PTI Technical Note K-167 Ovalene Fluorescence Standard

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Ovalene is a convenient standard used to test the functionality of a steady-state fluorescence spectroscopy system. The data collected through this step-by-step guide can be used for future reference to determine if your QuantaMaster system is functioning properly. Specifically, the K-167 standard can be used for testing of the wavelength calibration of the excitation and emission monochromators as well as the instrument long-term sensitivity. It is important that the measurement records are saved, so they can be used as the reference in the future. If you perform this ovalene standard protocol and do not get results similar to those seen in Figure 1 or notice a significant drop of intensity, please feel free to contact our toll-free customer support line at 877-784-4349 or by email at ptiservice@pti-nj.com.

- 1. Open FelixGX; Load Hardware configuration for QM400 steady-state fluorescence with the XCorr enabled.
- 2. Press 'Setup' to create a new acquisition and select 'Emission Scan'
- 3. Set the emission wavelength range from 400 nm to 585 nm and set the excitation wavelength to 342 nm.
- 4. Set the integration time 1 sec, and step size to 1 nm.
- 5. Set the excitation slits to 4 nm and the emission slits to 2 nm.
- 6. Enable 'Emission Correction' under the 'Real-Time Corrections' tab. Select the emcorr lookup table.
- 7. Save acquisition as 'OvaleneEm'.
- 8. Run the emission scan. You should see an emission spectrum like the one below.
- 9. Press 'Setup' to create a new acquisition and select 'Excitation Scan'
- 10. Set the excitation wavelength range from 280 nm to 390 nm and the emission wavelength to 482 nm.
- 11. As before, set the integration time to 0.1 sec, and step size to 1 nm.
- 12. Set the excitation slits to 2 nm and the emission slits to 4 nm.
- 13. Enable 'Excitation Correction' under the 'Real-Time Corrections' tab. Select the excorr lookup table.
- 14. Depending on your system, set the gain at 342 nm so the Xcorr signal is about 1.5V.
- 15. Save acquisition as 'OvaleneEx'.
- 16. Run the excitation scan. You should see an excitation spectrum like the one below.
- 17. Save this data as "Ovalene Spectra".

You have now collected the spectra for the ovalene standard. You can consult this data for future reference.

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Fig1. Corrected excitation and emission spectra for ovalene standard.

Experimental conditions:

Instrument:	QM-400 steady-state spectrofluorometer
Light source:	75 W continuous xenon lamp
Ex monochromator:	Single, 1200 line/mm, 300 nm blaze, slits 4 nm
Em monochromator:	Single, 1200 line/mm, 400 nm blaze, slits 2 nm
Detector:	914 with R928 PMT

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