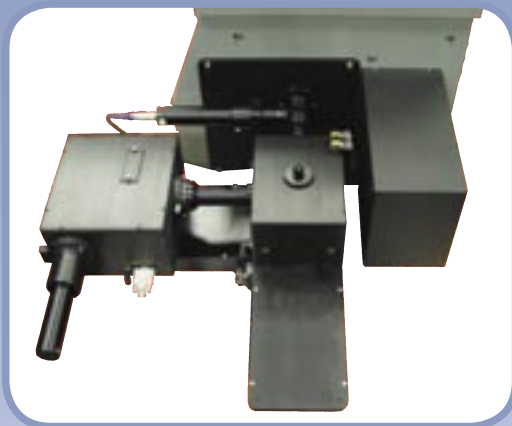


## Looking at NIR Fluorescence



## A Study of Key Parameters

Using weak and brightly emitting samples, we show illustrative results from an Olis spectrophotometer which is configured for NIR fluorimetry. The results will differ slightly among the Olis 17 UV/Vis/NIR, Olis DM 45, Olis DM 245, and Olis DM 445 fluorimeters.

Today's results were acquired on the model 17, comprised of a 150 watt xenon arc lamp running through an additive double monochromator (prism-grating version) with the emission traveling through an Olis single grating monochromator to an InGaAs 5  $\Omega$  TE cooled detector.

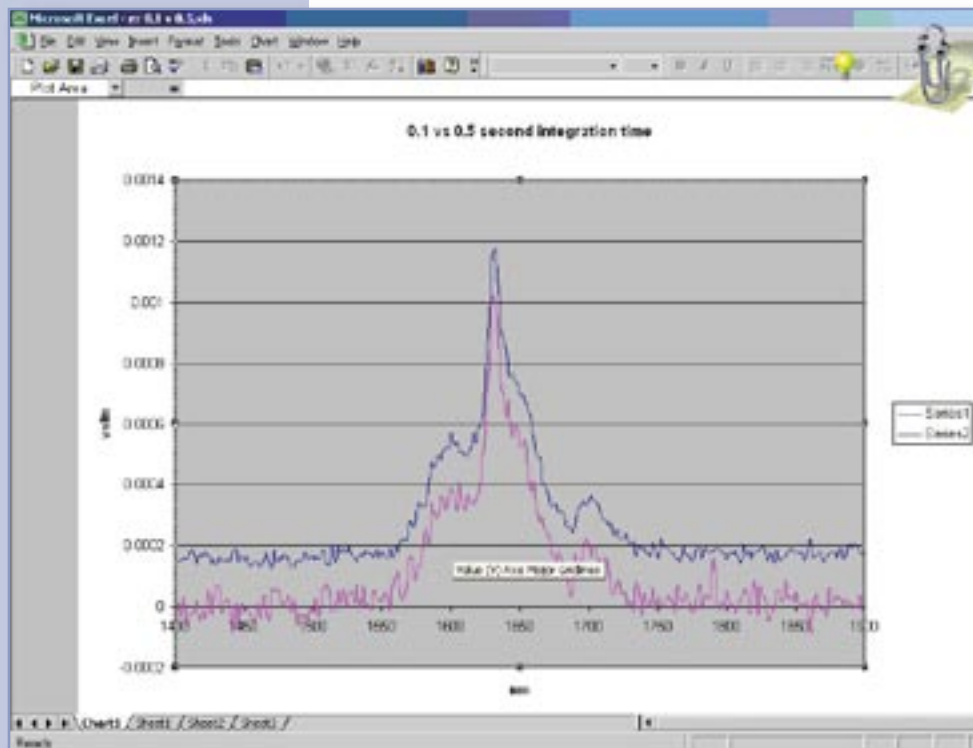
In addition to showing the excellent sensitivity, precision, linearity, and versatility of the Olis NIR fluorimeter, the cases remind us of the importance of measuring time and slit widths when designing experiments.

In many of these cases, we deliberately produced data with noise large enough to see, so that we could make our point. Given the optimal conditions, of course, one can have perfect S/N from Olis spectrofluorimeters.

# Integration Time and Slit Width

Let us start by comparing integration times. This example shows two integration times and one (narrow) slit width. The sample is an erbium doped glass. Er is a common NIR fluorescence standard. In the first case, the Olis instrument was set to acquire 250 points and to scan the emission from 1900 to 1400 nm. A 600 line/mm grating was used in the emission monochromator. On the excitation side, slit width was 1 mm and resulting bandwidth was 3.57 nm. On the emission side, the slits were 0.6 mm for a bandwidth of 4.8 nm.

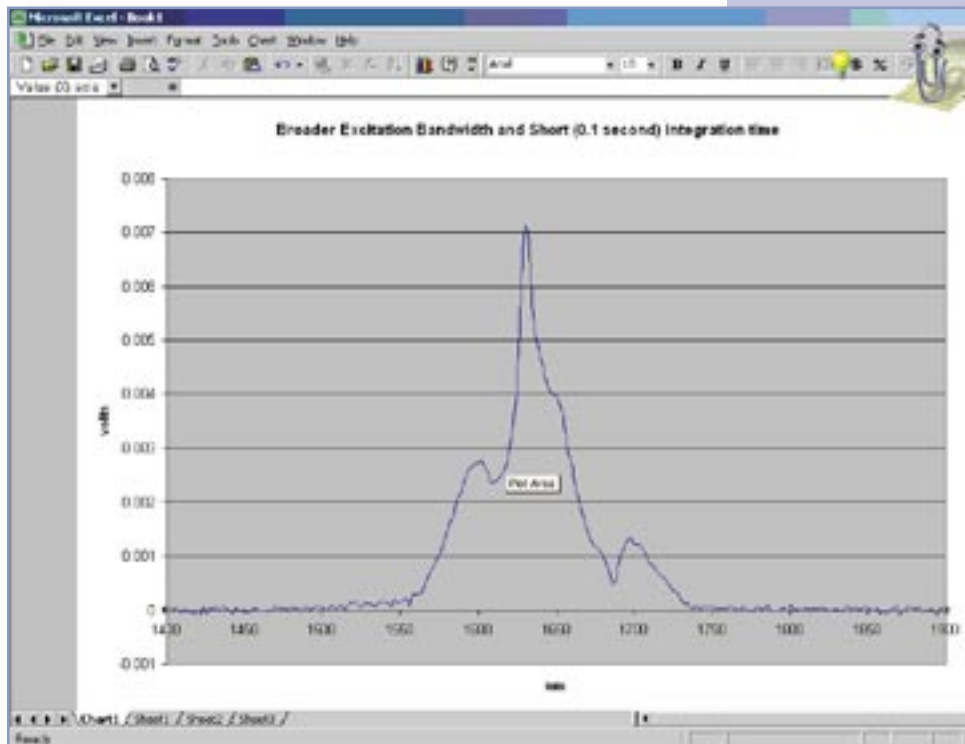
In one instance, the integration time was set to 0.1 second per point, which is relatively fast. Total time was 1 minute, 1 second (red line). Signal to noise is relatively low. Compare the S/N with the same sample, same conditions, but integration time five times longer (0.5 seconds per point), so that the scan took 2 minutes, 55 seconds (blue line). Now, the signal to noise is noticeably better.



# Open the slit and shorten the time

Let us now look at the effect of opening the slits to allow more light to pass through. In the first example, the excitation bandwidth was about 3.5 nm. This time, it is 10.71 nm. (Emission bandwidth remains 4.8 nm.)

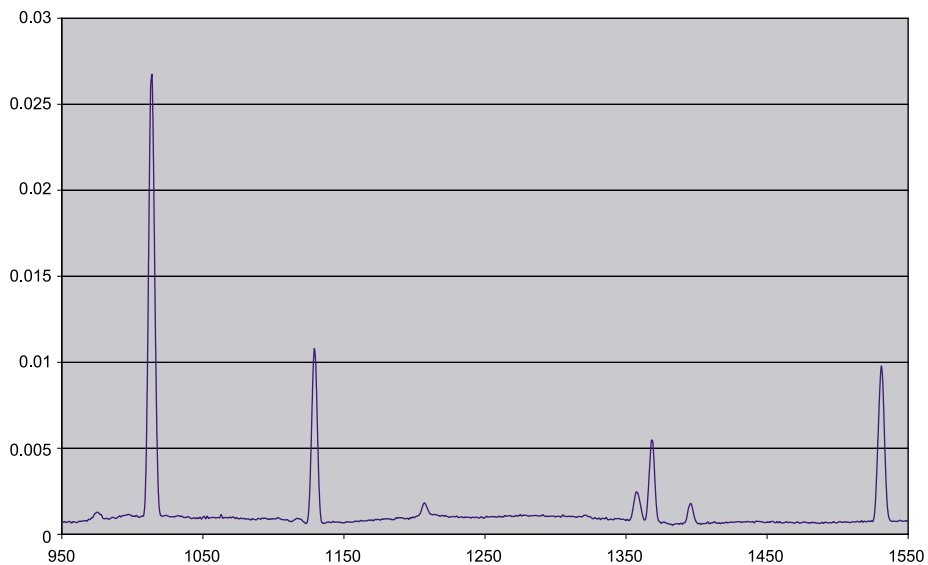
Even using the quick 0.1 second integration time, such that the scan took 1 minute, ten seconds, one sees far superior S/N than with the narrow slits, facing page, with little or no diminution in the resolution of the several features of the spectrum.



# Wavelength Linearity in the NIR

Using a standard available worldwide, fluorescent overhead lighting, we show the excellent wavelength linearity of the Olis NIR spectrofluorimeters. The peaks are correct to within 1.5 nm across this 600 nm span when compared with the theoretical values given for the mercury lines in the fluorescent lights determined in the 1950s. We find the first peak at 1014; theoretical wavelength is 1014. The second is 1029, previously presumed to be 1028.7 nm. The final peak, 500 nm from the first, is 1531 (earlier measured as 1529.9). Total scan time was 3 minutes, 19.5 seconds.

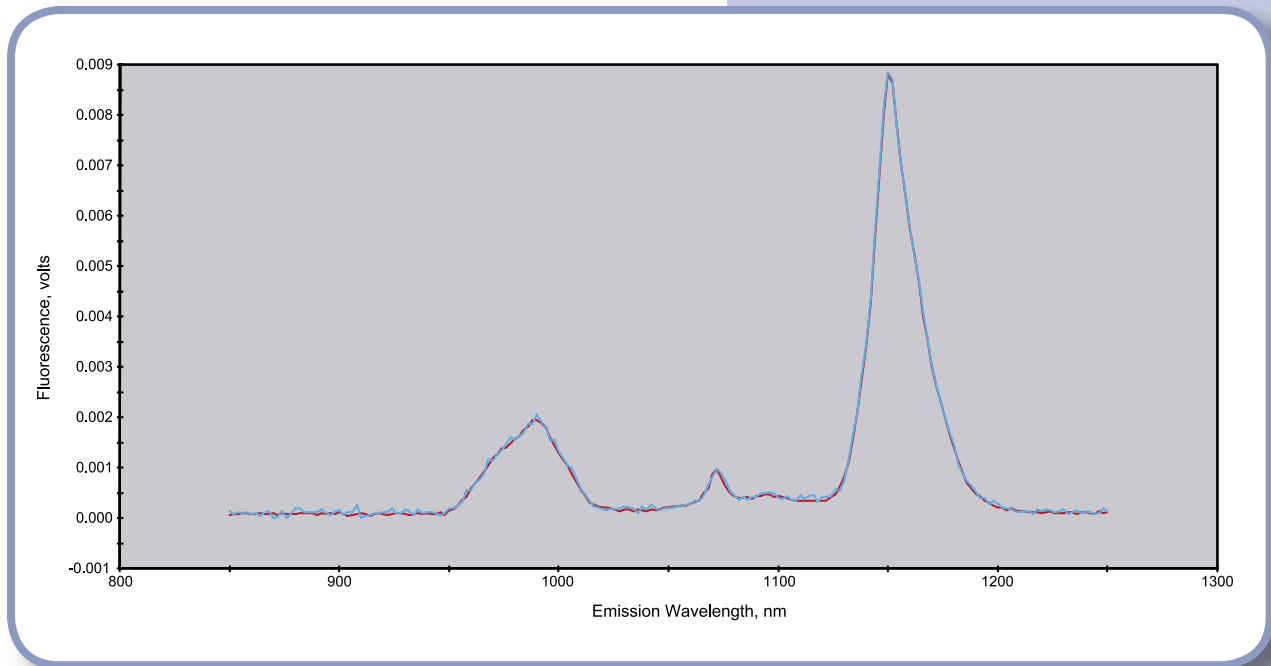
Hg NIR lines, 0.2 second integration time, 600 points in 3 min, 19.5 seconds



# Comparing 0.1 and 0.5 second Integration Times

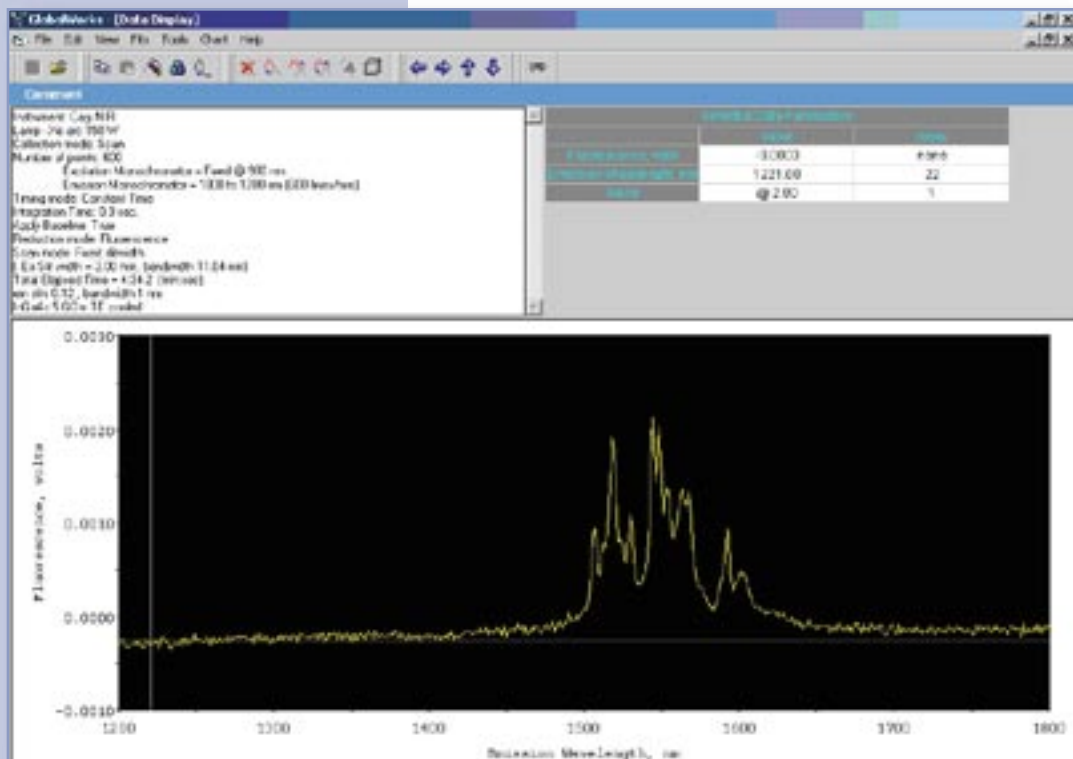
As we did earlier, here is another comparison of the importance of time when collecting a weak signal. The sample is neodymium glass. The excitation slit is 3 mm, with a resulting bandwidth of 11.11 nm. The emission slits are 0.6 mm, with a resulting bandwidth of 4.8 nm. Total collection time of the 200 points is 2 minutes, 12 seconds when the integration time is 0.5 seconds (blue line) and 55.6 seconds when the integration time is 0.1 second (red line).

While it is a little difficult to discern the improvement in S/N in this case, the red spectrum acquired with the 0.1 second integration time has considerably higher noise than the blue spectrum, acquired with the 0.5 second integration time.



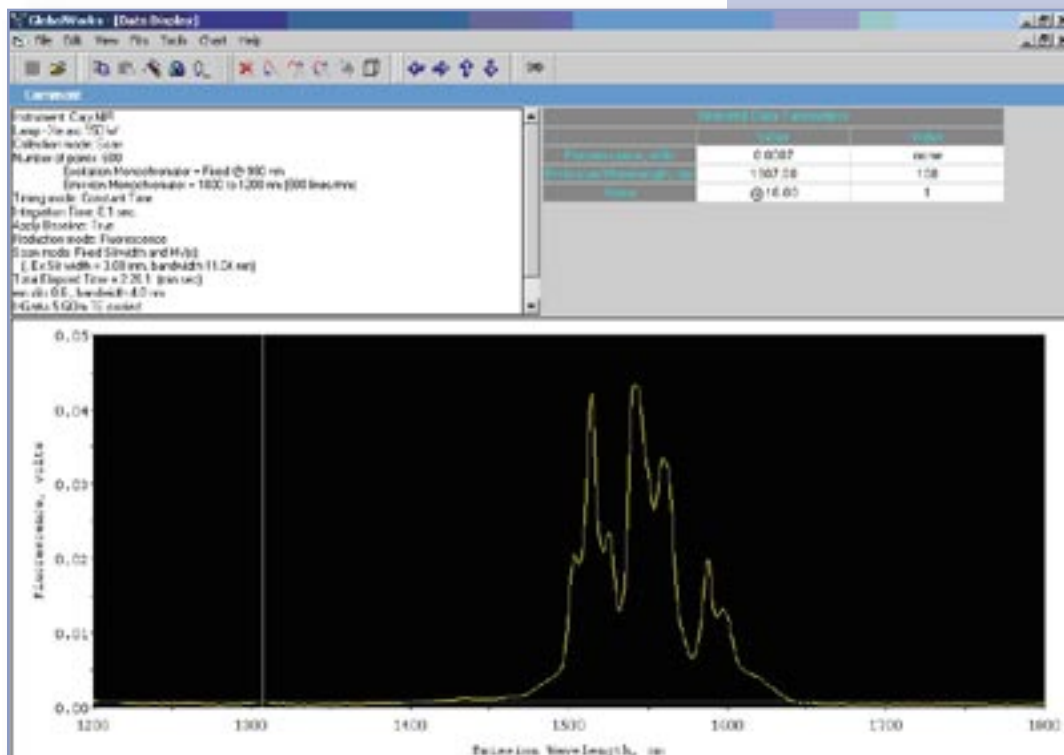
# Higher Spectral Resolution

When one uses very narrow slits on the emission monochromator, in this case 0.12 mm, one prevents much of the available emission light from reaching the detector, but what does arrive is of much high spectral resolution than through a wide slit (see next image). Narrow spectral resolution allows us to see 13 peaks from this sample, whereas the broad spectral resolution resolves only enough to see half that number. Interestingly, Olis, Inc. has viewed data from a competitor which has equivalent S/N as this scan but only the spectral resolution of the spectrum, facing page. In other words, even when our instrument is optimized for resolution and against sensitivity, we obtain better results than our nearest competitor!



# Lower Spectral Resolution

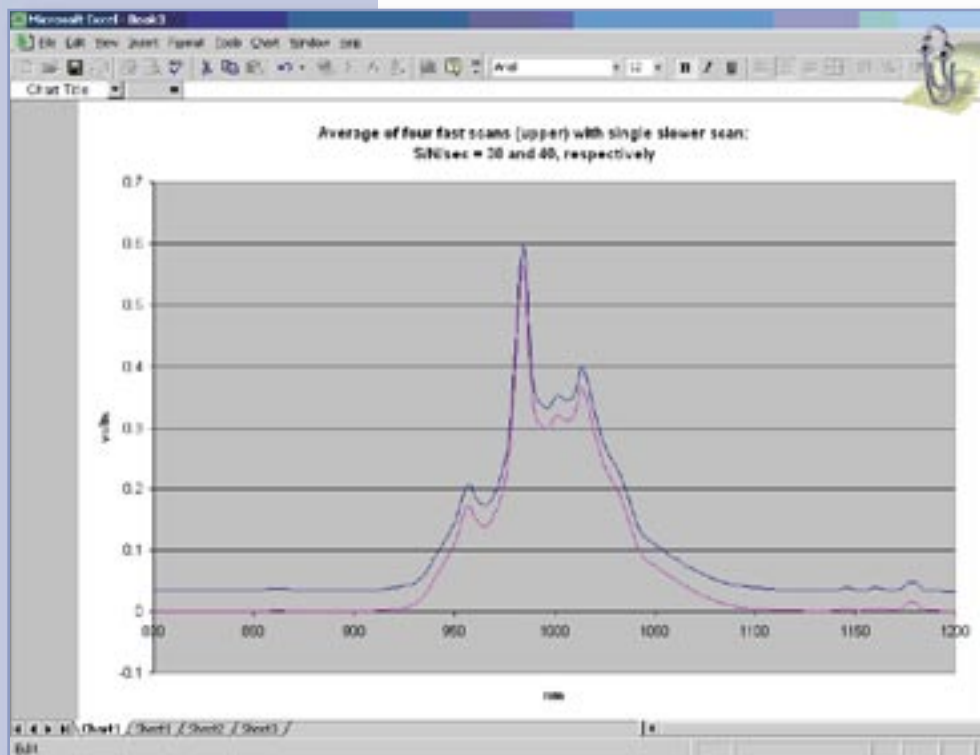
This spectrum, with its very low noise, looks beautiful. However, as the preceding spectrum confirms, these chosen slits are too wide, the spectral resolution is too low, so that some useful structural information is missed.



# To Average or to Scan Slower?

Measurements of ATX-O2 powder show that one can collect multiple scans quickly and average them to achieve the same S/N as one achieves by scanning slowly. The result of excellent S/N can be anticipated when the sample is undergoing changes that are monitored with the faster acquisition rate and then analyzed "globally" with the Olis 3D data fitting routines.

The blue spectrum represents the result of averaging four scans with a total collection time of 3 minutes 16 seconds. The red spectrum represents a single scan, with a much longer integration time and a total collection time of 3 minutes, 50 seconds. Short integration times are entirely usable when following a kinetic event, as they will produce results as accurate as those acquired slowly, if enough fast scans are collected and included in the kinetic analysis.

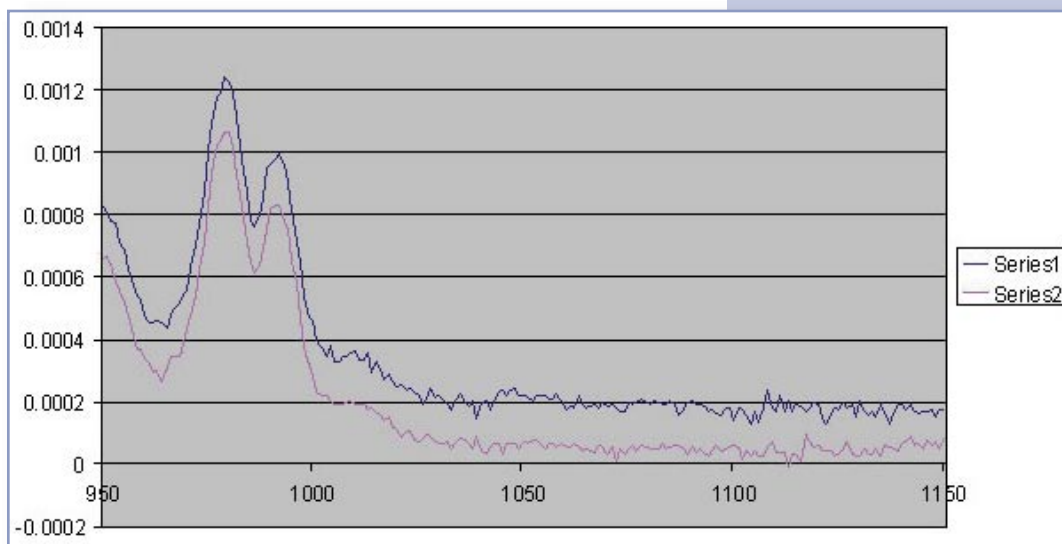


# S/N is Not an Absolute Means of Evaluating Spectral Quality

Finally, we would like to remind you the signal to noise (S/N) ratio alone is insufficient to understand and compare the sensitivity of a fluorimeter. Factors including the integration time, excitation light intensity (type and power of lamp used), and excitation and emission slit widths are some of them, as is the sample itself.

These data are of carbon nanotubes in a gel substrate. The excitation wavelength was 825 nm, the excitation slit was 3 mm and the emission slit was 1.24 mm. The spectrum in blue was acquired using an integration time of one second; the the total measuring time was 238 sec. The spectrum in red line was acquired half as fast, using an integration time of two seconds for a total time of 440 seconds.

The S/N ratio of the longer measurement is higher: 27 vs. 20. The S/N to time trade-off is obvious and expected. However, when one uses a more relevant measure – signal to noise adjusted for scan speed, i.e.,  $S/N \cdot \text{nm}/\text{sec}$  – to determine the quality of the scan per time spent measuring it, the numbers are 13 and 17 respectively, suggesting that the faster scan is more effective and should be used if time is an issue.



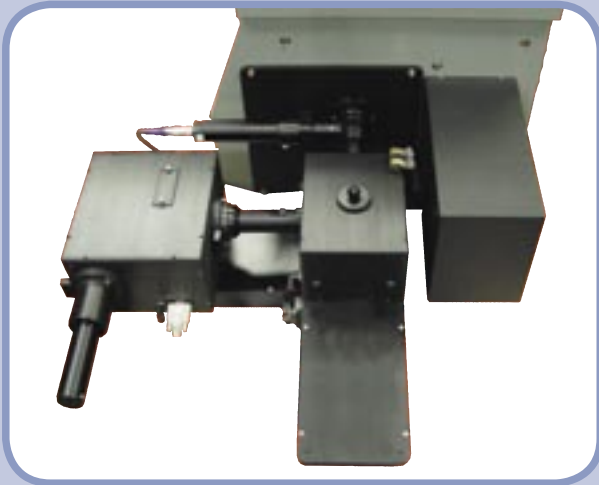
# Fluorescence Configurations

Fluorescence, anisotropy, and FDCD can be the primary or secondary measurements on nearly all Olis spectrophotometers. These photographs show our Olis 17 UV/Vis/NIR (in gray) with the Olis Scanning Emission Module (in black, upper photograph) and a lower cost, non-scanning version (black components, lower photograph).

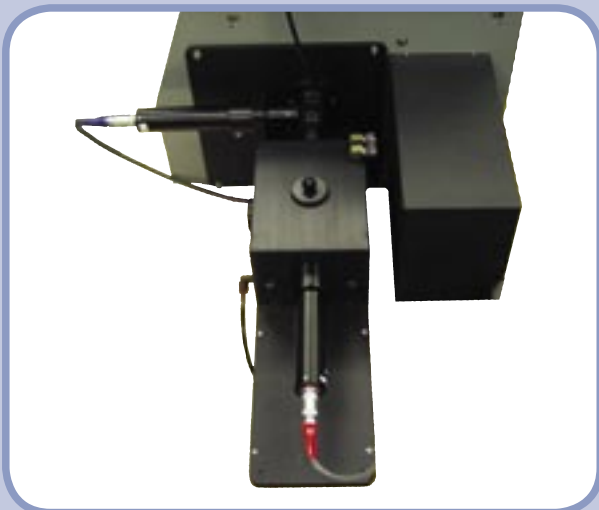
When the Cary monochromator is used as the excitation monochromator, its entire 185-2600 nanometer range is available for the excitation beam. When an Olis RSM 1000 or DB 620 or DSM CD monochromator is the excitation source, its spectral range will be determined by the gratings in place.

The emission monochromator can be the Olis single grating monochromator, shown here, or emission can be detected directly at the detector positioned 90 degrees to the excitation source (lower photo).

The emission wavelengths supported by the Olis single grating monochromator module will be UV/Vis, Vis/NIR, or NIR optimized. That is, the monochromator's grating will be selected for the emission wavelengths needed. The detector will be a UV/Vis or Vis/NIR photomultiplier tube, or a UV/Vis photon counting detector, or a high sensitivity InGaAs detector for NIR.

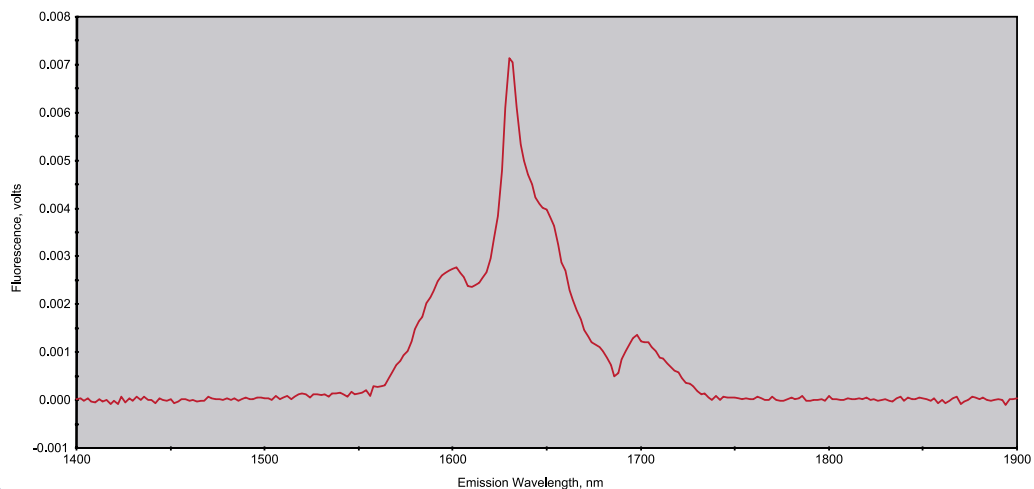


Emission through Monochromator



Emission through Filter

ND glass standard, 10.71 nm bandwidth, total time = 1 min, 10.6 seconds



# An All New Choice: The Olis DM 245 Spectrofluorimeter

Using the Olis Hummingbird, a subtractive double grating monochromator, on the excitation side and a single grating monochromator on the emission, we produce a research grade spectrofluorimeter with high resolution, low stray light excitation, and high throughput scanning emission. The measuring beam is homogeneous, which is a benefit on homogenous sample but especially so on heterogeneous ones.

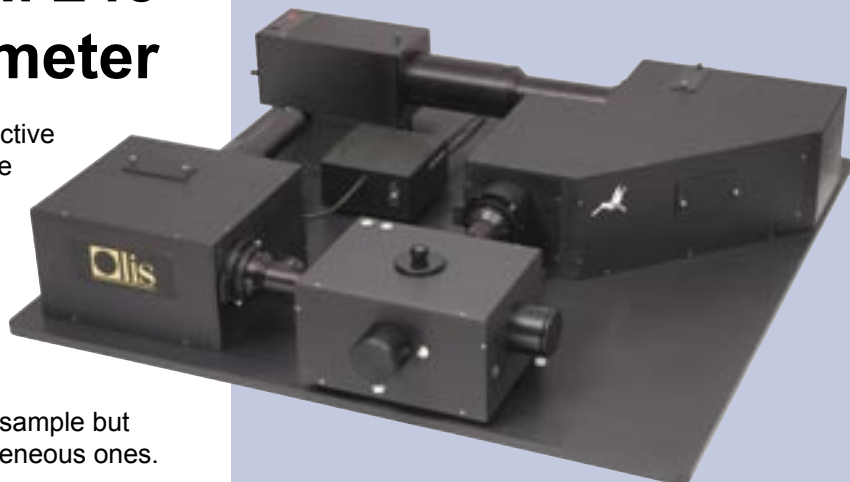
Our 150-watt xenon arc lamp in a specialized elliptical mirrored housing outputs the intensity of a typical 450-watt xenon arc. A high sensitivity photon counter or a high-speed photomultiplier tube is used for detection. Arbitrary amounts of signal averaging are possible with both detectors. The photon counter, with higher sensitivity, responds in 10 milliseconds to 2.5 second intervals; the photomultiplier tube provides for an uninterrupted stream of acquisition, the rate of which is limited only by the 20 MHz A/D card or user-supplied digital oscilloscope and available memory.

InGaAs detectors support emission detection to 2600 nm.

Olis software lets you scan the monochromators independently or in synchrony, so that scanning excitation, fixed wavelength excitation, scanning emission, fixed wavelength emission, and synchronous scanning of emission and excitation are supported.

These same components can be used for light scatter or luminescence measurements, as well as single or dual beam absorbance data. (Without the emission monochromator, the Olis DM 245 is an Olis DB 620 dual beam absorbance spectrophotometer.)

The standard sample compartment houses a single 1 cm<sup>2</sup> jacketed cell holder. Alternative cell holders include two, four, six, and ten position units, with water jacketed thermal regulation or Peltier electronic thermal regulation. Polarizers and shutters can be positioned anywhere in the optical train of the fluorimeter to support L-format, T-format, or variable retardance methods<sup>1</sup> of anisotropy measurements.

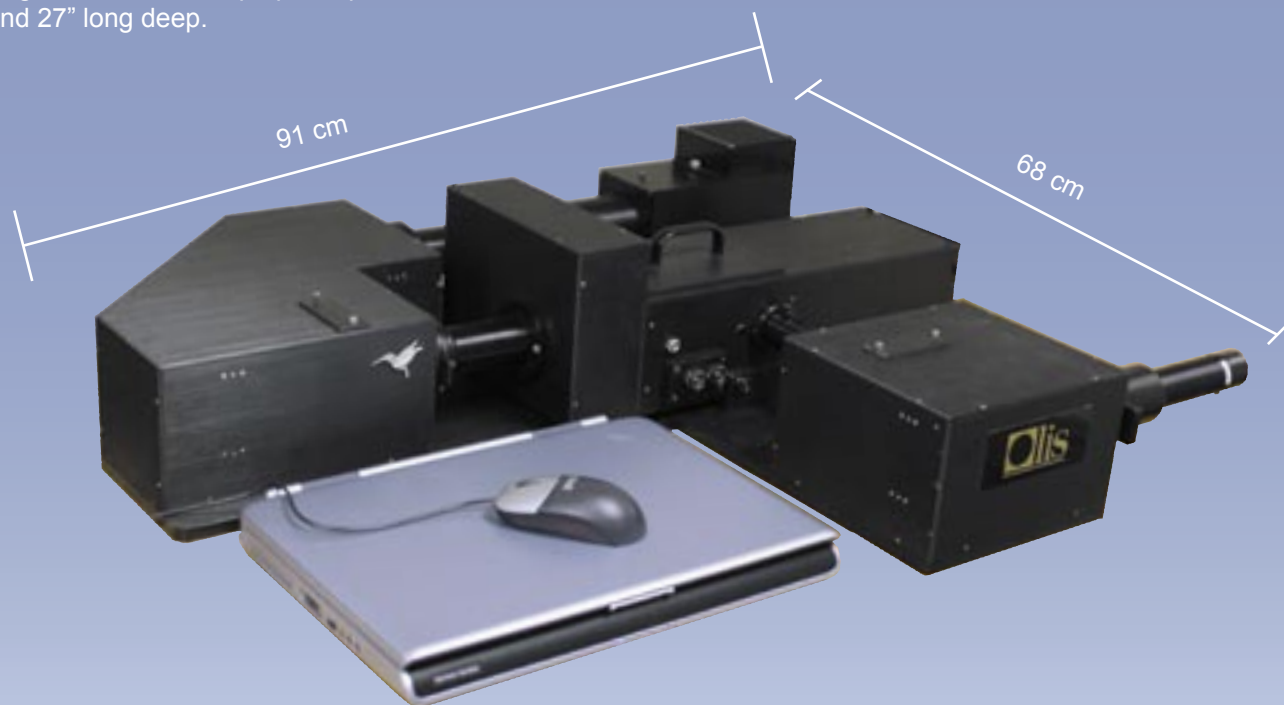


Without the Hummingbird, but with two single grating monochromators, we offer the Olis DM 45.

Alternately, and not shown here, two Hummingbirds can be used, producing the soon-to-be unveiled Olis DM 445.

1. DeSa, Richard. 2005. Device for Enabling Slow and Direct Measurement of Fluorescence Polarization. U.S. Patent 6,970,241 B1, filed August 24, 2004, and issued November 29, 2005.

This unit, with Hummingbird monochromator, the CD module, and the Scanning Emission module is equally an "Olis DM 245 with a CD module" and an "Olis DSM 20 CD spectrophotometer fitted with the Scanning Emission Module." The footprint, including the 17" screen laptop computer, is 36" long and 27" long deep.



**For more information on this and other Olis products:**

Visit [www.olisweb.com](http://www.olisweb.com)

Write [sales@olisweb.com](mailto:sales@olisweb.com)

Call **1-800-852-3504** in the US & Canada  
**1-706-353-6547** worldwide