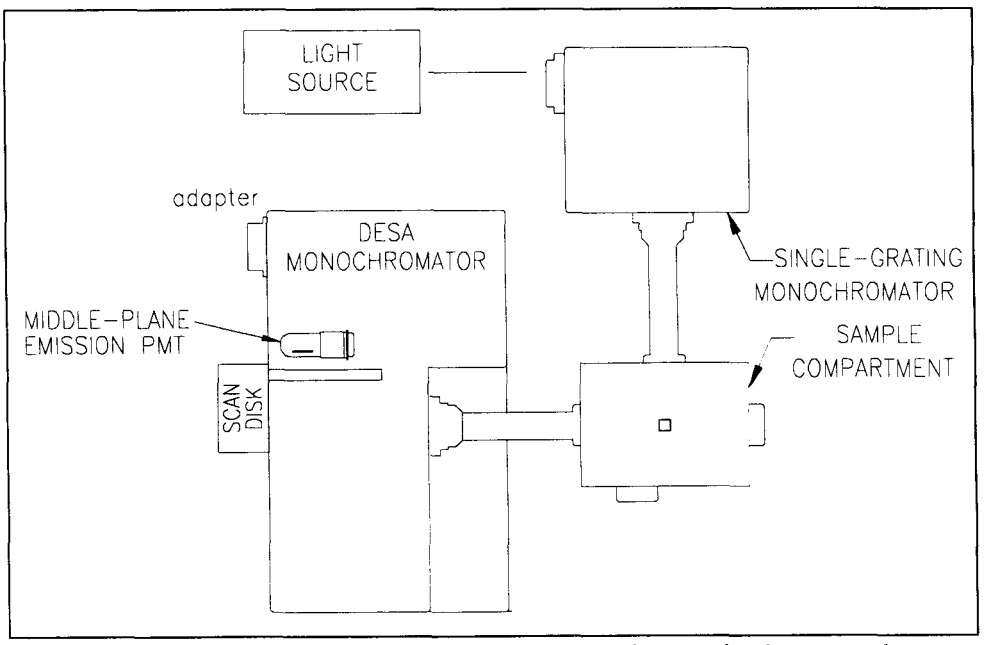
For optimum fluorescence measurement, the *OLIS* RSM 1000F (the RSM spectrofluorimeter) is recommended. In the RSM 1000F, the DeSa monochromator is coupled with the *OLIS* single-grating monochromator to produce **three different fluorescence configurations:**

Using the Entire DeSa Monochromator as Emission Monochromator

To use the DeSa monochromator to collect rapid snapshots (scans) of a changing fluorescent sample, the *OLIS* single-grating monochromator is used to excite sample fluorescence and the sample PMT is placed in the lamp port of the DeSa monochromator.



Using Half of the DeSa Monochromator as Emission Monochromator

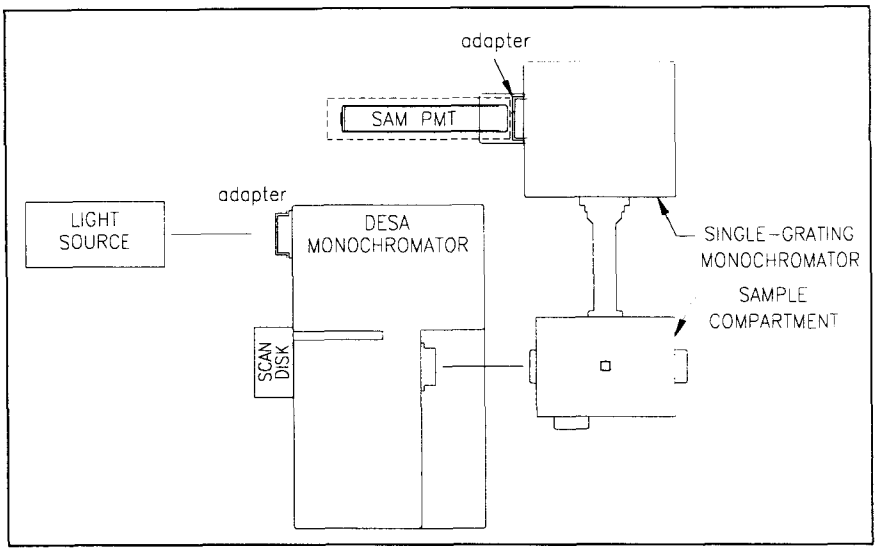


To increase the RSM's sensitivity during fluorescence measurements, the *OLIS* single-grating monochromator is used to excite sample fluorescence and the sample PMT is placed just beyond the ScanDisk cartridge; a mid-plane, side-window, fluorescence-optimized PMT is used (see page 44 for more about the *OLIS* mid-plane PMT). Sensitivity is increased substantially because this setup turns the DeSa (double) monochromator into a single monochromator, eliminating two mirrors and a grating from the path of the emission beam.

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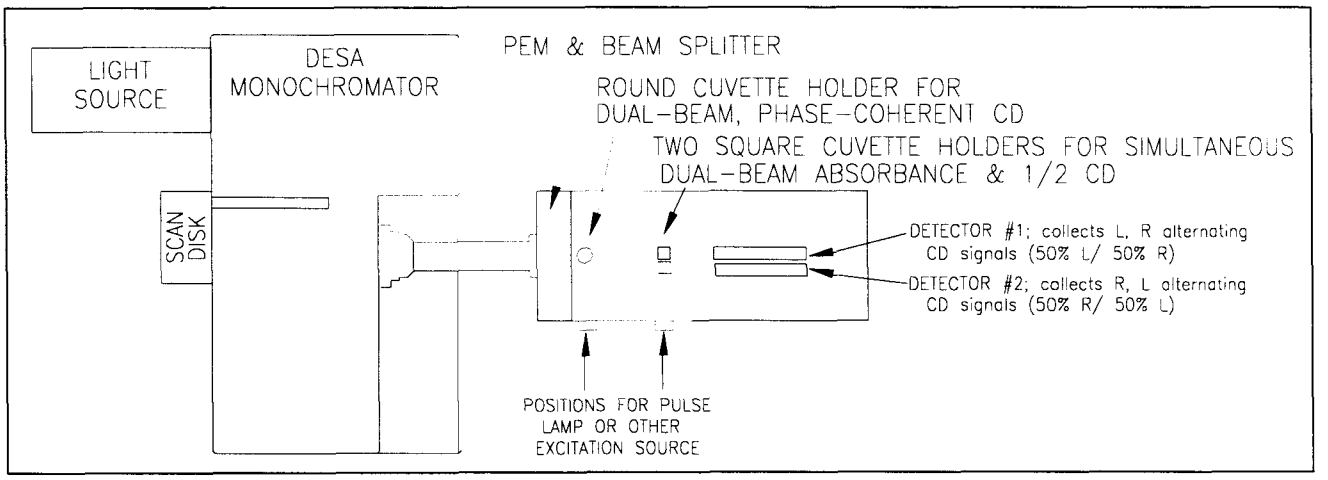
Using the DeSa as Fluorescence-Excitation Monochromator

To use the DeSa monochromator as the excitation monochromator, the PMT is placed (using an adapter) in the lamp port of the *OLIS* single-grating monochromator to capture the fluorescence emission excited by the DeSa monochromator. Rapid-scanning or single-wavelength excitation is produced, depending on the type of ScanDisk used.

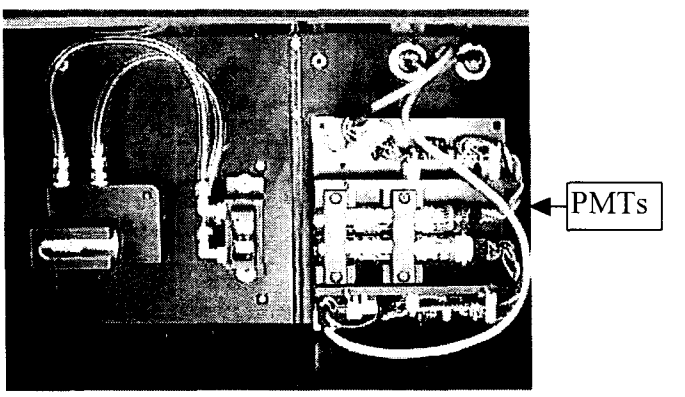


CD Configuration with Parallel PMTs

In the RSM CD, the two PMTs are parallel to each other, as shown in the diagram and photo of the CD sample compartment below.



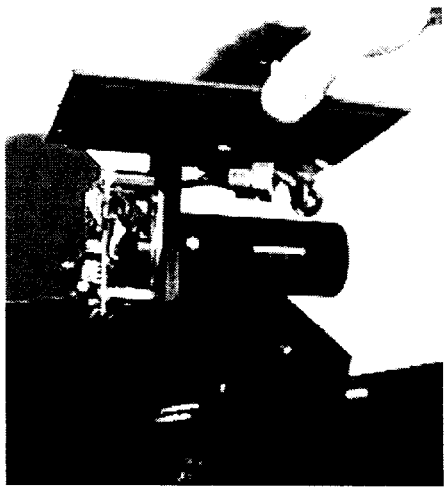
Unlike the RSM absorbance and fluorescence configurations, in which one PMT measures the lamp output and the other measures the beam after it is effected by the sample, the two PMTs in the RSM CD configuration both see the effect of the sample on the phase-coherent beams; one PMT measures the L/R circularly polarized beam and the other PMT measures the R/L circularly polarized beam.



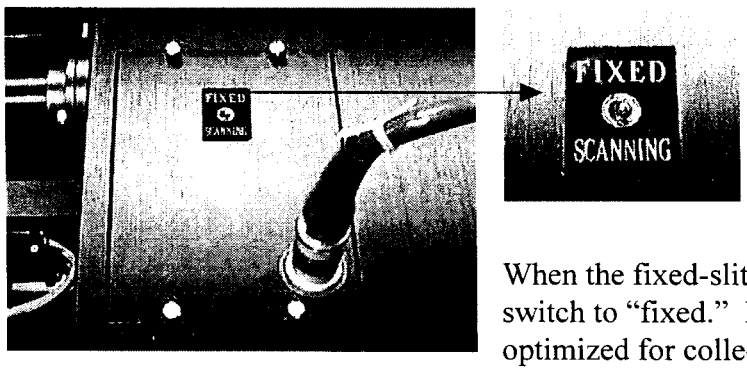
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OLIS MIDDLE-PLANE PMT

As explained and illustrated on page 41, for fluorescence work involving a sample with a weak emission signal, a mid-plane, side-window, fluorescence-optimized sample PMT can be used to increase the sensitivity of your RSM. The *OLIS* single-grating monochromator is used to excite sample fluorescence and the mid-plane PMT is placed just beyond the ScanDisk cartridge, turning the DeSa (double) monochromator into a single monochromator.



Changing Data-Collection Modes



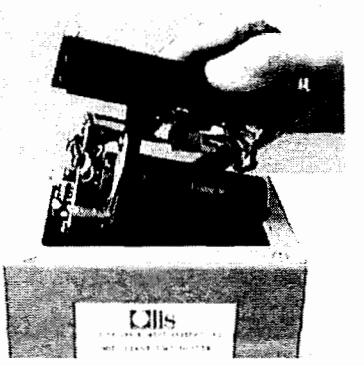
The *OLIS* Middle-Plane PMT has two modes for collecting data. A switch mounted on the mid-plane PMT lid allows the user to select the mode appropriate to the type of ScanDisk cartridge being used and the desired S/N.

When the fixed-slit ScanDisk is being used, flip the mode switch to "fixed." In fixed mode, the mid-plane PMT is optimized for collecting the large number of photons characteristic of single-wavelength detection using the *OLIS* RSM.

When the RSM is used in its rapid-scanning mode, fewer photons pass through the RSM's intermediate slit than when a single wavelength is monitored. When a scanning ScanDisk is being used, you may choose to flip the mode switch to "scanning." In scanning mode, the mid-plane PMT utilizes a resistor that increases the PMT's sensitivity, enabling the PMT to detect the maximum number of photons passing through the RSM's spinning intermediate slit. Because increasing sensitivity necessarily increases the amount of noise, the decision to utilize the PMT's scanning mode will be based on the S/N needed by the user.

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Handling & Storage



When inserting and removing the mid-plane PMT, purge the DeSa monochromator with nitrogen during the process to maintain positive pressure and prevent dust from entering the RSM.

Handle the *OLIS* Middle-Plane PMT very carefully. When not in use, store it safely in the container provided with purchase. Use care when installing the mid-plane PMT to ensure that it does not bump against the edges of the opening. After removing the mid-plane PMT from the RSM, put the PMT in its storage container immediately, using care to avoid bumping the PMT against the edges of the container opening.

PMT PURCHASE/ MAINTENANCE RECORD

Additional notes:

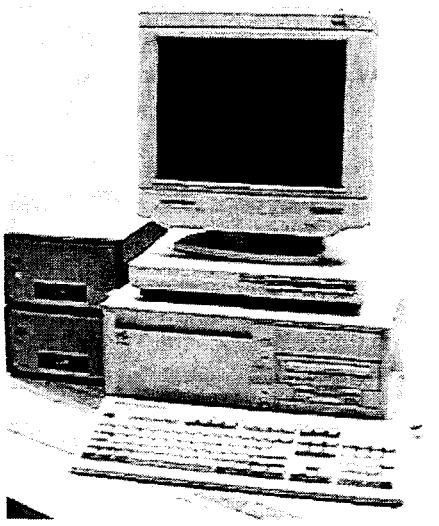
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COMPUTER

OLIS always supplies the current state-of-the-art computer system; therefore, the control unit detailed below will change as more advanced computer hardware becomes available and is confirmed compatible with our software.

COMPONENTS

The control unit currently supplied with all RSM systems includes a P5 133 computer with 16 megabytes of RAM, a 1.6-gigabyte hard drive, MS-DOS version 6.2 or higher, two RS-232 and one parallel port, a high-resolution graphics monitor, and a printer.



Other computer components include:

- Timer I/O board
- DAC board
- 12-bit A/D converter (some temperature-control instruments have an additional A/D converter or I/O card)
- Optional tape or zip drive for storage of RSM data

Refer to manufacturers' manuals for normal troubleshooting for your computer and peripherals. These manuals are provided at installation. If your computer hardware was purchased from *OLIS*, we will help you with diagnostics and repair.

Addresses used by the RSM software:	A/D board	360 - 36E	Hex
	Timer & I/O board	IA0 - IA3	Hex
	DAC board	300 - 30F	Hex

SAFETY CONSIDERATIONS

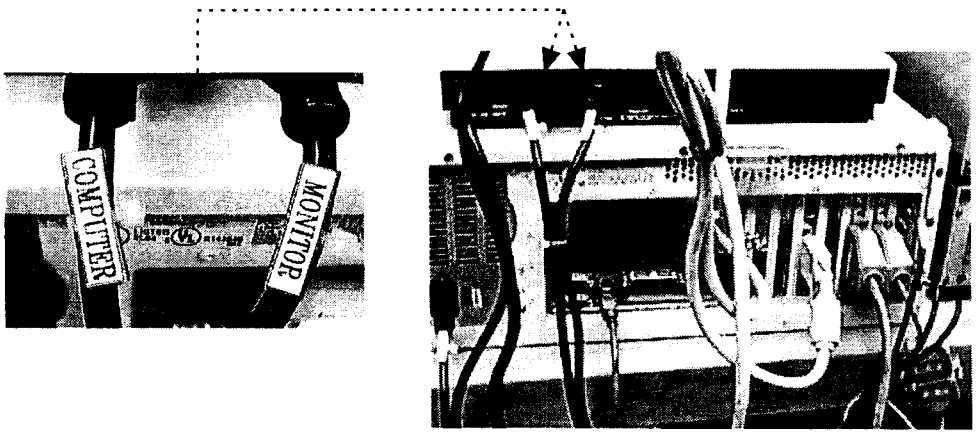
All components of the RSM system are sensitive to static charges and should never be connected or disconnected unless all power supplies associated with the RSM system are off and adequate measures have been taken to eliminate static electricity.

The computer is an essential component of the RSM system; treat it as such. Never swap the computer from your RSM system with any other computer in your lab. The performance of the RSM system is guaranteed only when it is used with the computer and operating system provided at installation.

Never start the lamp with the computer or any associated system electronic devices on; start the lamp first, then turn on the computer and other electronic devices.

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Note that every cable and outlet/ port are labeled to ensure correct connections. Do not remove labels.



COMPUTER PURCHASE/ MAINTENANCE RECORD

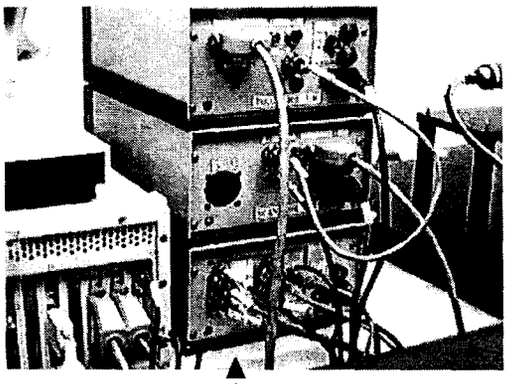
Additional notes:

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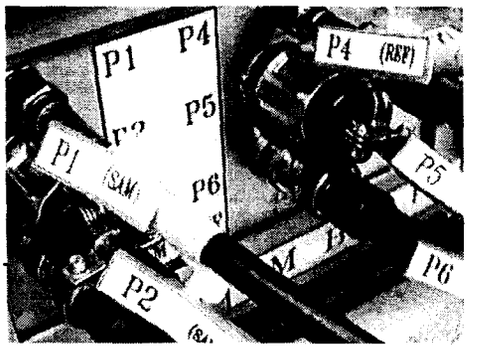
ELECTRONIC BOXES

Do not open and attempt to adjust or fix the electronic components without first calling *OLIS*. The primary reasons are the risk of electric shock and the potential for damaging the electronic components. In addition, unauthorized repair attempts will void your warranty.

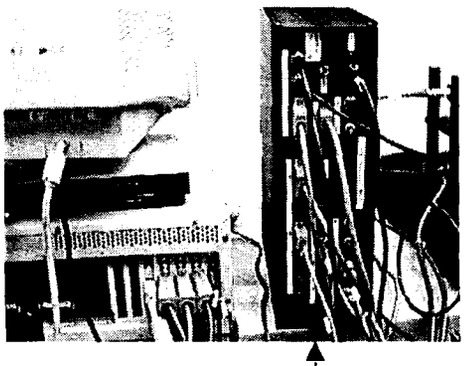
Note that all cables and outlets are labeled to ensure correct connections. Do not remove labels.

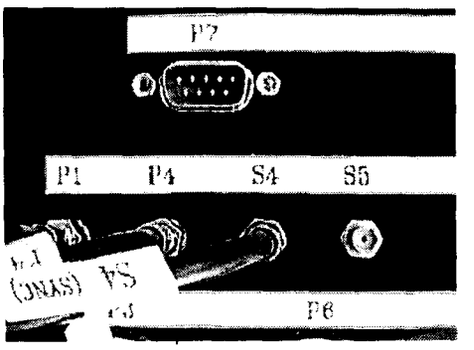


RSM systems purchased prior to June 1996 have two or more stacked electronic boxes; these are pictured at left, with a closeup of the labels pictured below.



A single electronic box comes with RSM systems purchased during or after June 1996, when all of the electronic components were condensed into a single electronic box, which is pictured below along with an enlarged photo of the labels.





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ELECTRONIC BOX PURCHASE/MAINTENANCE RECORD

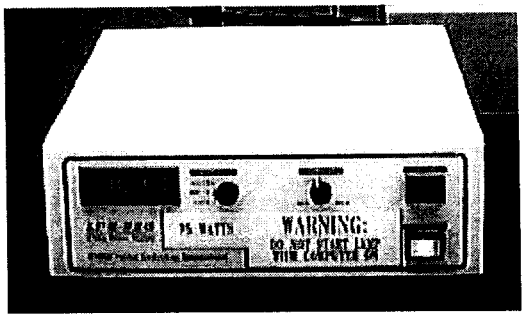
Additional notes:

Additional notes:

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POWER SUPPLY

The power supplies for the 75/150-watt lamp and the 450-watt lamp are pictured below. A power supply is also available for 1000-watt lamps (not pictured).



Power supply for a 75- and 150-watt xenon arc lamp



Power supply for a 450-watt xenon arc lamp

Prominently displayed on the front of each *OLIS* power supply is an important warning: **Do not start the lamp with the computer on. Always turn the lamp on first, then turn on the computer and other electronics.** The importance of this warning cannot be overstated; if you start your computer while the lamp is on, the resulting power surge will instantly melt every circuit in your computer.

WARNING:
DO NOT START LAMP
WITH COMPUTER ON

When your RSM system is installed, *OLIS* provides you with the owner's manual that comes from the manufacturer of your particular power supply. Refer to the owner's manual if you have problems with your power supply.

POWER SUPPLY PURCHASE/ MAINTENANCE RECORD

Additional notes:

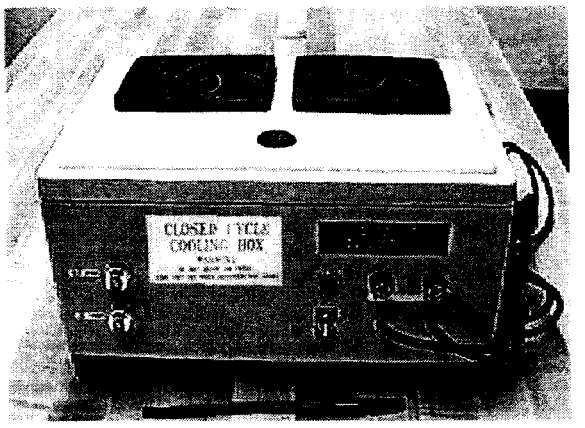
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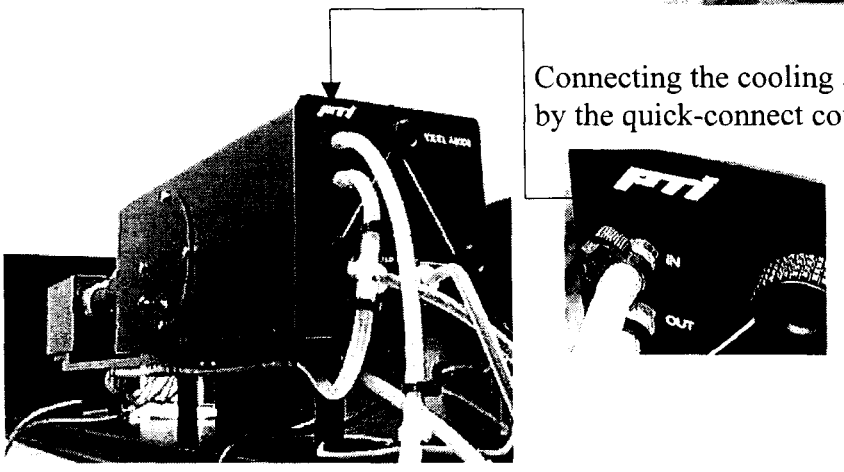
WATER-COOLING DEVICES

OLIS offers three cooling devices, two for cooling lamps and one for cooling the light beam.

OLIS CLOSED-CYCLE COOLING SYSTEM

The *OLIS* closed-cycle cooling system is a stand-alone lamp cooling box that is fitted with a pump, a heat exchange coil, and two fans to dissipate the heat. It provides sufficient heat-exchange capability for lamps to 1,000 watts, and is required for lamps 150 watts and greater. The closed-cycle cooling system is 7½" H x 13" W x 11 ¼" D, and weighs 14 lb. The ball-point pen in the photo foreground provides scale.





Connecting the cooling system to your lamp is facilitated by the quick-connect couplers. All hoses are labeled, as are the corresponding ports on the lamp housing; simply connect each hose to its appropriate port. Plastic clamps are provided for securing the hoses in place along the underside of the lamp housing.

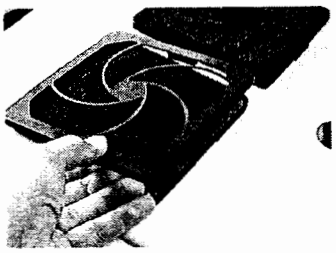
A flow sensor and a safety interlock system ensure that the lamp being cooled cannot be operated unless the cooling box is connected and operating correctly. This built-in safety feature also ensures that accidental disruption of the water flow during operation—due to hose damage, disconnection, or any other unanticipated situation—will not cause your lamp to explode; the lamp automatically shuts off if the cooling system is compromised in any way. Therefore, if your lamp fails to start or shuts off during operation, check the cooling system connections.

The circulating fluid—which is a mixture of antifreeze and water—remains pure, which prevents clogged hoses and corrosion of metal parts. The fluid never needs replacing.

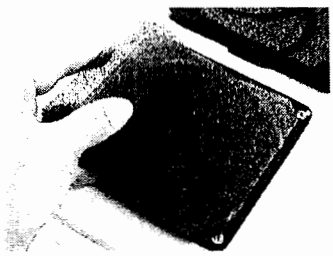
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Cleaning the Filters

The cooling system filters should be cleaned regularly to ensure the efficient operation of the system.



Step 1



Step 2

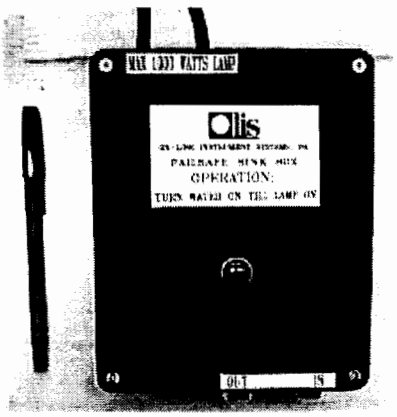
1. Pry off the snap-on filter cover.
2. Remove filter. Shaking the filter will remove most of the dust. If necessary, remove stubborn dust by holding the filter under running water; allow filter to dry before replacing.
3. Replace filter; replace filter cover by pressing down on all sides till you hear it snap into place.

OLIS FAILSAFE SINK BOX

The *OLIS* Failsafe Sink Box requires an external water source; in every other way, it operates in the same way as the *OLIS* Closed-Cycle Cooling System.

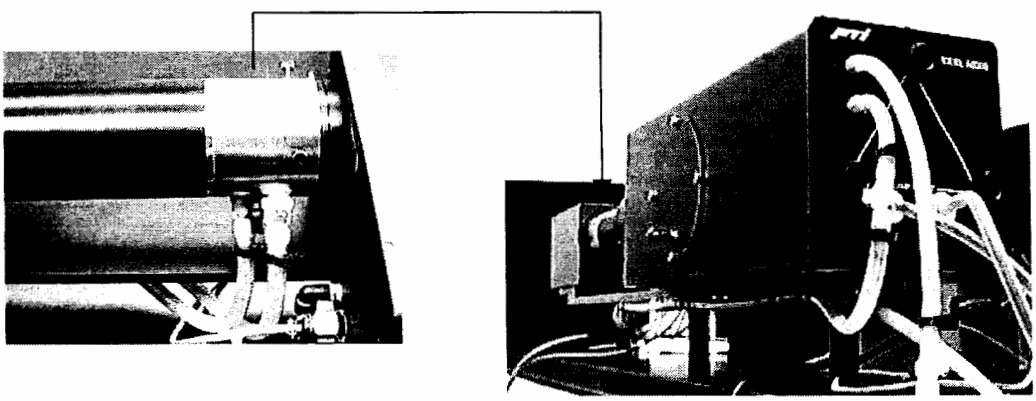
The Failsafe Sink Box is 2½" H x 7"W x 5 ¼" D, and weighs only 2 lb. The photo at right shows the sink box next to a ball-point pen for scale.

The internal filter inside the Failsafe Sink Box will remain clean for years, depending on the amount of sediment in your water source.



OLIS BEAM-COOLING ACCESSORY

The *OLIS* Beam-Cooling Accessory removes the IR from the lamp output beam, offering protection for photolabile samples and the RSM's mirrors, gratings, and filters.



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Ideally, you will use the *OLIS* beam-cooling accessory in conjunction with the *OLIS* closed-cycle cooling system or the *OLIS* failsafe sink box because doing so conveniently provides cooling for both the lamp and the beam while offering the safety feature of both *OLIS* lamp-cooling accessories.

OTHER MANUFACTURERS' COOLING DEVICES

Other cooling devices can be used with *OLIS* instruments, including Dewar cells. Hardware customization of the sample compartment may be necessary, but is available at a reasonable cost.

If you are using a water-cooling device for the cuvette, be aware that the fluid you are using to cool the cuvette will condensate so you must keep the cuvette holder dry by purging it with nitrogen gas.

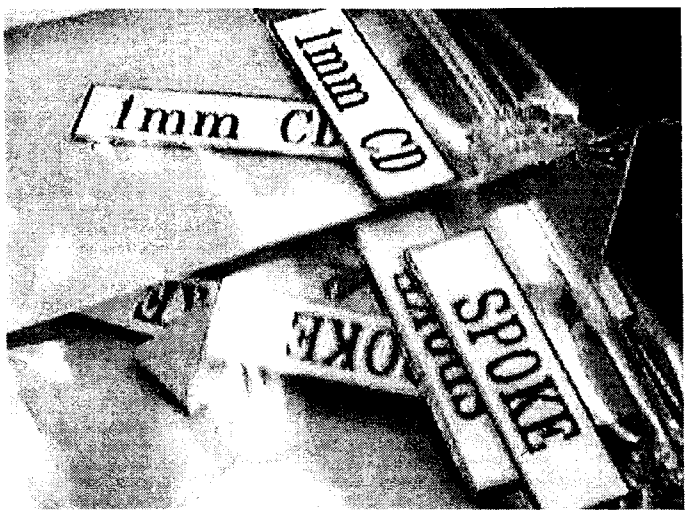
COOLING DEVICE PURCHASE/MAINTENANCE RECORD

Additional notes:

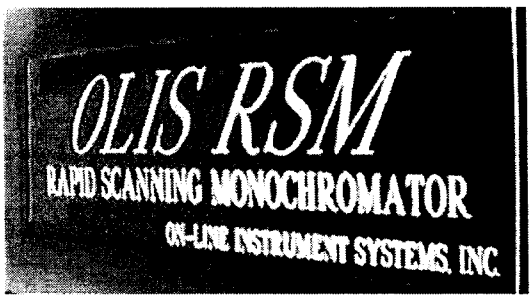
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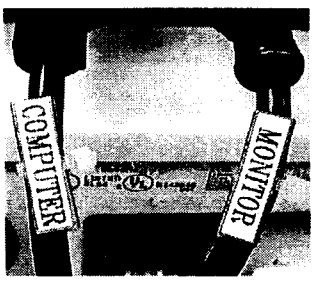
LABELS, LABELS, LABELS!

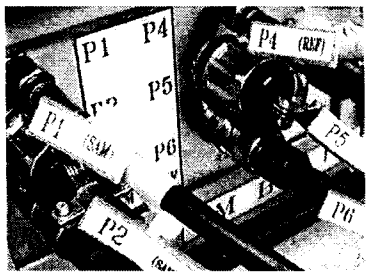
We love labels because they make life easier for both you and the *OLIS* staff! Labels mean that you do not have to guess about important connections. Labels also serve as a 'common language' for communications between you and the *OLIS* staff.



You will find labels everywhere on your RSM system; please do not remove the labels—they are your friends!











The serial number of your RSM system can be found on a plaque on the front of the DeSa monochromator, as shown below. (NOTE: this RSM belongs to the enlightened, bold scientist who bought the very first RSM! Dr. Robert Blake of Xavier University in New Orleans recently returned his system for the upgrade to support polarization of fluorescence.)



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IMPORTANT INFORMATION

Serial Number of your RSM: _____

RSM Model: _____

Date your RSM was installed: _____

Components included with your RSM at time of original purchase/ installation:

Component	Type/ Size
Monochromator	
Monochromator	
Filter	
Filter	
Entrance Slit	
Exit Slit	
ScanDisk	
ScanDisk	
Lamp	
Lamp	
Power Supply 75-watt	
Power Supply 450-watt	
Power Supply 1000-watt	
Gratings	
Gratings	
Sample Chamber/ Cuvette Holder	
Sample Chamber/ Cuvette Holder	
PMTs	
PMTs	
Computer	
Electronic Box(es)	
Water-cooling device	

OLIS address and phone numbers: 130 Conway Drive, Suites A & B
Bogart, GA 30622-1724
800/852-3504
706/353-6547
Fax: 706/353-1972
E-mail: olis@mindspring.com
Website address: www.olisweb.com

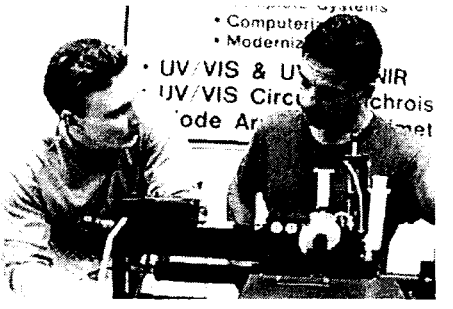
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TROUBLESHOOTING GUIDE

To assist you in diagnosing minor problems that may occur with your RSM system, this troubleshooting guide provides symptoms of potential problems and appropriate remedial adjustments. Of course, there is no substitute for the quality, expert assistance that the *OLIS* staff can provide via telephone.



If it is necessary to return any part of your RSM system to *OLIS* for repairs, we will replace the ailing item with a "loaner" while repairs are being made so that your important work is not interrupted. If you have any qualms about making minor adjustments to your instrument, or if equipment maintenance is not allowed by your institution, please do not hesitate to call *OLIS*.



Please help us help other RSM owners by reporting problems that do not appear in this troubleshooting guide so that we may add them to future guides.

SAVING FACE

It happens to everyone at one time or another: you call *OLIS* to report a problem you are having with your RSM. A friendly staff member asks a few questions and your responses suggest a simple and—in retrospect, obvious—solution. Perhaps your gratings were not properly seated, making it impossible for you to reach the spectral region you have targeted. Perhaps you forgot to turn on your *OLIS* lamp-cooling device, making it impossible for you to turn on your lamp.

It is easy to overlook "obvious" details when your mind is focused on an important experiment. It is also easy to skip over the simple causes and assume that something more serious may be causing a problem you are having with your RSM. Taking a few moments to think about the problem logically may save you time, frustration, and—in the case that you overlooked the obvious solution—face! For example:

1. When you change gratings or switch between slit and spoke ScanDisks, be sure to update your data-collection and monochromator parameters to the components you are using.
2. Ensure that your gratings are a matched pair and that they are properly seated and facing in the correct direction.
3. Keeping your stopped-flow clean is essential to obtaining accurate results; e.g., residue in the stopped-flow can decrease the light throughput of your RSM.
4. 'In the beginning, there should be light:' at the beginning of each session with your RSM, turn on the light (lamp). THEN, turn on the computer. Never fire the lamp when the computer is on! And remember that the lamp will not fire unless the cooling device (if you are using one) is on.
5. Be sure that your instrument is plugged in all cables are fully engaged.

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6. Make sure that the entrance and exit slits are correctly positioned.
7. If someone else used the RSM before you, check for filters at the PMTs which would decrease the light throughput and could prevent you from reaching the wavelength range you are targeting.

ANOMALOUS DISPLAYS

Wobbling Spectra

When you first start up your RSM, you may notice that spectra are wobbling, vibrating, or jumping slightly (1–2 nm) from side to side. This wobble is caused by instability of the ScanDisk motor, which corrects itself within a minute or two of starting the RSM program. If the wobble does not correct itself within a couple of minutes, call *OLIS*. However, if the wobble is greater than a couple of nanometers, see the section below on "Sliding Spectra."

Sliding Spectra

If, upon entering the RSM program, you observe spectra sliding from one side of the screen to the other, or sliding off of the screen, it is possible that your A/D board is out of sync or not accurately sensing the wheel (ScanDisk) speed. To correct this, you will need to check the connections between the computer and the electronic box.

First, check the cable labeled "C5 (SYNC)" on the back of your computer (see photo below left). The other end of the sync cable connects to the "SYNC" outlet on the back of the electronic box (see photo below right). Unplug and reconnect both ends of the cable to ensure proper connections to the computer and the electronic box.





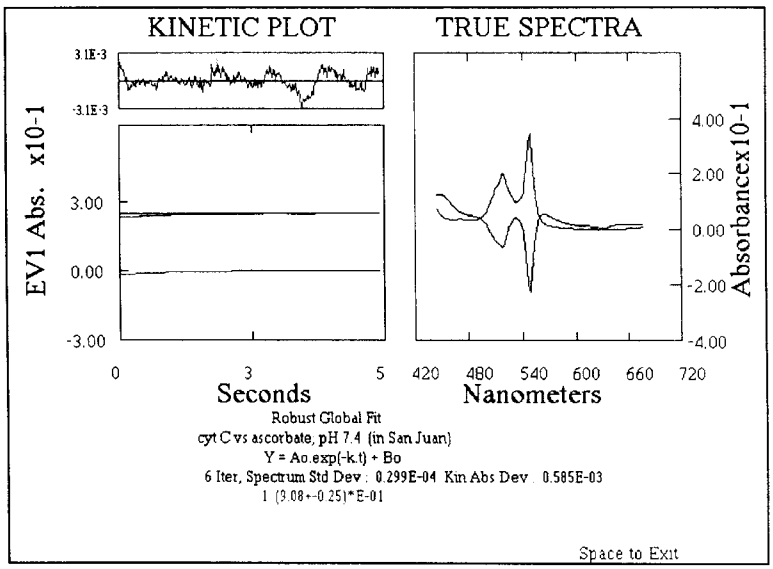
If this procedure does not rectify the problem, call *OLIS*.

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Funky Spectra

Remember that the Robust Global Fitting (RGF) program reconstructs the spectra of the reaction species based on the mechanism the user believes describes the chemical process occurring during the reaction being monitored. If the user chooses the incorrect mechanism for spectral reconstruction, the program produces spectra based on the incorrect mechanism. The results are negative absorbance spectra, upside-down spectra, mirror-image spectra, and other odd forms that do not occur in the real world. These unbelievable spectra are a tool the user employs to evaluate fits.

For example, among the *OLIS* software's demonstration files is a reaction involving cytochrome C and ascorbate; when the user directs the RGF program to reconstruct the spectra of the species involved in this one-exponential reaction based on the mechanism "first order with background," the screen shown at right is displayed. Because the mechanism is actually a "first order growing in" process, the software is forced to draw the spectra of cyt C and ASC based on a mechanism that does NOT describe the species involved. The result: mirror-image spectra, indicative that the wrong mechanism was chosen.



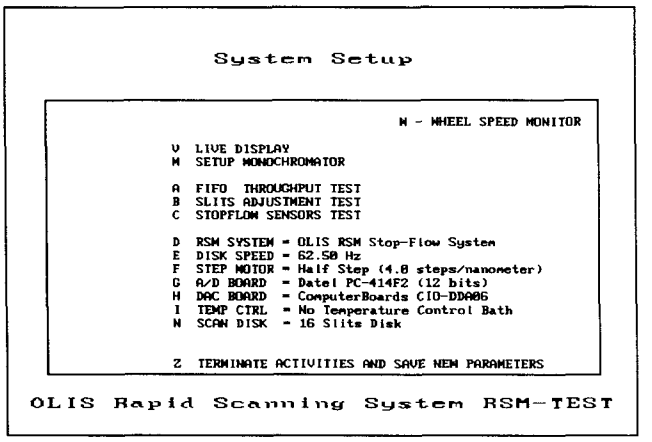
Aside from the unbelievable spectra that will be displayed when the wrong mechanism is chosen for spectral reconstruction, anomalous displays may be a sign that something is not quite right with your RSM.

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FIFO THROUGHPUT TEST

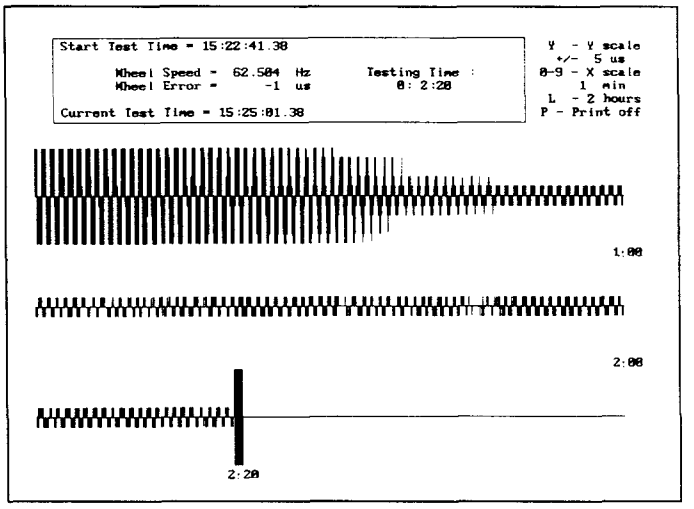
The RSM 1000 and its associated computer software must be correctly configured to ensure that data can be collected at full speed (1000 scans per second). We have observed that similar computers can be different enough that some will not be able to collect data at full speed. The software OLIS has provided is configured for a 133 MHz Pentium and the PC414F A/D board. The purpose of this procedure is to find data collection parameters for a particular combination of **your** computer and A/D board. These tests are **not** routine; they are necessary **only** during initial setup and calibration and after changing computers or other electronic hardware. However, should you suspect that your RSM is not performing correctly, this procedure can be used to help you analyze your computer and A/D board and adjust the software to ensure correct data collection.

1. Enter the RSM test program by typing "RSM-TEST" at the MS-DOS prompt. Screen #1 will appear.



Screen #1

2. Select option "W-Wheel speed monitor." This display shows the error (in µs) of the spinning ScanDisk. The nominal frequency is 62.5 Hz. When one enters the RSM-TEST program (and the RSM 1000 program), the error will be large at first. After a few minutes, the spinning of the ScanDisk will stabilize with an error of only ±1 µs, which is one part in 16,000 (screen #2). If the ScanDisk has a larger error, please contact an OLIS staff member.



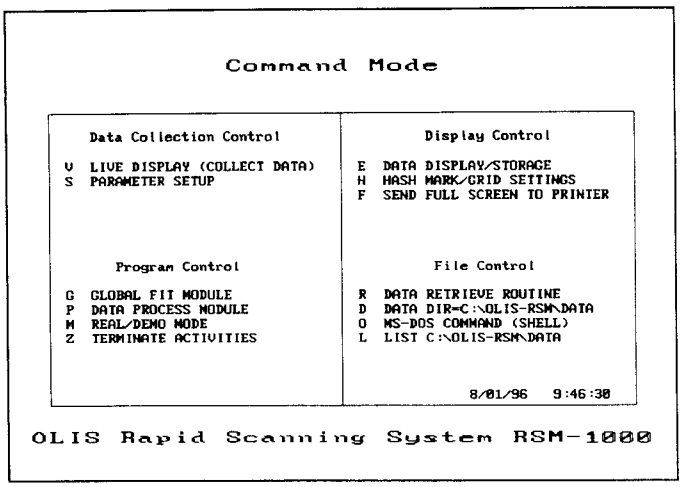
Screen #2

3. From the main System Setup screen, select option "A-FIFO throughput test." This test will determine the specific data transfer parameters (from the FIFO buffer on the A/D board to the computer's memory) for your computer. It is not necessary to run this test at 1000 kHz. If the FIFO throughput test fails before completion, contact an OLIS staff member.

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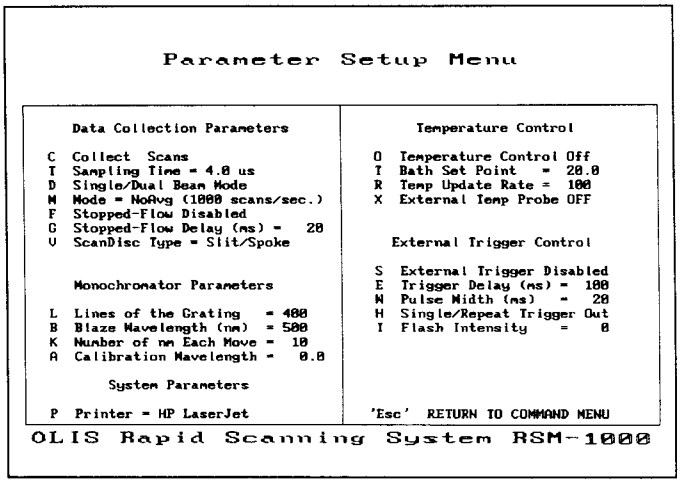
4. Exiting the RSM-test program (by pressing "Z") will write the new parameters to a file ("RSM-HDW.CFG"—RSM Hardware Configuration).

5. Type "RSM" at the MS-DOS prompt to enter the RSM-1000 program. After the automatic monochromator calibration, you will be presented with the "Command Mode" screen (screen #3).



Screen #3

6. Select option "S-Parameter Setup." The parameter setup menu will be presented (screen #4). Make sure that the "Lines of the Grating" and the "Blaze Wavelength" settings in the "Monochromator Parameters" block are set according to the gratings you are using. Specifically, make sure that:
   a) the External Trigger is disabled;
   b) the Stopped-Flow is disabled;
   c) the ScanDisk type is set to "slit;"
   d) the Mode is set to "No Avg
   e) (1000 scans/sec)"
   f) Dual-Beam Mode is selected;
   g) "Collect Scans" is set.



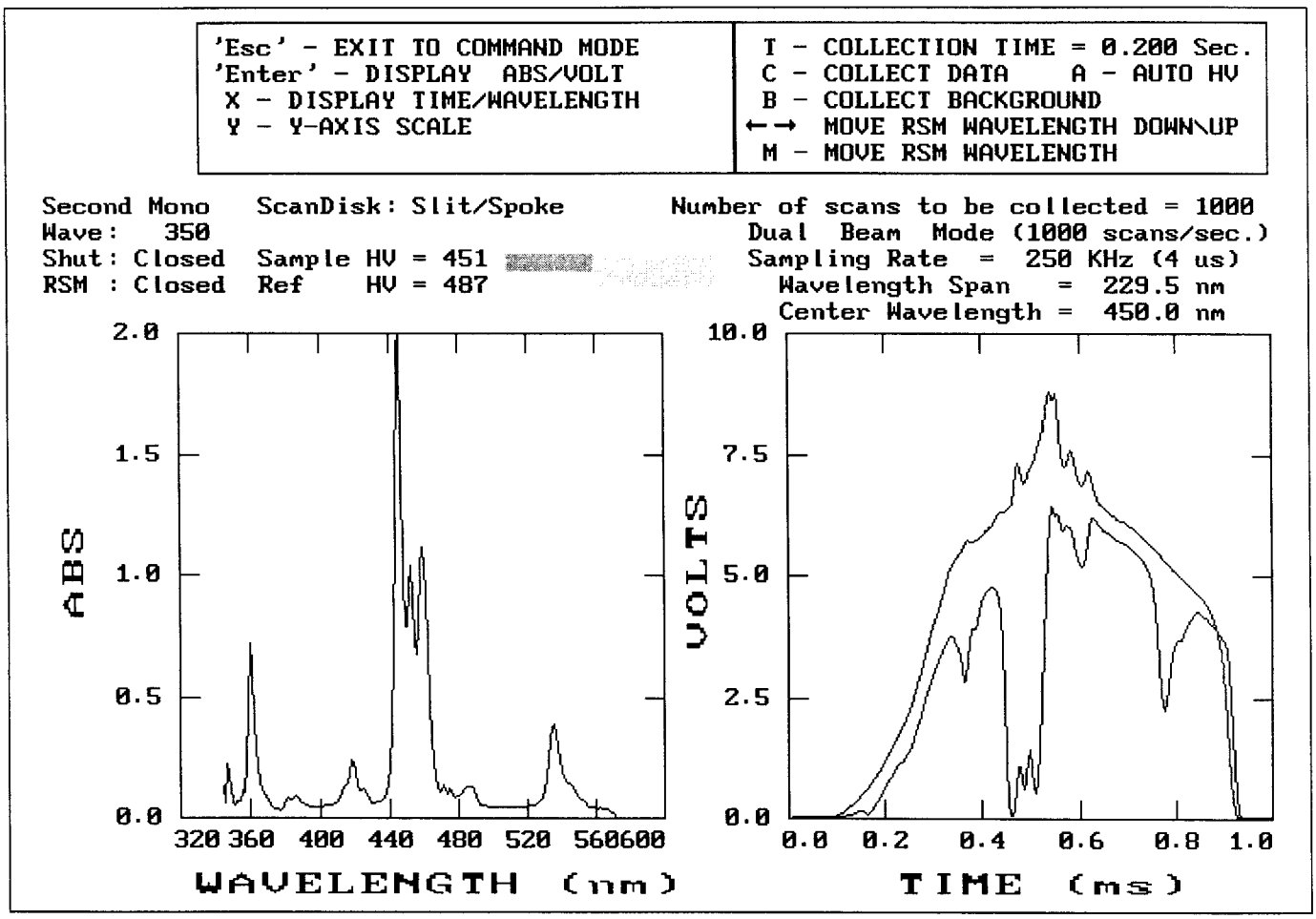
Screen #4

7. Return to Command Mode by pressing "Esc" in the setup parameters menu.

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8. Enter Live Display by pressing "V." In Live Display:
   a) use "M-Move RSM Wavelength" option to select 450 nm as the center wavelength (make sure that the RSM shutter is open, if appropriate);
   b) adjust the High Voltage on the PMTs using the "A-Auto HV" option;
   c) insert the holmium oxide filter in the light beam path; live display should look like screen #5;
   d) set the Collection time (option "T") to 0.2 sec;
   e) collect data.

Note: Do not collect Baseline in this case, since the absolute spectrum is of no particular interest here.



Screen #5

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9. Examine the data in the 3-D display (option "T-Create/display 3-D data"). Since the sample is not changing during data collection, all scans should be identical. Press "shift–" (shift key and minus sign simultaneously) or "–" (minus sign) to adjust the Z skip for better display, if necessary.

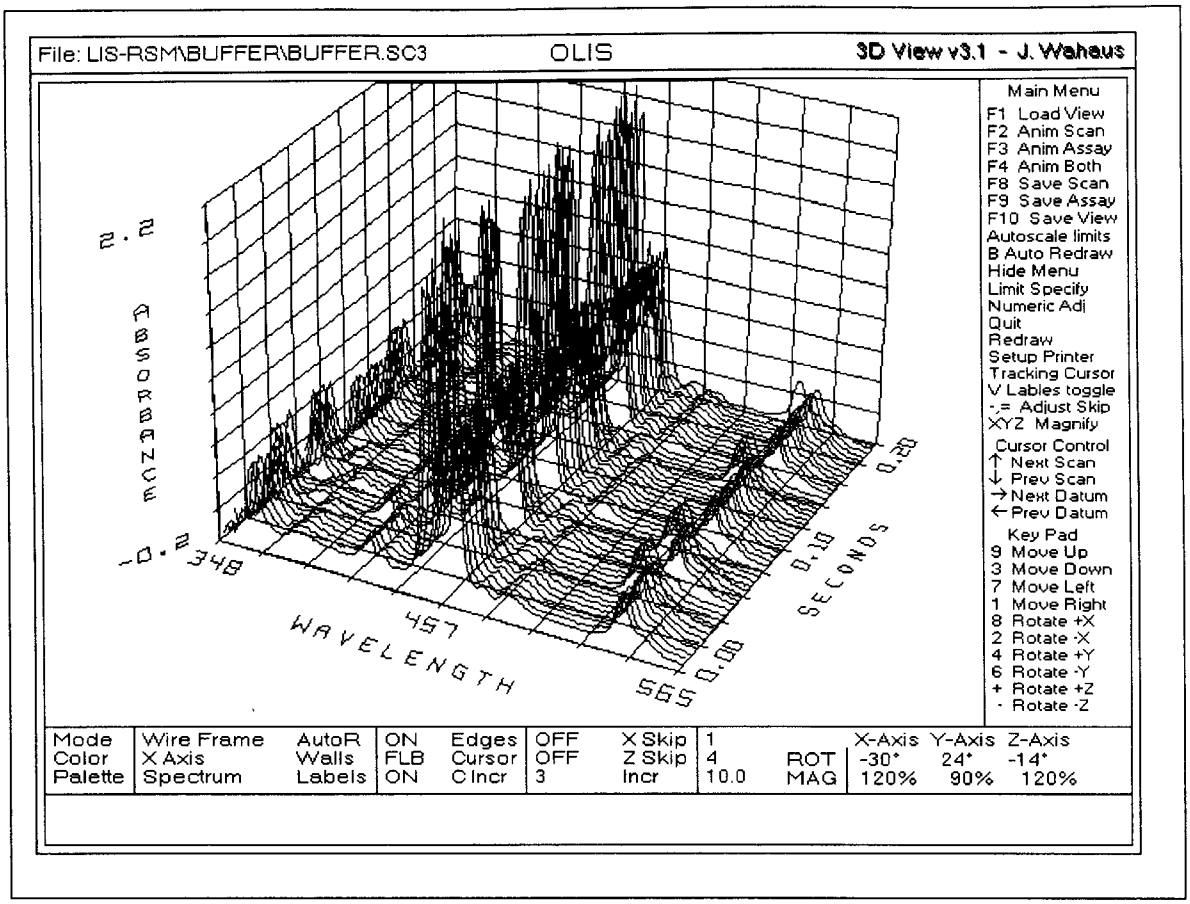
10. The problems one should look for are:
    a) "Error: Check FIFO speed" message appearing immediately after data collection (even though data itself looks good);
    b) "gaps" or wavelength shifts of some scans relative to the rest (screen #6.3);
    c) noise spikes on some scans, especially in the red end of the scans (screens #7.3 and #8.3—the only difference between these data is the amount of noise; as the adjustment approaches the correct values, the noise becomes less evident);

The same problems can be seen in the 2-D slices of the data set (screens #6.2, 7.2, and 8.2; these are 2-D displays of the same data shown in 3-D display in screens #6.3–8.3). However, because only 9 scans are shown in the 2-D display, the effected scans would have to be among the 9 displayed in order for one to see a problem. One can use the "D–Extract/Display 2-D data" option to select scans for display from the entire data set.

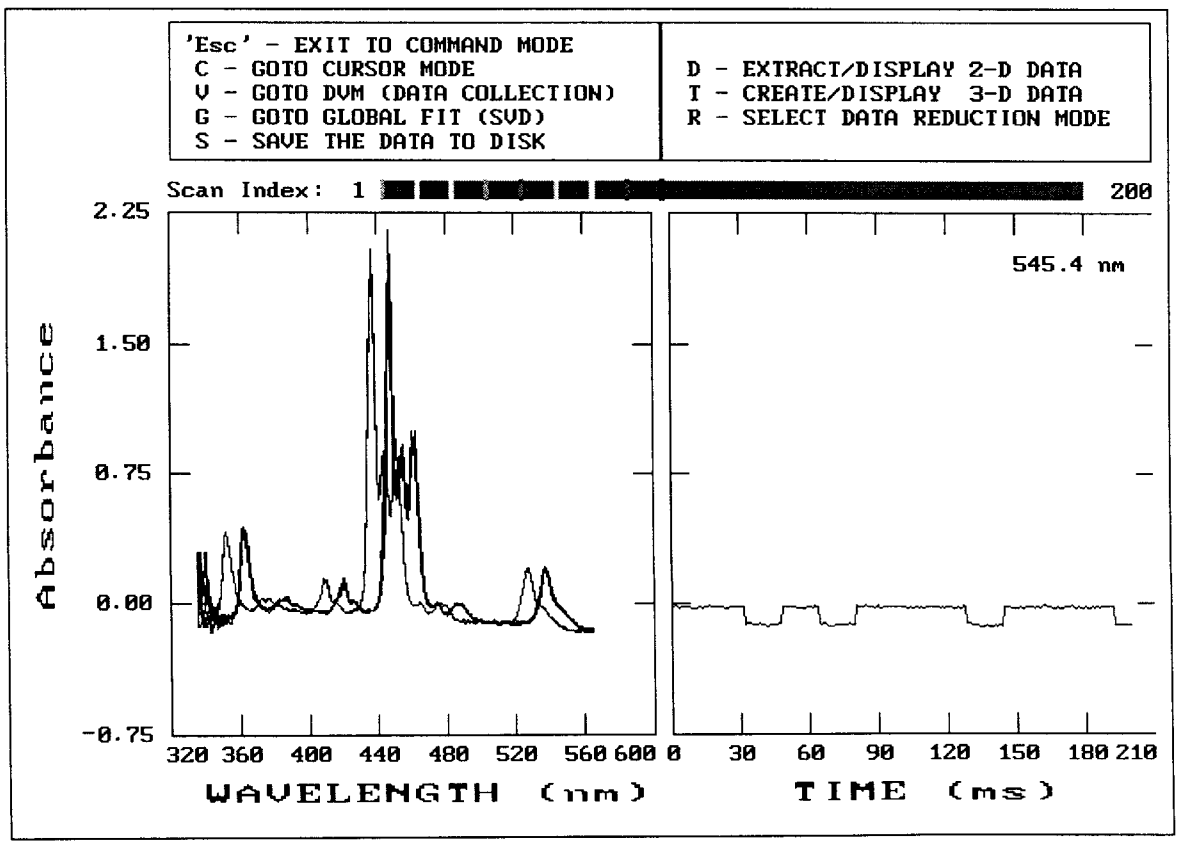
Similarly, the kinetic trace displayed in 2-D view must be extracted at a wavelength at which problems exist. One can select kinetic data for display by using the "K–Extract kinetic trace" option in the Cursor Mode screen.

For these reasons, it is often easier to spot the problems using the 3-D display.

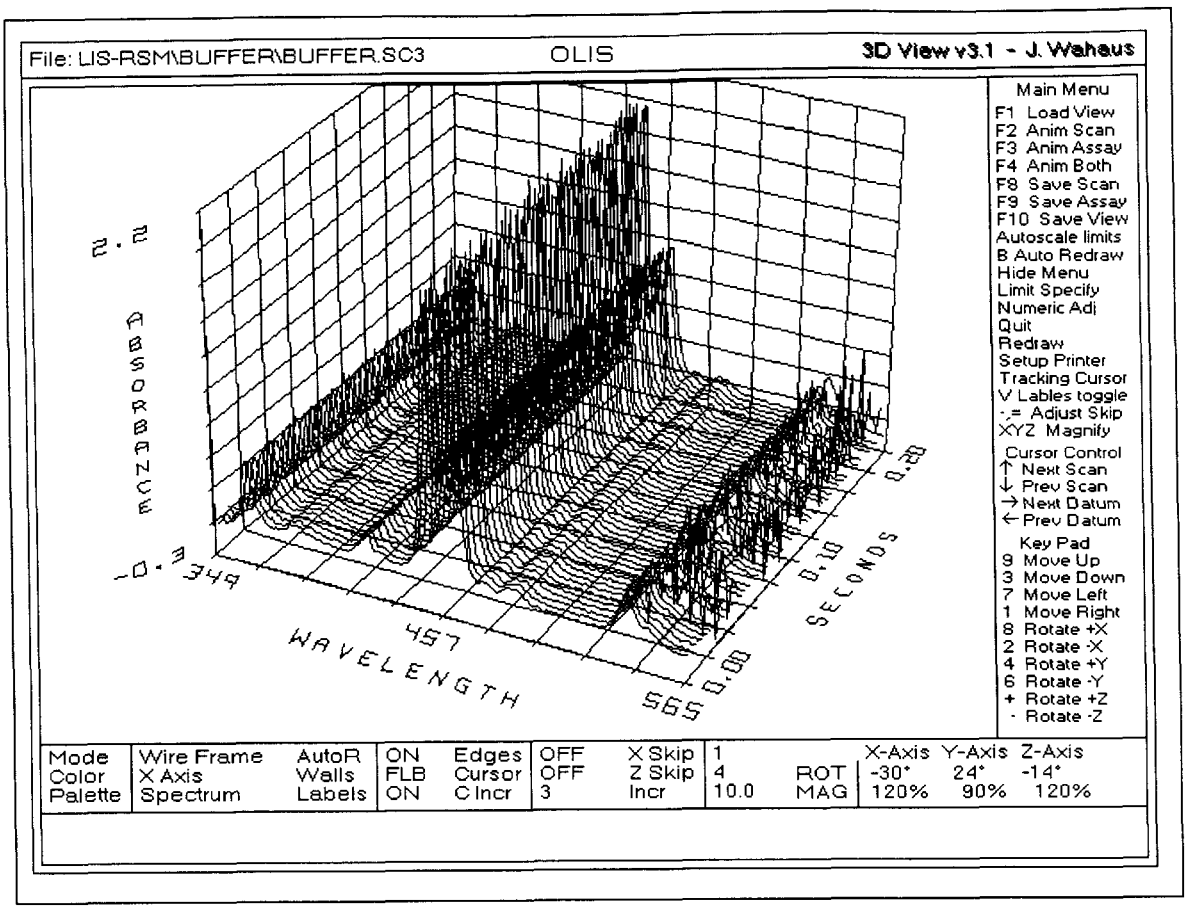
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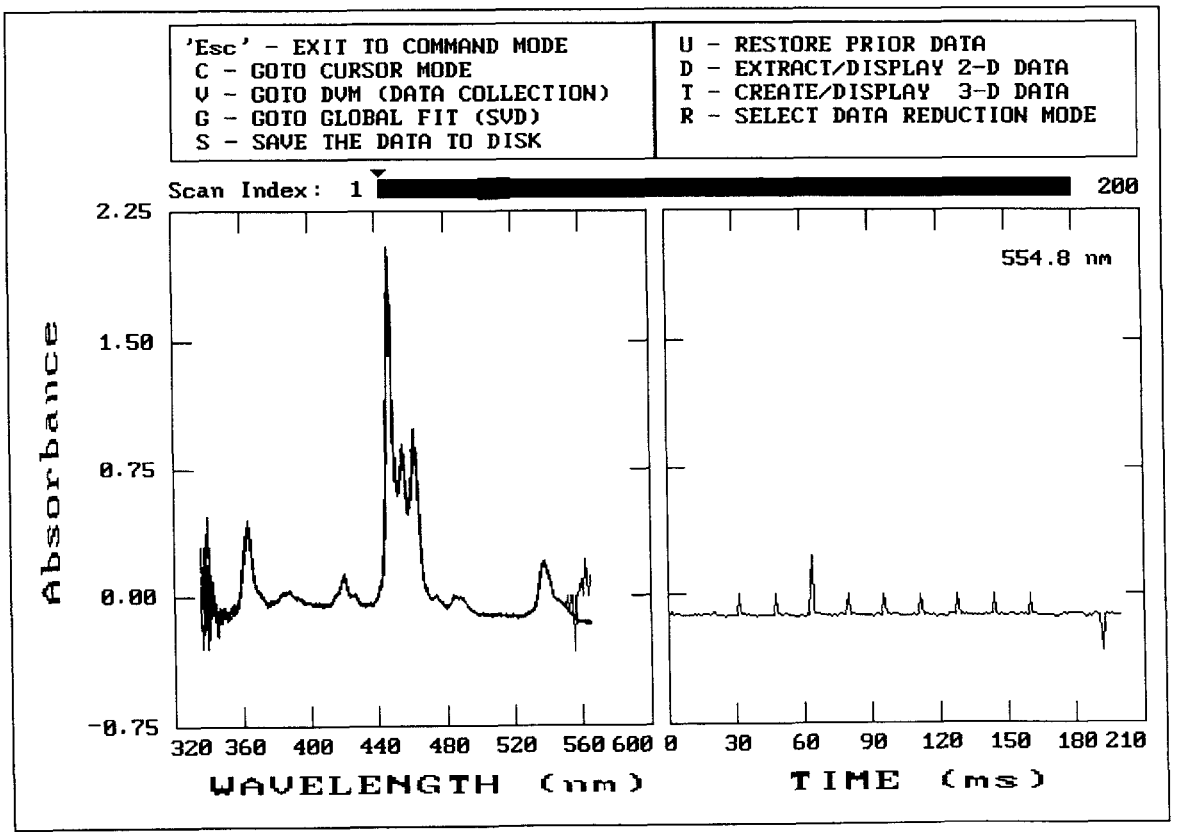
Screen #6.3↑ Screen #6.2↓



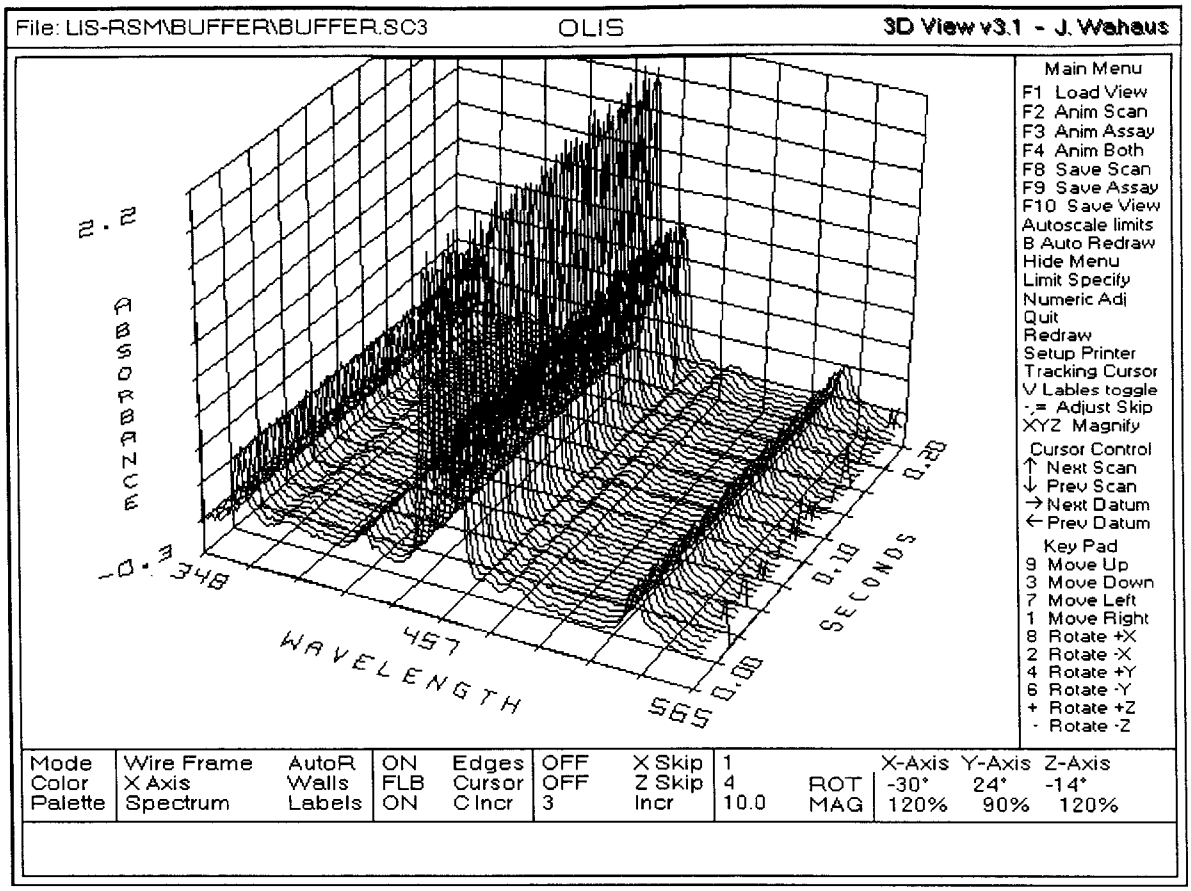
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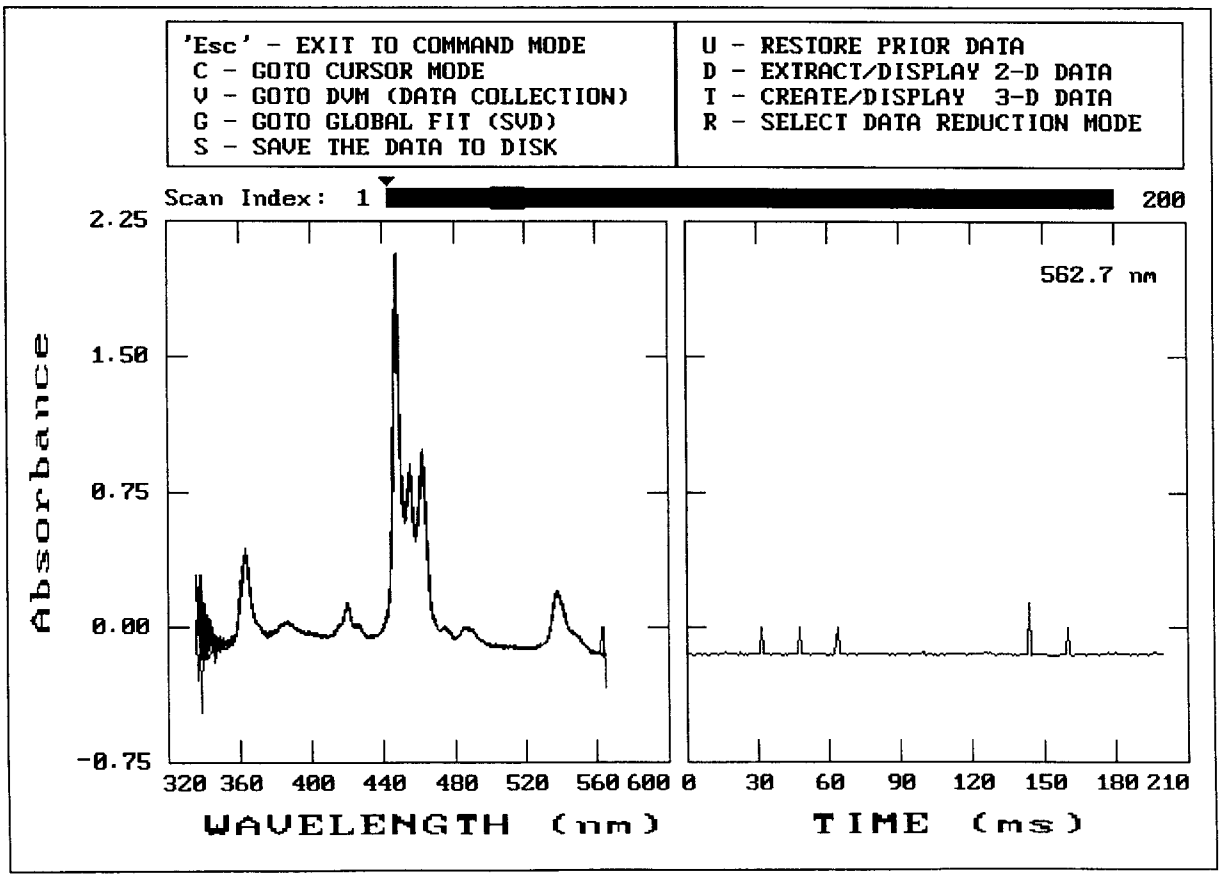
Screen #7.3↑ Screen #7.2↓



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Screen #8.3↑ Screen 8.2↓



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11. The problems described above can be corrected by fine-tuning a critical data-collection parameter called "FIFO Adjustment." To change this parameter, enter the Parameter Setup menu by choosing option "S–Parameter Setup" from the Command Mode screen. The FIFO adjustment option is not displayed among the Parameter Setup options—it is a hidden command. To access the hidden FIFO adjustment option, press "Ctrl" and "F" simultaneously. A warning message will be displayed. Press "Ctrl" and "F" simultaneously again. Enter a new value for the FIFO adjustment parameter. The value can be set in the ±50 range. Having entered a new value, exit from the Parameter Setup menu by pressing "Esc." Collect data as described previously and examine the data. Repeat the adjustment process as necessary to eliminate the problems described in step #10. Once the proper data collection parameters are found and there are no problems, the FIFO adjustment should not be changed.

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