

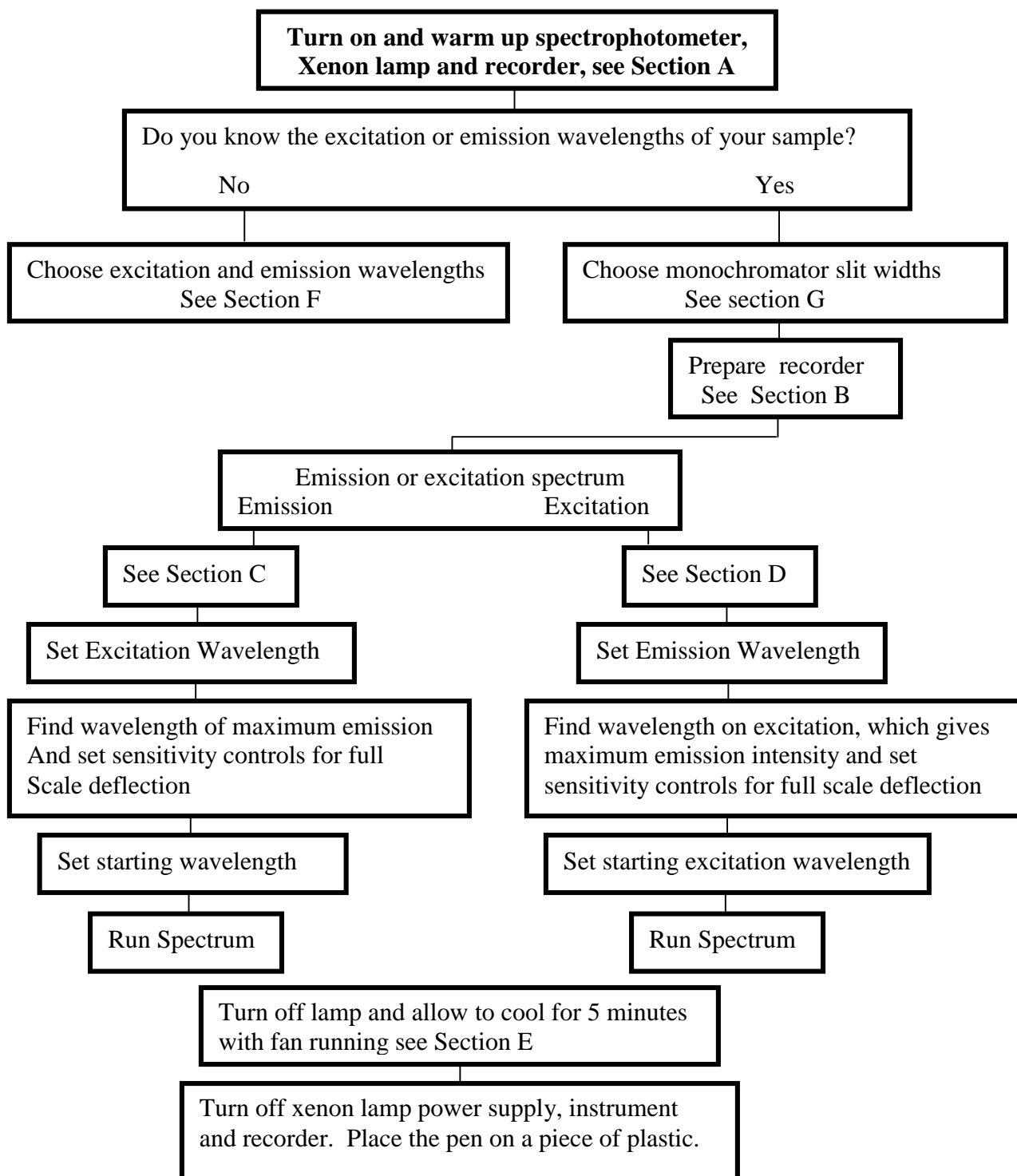
OPERATING INSTRUCTIONS

for the

Perkin Elmer Model 650-10S

Fluorescence Spectrophotometer

The following flow diagram should help to guide you through the steps necessary to run a fluorescence spectrum. For routine fluorescence spectra sections A, B, C, and E should be consulted. The less routine instructions are left for later sections; these may usually be skipped for an introductory laboratory using the instrument.



A. Preparatory Steps

1. Turn the POWER switch on the xenon lamp power supply to ON.
2. Make certain that the fan in the light source compartment is operating properly and ten or fifteen seconds after turning the POWER switch to ON depress the START switch on the xenon lamp power supply.
3. Turn on the POWER switch on the spectrophotometer.
4. Turn the recorder POWER switch from the OFF position to the AMP position. This allows the recorder to warm up. Set the recorder switch box to fluorescence.

WARNING: High voltage is applied when lighting the xenon lamp and the lamp is kept at a high temperature when lit. Do not touch the lamp compartment during operation of the lamp.

5. If stable analytical data is to be obtained, wait about 20 minutes before making sample runs.
6. Preliminary Control Settings: Confirm that the switches and knobs listed below are set as indicated.

Wavelength SCAN switch: STOP
MODE switch: NORMAl
ZERO SUPPRESSION Knob: OFF
PM GAIN Control: NORMAl
Shutter: Closed. (Out)

RESPONSE: NORM
SCAN SPEED: 120 nm/min
On recorder:
POLARITY: +
PHOTOMETRIC RANGE: 1 (100% T)
SCAN SPEED: 120 nm/min

B. Recorder Preparation

1. Turn the recorder POWER switch from the AMP position to the SERVO position.
2. Set the MEAS/ZERO switch at the ZERO position and adjust the recorder pen to the zero position on the chart by turning the ZERO control. (The zero level is normally located on the left side of the chart at a convenient line.)
3. Release the recorder MEAS/ZERO switch to the MEAS position and adjust the recorder pen exactly to the zero position on the chart by turning the ZERO ADJUST Knob on the instrument.

Note: Once the zero level has been adjusted properly, zero adjustment is not required for subsequent measurements.

The recorder is now ready to operate. The recorder chart drive will operate when the instrument SCAN switch is set to EX or EM, and the chart will move at a speed set with the recorder CHART SPEED control.

C. Measuring Procedures, Emission Spectra

The ratio mode (MODE switch in NORMAL position) should be used for all spectra and data collection. (The ratio mode provides stable performance for extended periods, permitting quantitative determinations.) The energy mode (MODE switch in ENERGY position) is primarily for focus and alignment of the source.

Note: To check zero, pull out (close) the sample shutter. (When it is not required to pass light through the sample, the shutter should be closed to prevent photo decomposition of the sample, which could occur because of excessive irradiation.)

- 1) Perform the preparatory steps as instructed in Sec. A and B.
- 2) Make certain that the MODE switch is in the NORMAL position.
- 3) Fill a cell with sample and place the cell in the cell holder. Make certain that the cell sits properly in the holder.
- 4) Set the PM GAIN switch to the NORMAL position.
- 5) Set the EXCITATION and EMISSION WAVELENGTH controls to the optimum wavelengths position for the sample (see Sec. F).
- 6) Push in (open) the sample shutter.
- 7) With the SENSITIVITY RANGE and SENSITIVITY FINE controls, bring the recorder pen to between 90 and 100%.
- 8) Rotate the EMISSION WAVELENGTH control to the shortest wavelength in the wavelength range in which spectral recording is to take place.
- 9) Set the instrument SCAN SPEED switch and the recorder chart speed selector switch for the desired speeds. Setting both to the same speed gives a presentation of 10 nm per division. See Sec. H.
- 10) Bring the recorder pen in contact with the chart paper.
- 11) Turn the wavelength SCAN switch from the STOP to the EM position.
- 12) After recording the spectrum, set the wavelength SCAN switch at the STOP position.
- 13) Raise the recorder pen.
- 14) Pull out (close) the sample shutter.

D. Excitation Spectra

- 1) Perform steps 1-8 of Sec. C.
- 2) Set the EXCITATION WAVELENGTH control for the shortest wavelength range intended for recording.
- 3) Set the instrument SCAN SPEED switch and the recorder chart speed selector switch for the desired speeds.
- 4) Bring the recorder pen in contact with the chart paper.
- 5) Turn the wavelength SCAN switch from the STOP to the EX position.
- 6) After recording the spectrum, set the wavelength SCAN switch at the STOP position.
- 7) Raise the recorder pen and marker.
- 8) Pull out (close) the sample shutter.

E. Shutdown Procedure

To shutdown the instrument proceed as follows.

- 1) Set the Wavelength SCAN switch at STOP.
- 2) Close the shutter.
- 3) Turn off the xenon lamp power supply and then quickly turn it back on again. This turns the lamp off but allows the lamp compartment fan to continue running to cool the xenon lamp. Let the fan run for at least 5 minutes.
- 4) Set the recorder POWER switch to OFF.

- 5) Turn the spectrophotometer OFF.
- 6) When the lamp compartment is cool turn off the lamp power supply.
- 7) Sign out in the log book. Place the recorder pin on a piece of plastic.

IMPORTANT

Notes for Correct Measurement

- 1) When setting wavelengths, always turn the WAVELENGTH dials from shorter to longer wavelengths.
- 2) When setting slit widths, always turn the SLIT controls from narrower to wider slit widths.
- 3) When a cell is put into the cell holder, it should always be oriented in the same direction.
- 4) The shutter should be closed (pulled out) as long as it is not necessary to open it for sample irradiation.
- 5) When a cell is filled with a volatile sample, cover it with the cover plate furnished.

F. Determining Excitation and Emission Wavelengths

When either the excitation or the emission wavelength, or both, are unknown, they can be determined by following the instructions given below.

1) Excitation Wavelength

Record an excitation spectrum of the unknown sample with the emission monochromator set at zero order on the EMISSION WAVELENGTH dial. The zero order setting lets all the fluorescence fall on the detector. The wavelengths at which peaks appear correspond to the excitation wavelengths for the sample. Choose the excitation wavelength that gives the maximum fluorescence emission signal.

2) Emission Wavelength

Record an emission spectrum of the unknown sample with the excitation monochromator set at the wavelength chosen in Section F1. The zero order setting allows white light to fall on the sample. The wavelengths at which peaks appear correspond to the emission wavelengths of the sample.

Notes: For measuring samples where the excitation wavelength and emission wavelength are very close to each other, it will be advantageous to shift the excitation wavelength to the shorter wavelength side of the band to avoid overlap and minimize the effect of Rayleigh scattering on the wavelength of interest.

The monochromators may be turned by hand if the SCAN switch is in the STOP position. Scanning by hand will save time.

G. How to Select Proper Slit Width

Analytical results can be optimized by selecting the proper slit width or spectral bandpass. The factors, which are influenced by slit width, are:

- a) the narrower the slits the better the resolution.
- b) the wider the slits the better the signal to noise, and therefore the more sensitive the method.

1. If the optimum slit widths are not known, set the EXCITATION and EMISSION SLIT dials at 10 nm.
2. The table following includes recommended slit widths for various analytical purposes.

RECOMMENDED SLIT WIDTHS FOR VARIOUS ANALYTICAL PURPOSES

Analytical Purpose	Excitation Slit	Emission Slit	Remarks
Excitation Spectra	Narrow	Wide	For measuring broad band spectra, both the slits can be wide.
Emission Spectra	Wide	Narrow	
Quantitative	Wide	Wide	For obtaining high S/N Ratio, slit width should be As wide as possible.
Analysis of Photochemically reactive samples	Narrow	Wide	Select excitation light as weak as possible and open the shutter only while the samples are to be irradiated.

3. To test if the slit widths are narrow enough for adequate resolution run a spectrum then, decrease the slit width and run the spectrum again. If there is no significant change, the slit widths are adequate.

H. Determining Wavelength Scanning Speed

It is generally advantageous to select fast scanning speeds for samples showing broad spectra, and slow scanning speeds for resolution. A high scanning speed is possible for quick analyses. Care should be taken that a fast enough pen response is used at fast speeds to follow bands accurately.

USE OF RESPONSE SWITCH

The RESPONSE switch should be set as specified below for the conditions listed:

- FAST - for recording sharp spectra, measuring phosphorescence lifetime, and quickly tracing spectra.
- NORM - for (most) general measurements.
- SLOW - for quantitative analysis and analysis requiring exceptional stability.

The table below summarizes settings of the SLIT control, SCAN SPEED selector, and RESPONSE switch (A * indicates a rarely used combination of settings.)

GENERAL GUIDE FOR RESPONSE SETTING

Scan Speed	Slit	3 nm or narrower	3 nm ~ 6 nm	6 nm ~10 nm	10 nm or -wider
15 nm/min		SLOW NORM	SLOW	SLOW	SLOW
30 nm/min		NORM	NORM SLOW	NORM SLOW	NORM SLOW
60 nm/min		NORM FAST	NORM	NORM	NORM
120 nm/min		FAST	NORM FAST	NORM	NORM
240 nm/min		*	FAST	FAST	NORM FAST
480 nm/min		*	*	FAST	FAST
