Fluorescence Spectrophotometer Jasco FP6500

Validation and Correction

30th March, 2014

Ankit Raj

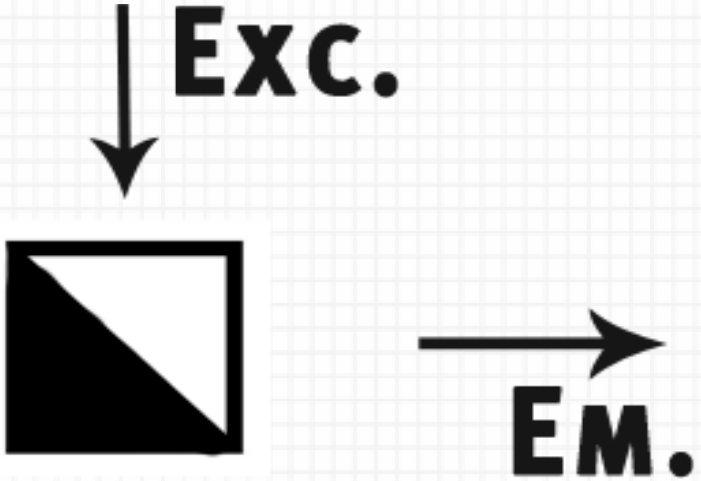
1. Validation
(by inbuilt software and given accessories)

Includes

- Wavelength accuracy
- Wavelength repeatability
- Resolution
- Stray light
- Minimum detectable amount
- Photometric stability

1.1 Wavelength accuracy

- Various wavelength light is sent via diffusion plate, and recorded as emission.



Diffusion Plate
white plate, no window

1.1 Wavelength accuracy

INSPECTION SHEET

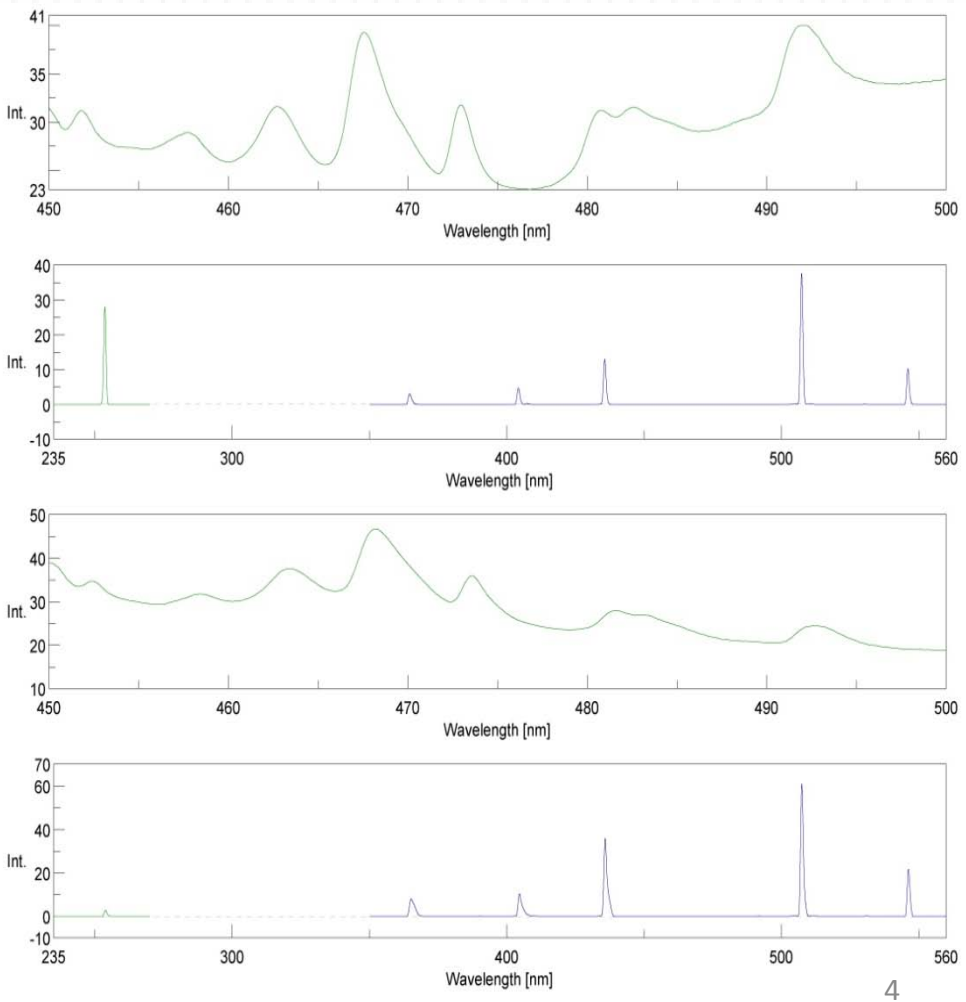
Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014
Room temp.: 24.9 C
Humidity: 41 %
Operator: Ankit
Inspected by:

Wavelength accuracy
Overall Pass/Fail: Pass

Acceptance criterion
(Ex) 253.7 +/-1.5 nm, 365.0 +/-1.5 nm, 435.8 +/-1.5 nm, 546.1 +/-1.5 nm
(Em) 253.7 +/-1.5 nm, 365.0 +/-1.5 nm, 435.8 +/-1.5 nm, 546.1 +/-1.5 nm

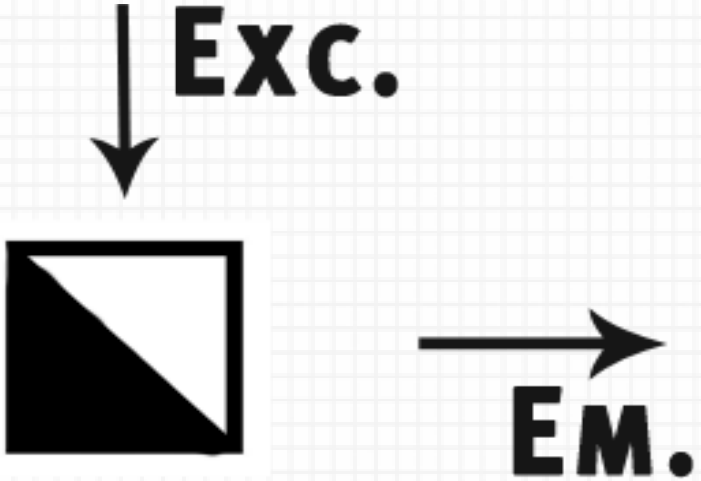
Result(s)

Standard value	Measured value	Difference
468.5nm(Ex)	467.6 nm	-0.9 nm
253.7nm(Ex)	253.7 nm	-0.0 nm
365.0nm(Ex)	364.6 nm	-0.4 nm
435.8nm(Ex)	435.6 nm	-0.2 nm
546.1nm(Ex)	546.1 nm	-0.0 nm
468.5nm(Em)	468.2 nm	-0.3 nm
253.7nm(Em)	253.9 nm	0.2 nm
365.0nm(Em)	365.1 nm	0.1 nm
435.8nm(Em)	435.8 nm	-0.0 nm
546.1nm(Em)	546.3 nm	0.2 nm



1.2 Wavelength repeatability

- 546.1 nm light is sent via diffusion plate, and recorded as emission. The repeatibility of the light at 546.1nm is tested.



Diffusion Plate
white plate, no window

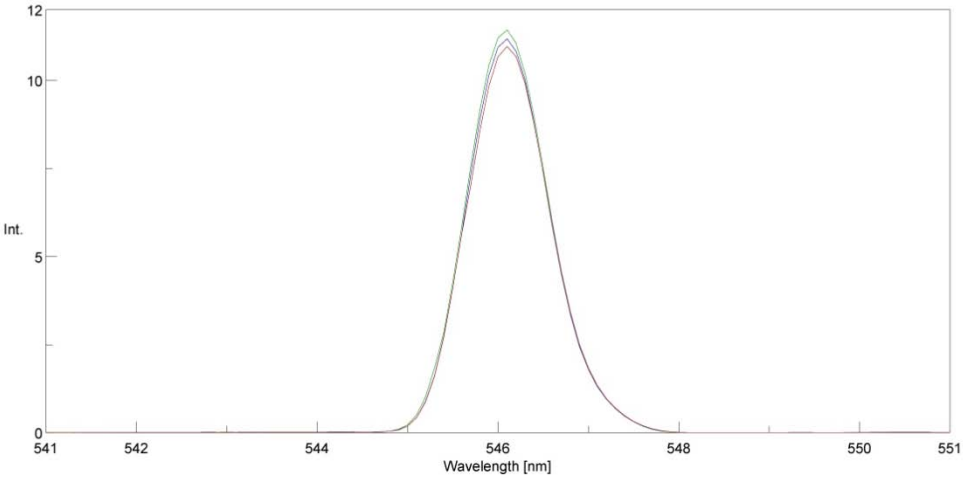
1.2 Wavelength repeatability

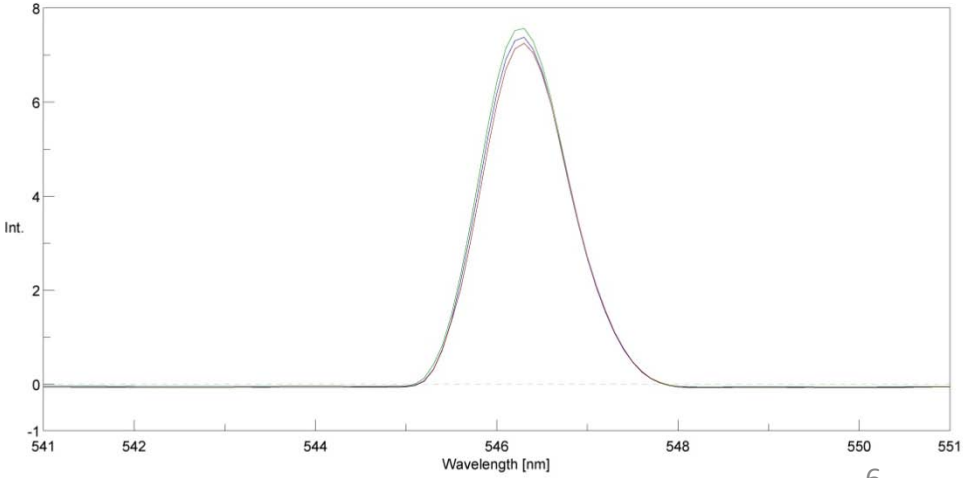
INSPECTION SHEET

Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014 Room temp.: 24.9 C Inspected by:
Humidity: 41 %
Operator: Ankit

Wavelength repeatability Overall Pass/Fail: Pass
Acceptance criterion (Ex) +/-0.3 nm (Em) +/-0.3 nm
Result(s)

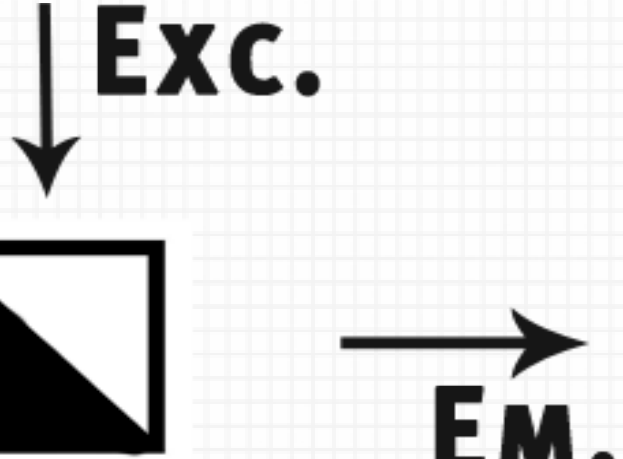
					Mid point.
(Ex)	Measured value	546.1 nm	546.1 nm	546.1 nm	546.1 nm
	Difference	0.0 nm	0.0 nm	0.0 nm	
(Em)	Measured value	546.3 nm	546.3 nm	546.3 nm	546.3 nm
	Difference	0.0 nm	0.0 nm	0.0 nm	





1.3 Resolution

- 546.1 nm wavelength is used
- FWHM of the peak is taken as the factor for the test.



Diffusion Plate mirror type

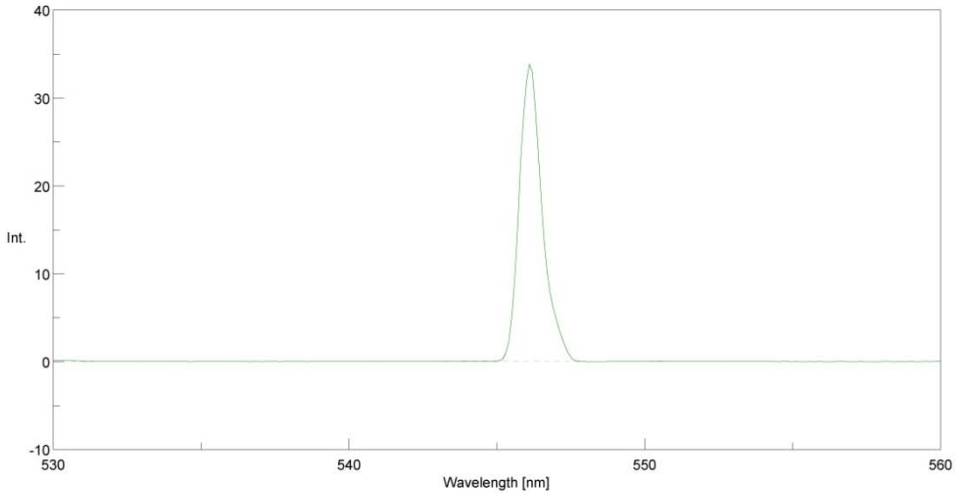
1.3 Resolution

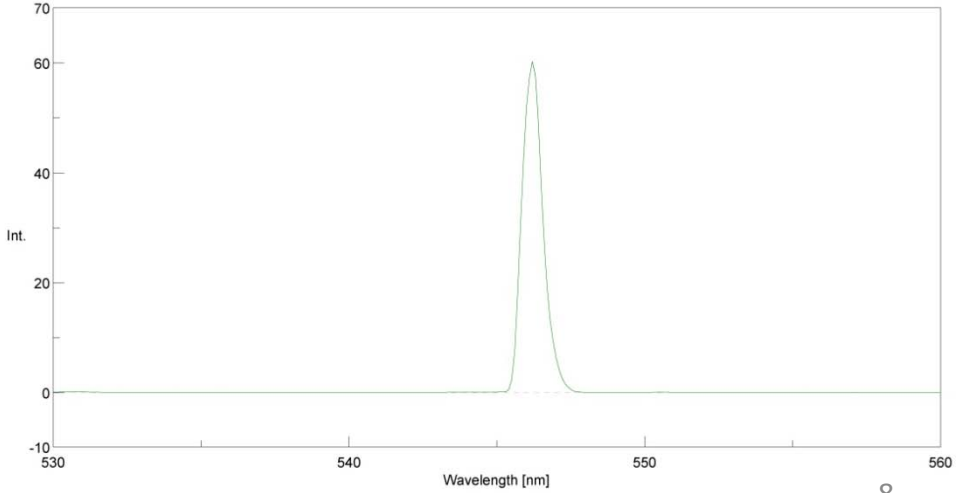
INSPECTION SHEET

Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014
Room temp.: 24.9 C
Humidity: 41 %
Operator: Ankit

Resolution — Overall Pass/Fail: Pass

Acceptance criterion	(Ex) 1.10 nm
	(Em) 1.10 nm
Result(s)	(Ex) 0.81 nm
	(Em) 0.77 nm

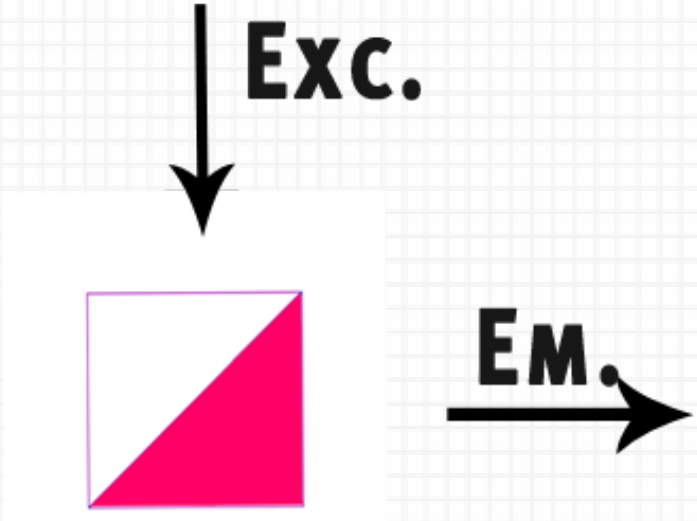




1.4 Stray light

- Triangular cell with Rhodamine B dye used.
- In step 1 Rhodamine B is tested. [for emission]
- In step 2, only Diffusion plate is kept, no sample.

[for Excitation]



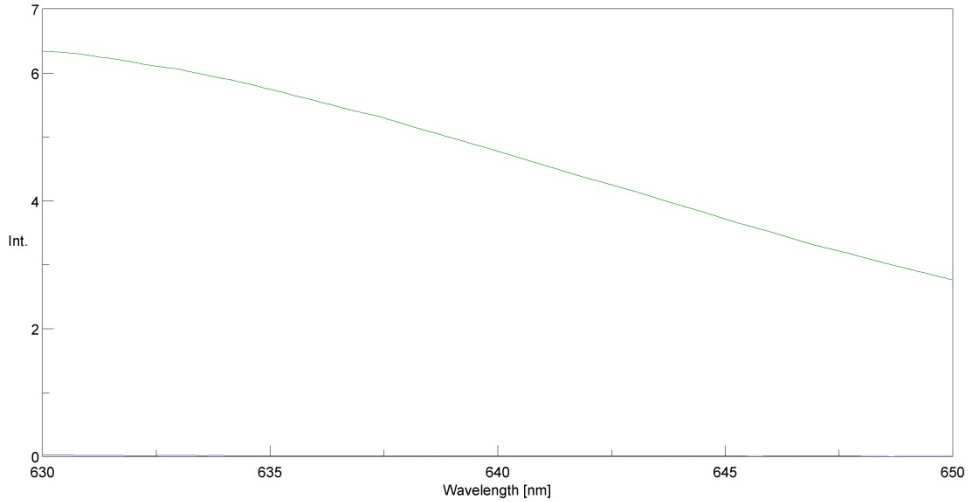
Rhodamine B Solution

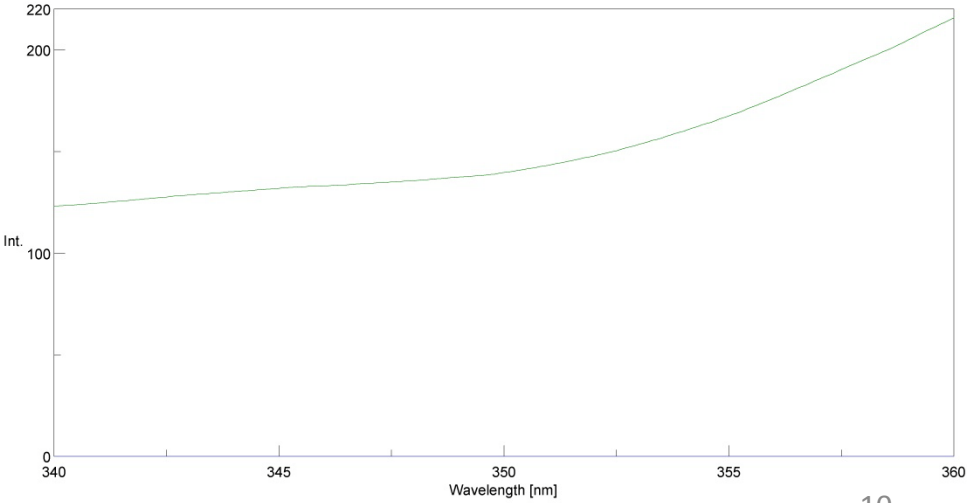
1.4 Stray light

INSPECTION SHEET

Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014
Room temp.: 25.0 C
Humidity: 41 %
Operator: Ankit

Stray light — Overall Pass/Fail: Pass
Acceptance criterion — (Ex) Less than 3 %
(Em) Less than 1 %
Result(s) — (Ex) 0.30 %
(Em) 0.03 %



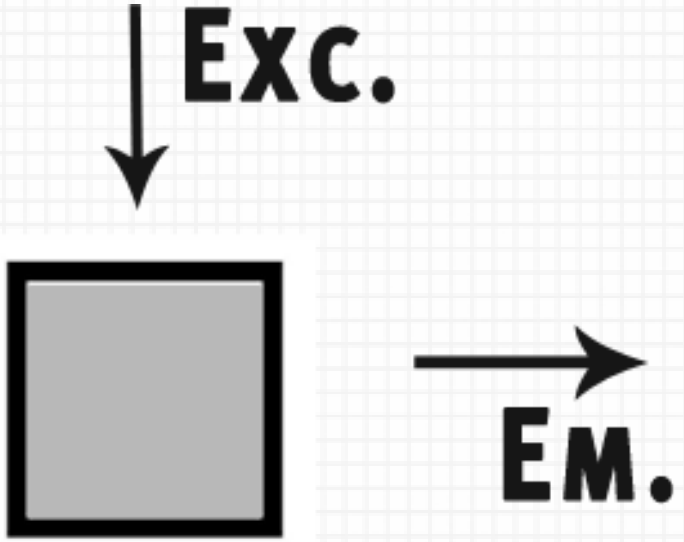


1.5 Minimum Detectable Amount

- Rectangular cell with Distilled water.
- We measure the time-course Raman peak at 398.4nm

Excitation at 350 nm
Emission at 397nm.

In wavenumbers,
we measure the Raman peak at
3380cm-1 (O-H stretching)



Distilled water

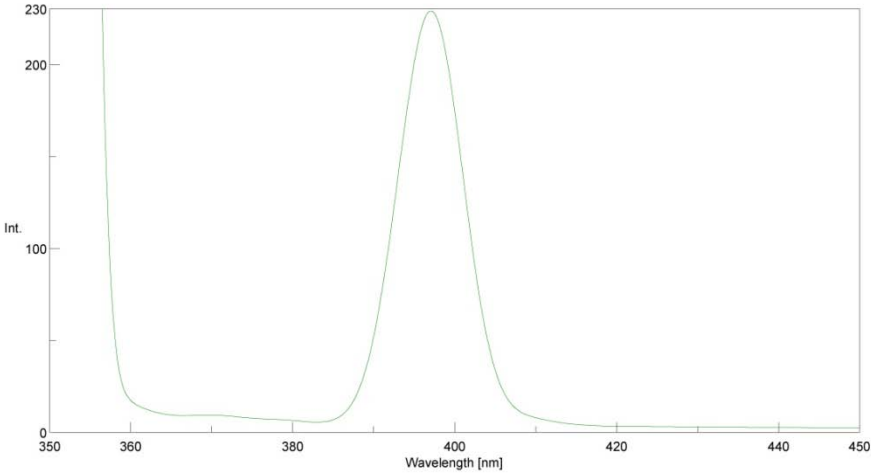
S/n ratio is calculated after taking 10 measurements. The S/n ratio is the criteria for the test.

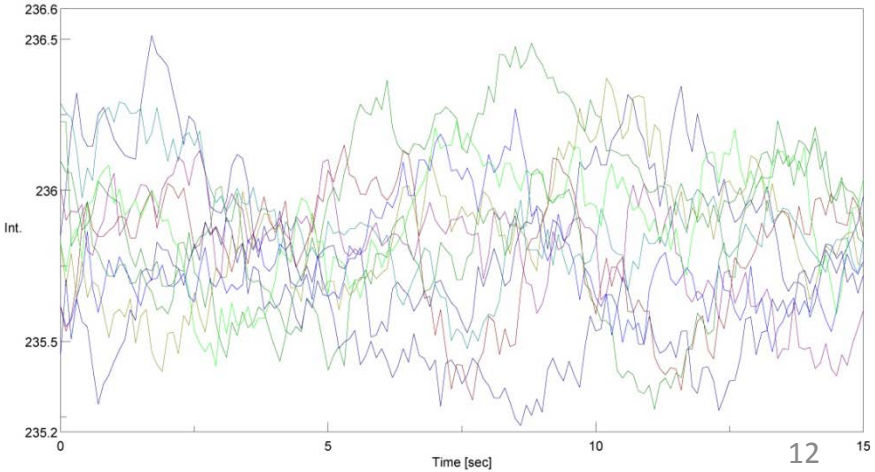
1.5 Minimum Detectable Amount

INSPECTION SHEET

Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014 Room temp.: 25.0 C
Humidity: 41 %
Operator: Ankit

Detection limit(Minimum detectable amount) Overall Pass/Fail: Pass
Acceptance criterion SN Ratio:More than 200(Ex 350 nm, Em 397 nm
Result(s) SN Ratio: 265.174 Noise: 0.87





The intensity of the peak at 3380cm^{-1} is recorded. Its stability over 15 sec for 10 cycles of measurement is analyzed.

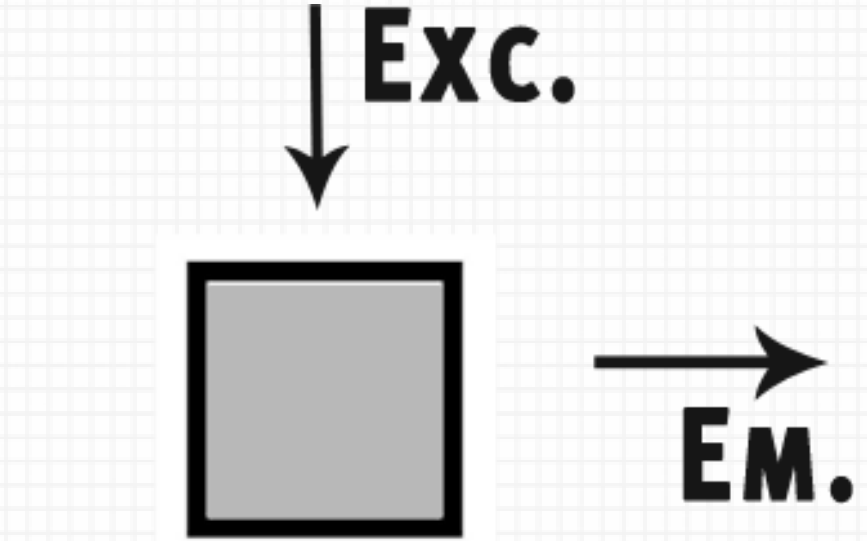
1.6 Photometric Stability

- Rectangular cell with Distilled water.
- We measure the time-course Raman peak at 398.4nm

Excitation at 350 nm
Emission at 397.1 nm measured over 1 hour.

In wavenumbers,
we measure the peak at 3380cm-1.

Variation in the signal intensity is recorded.



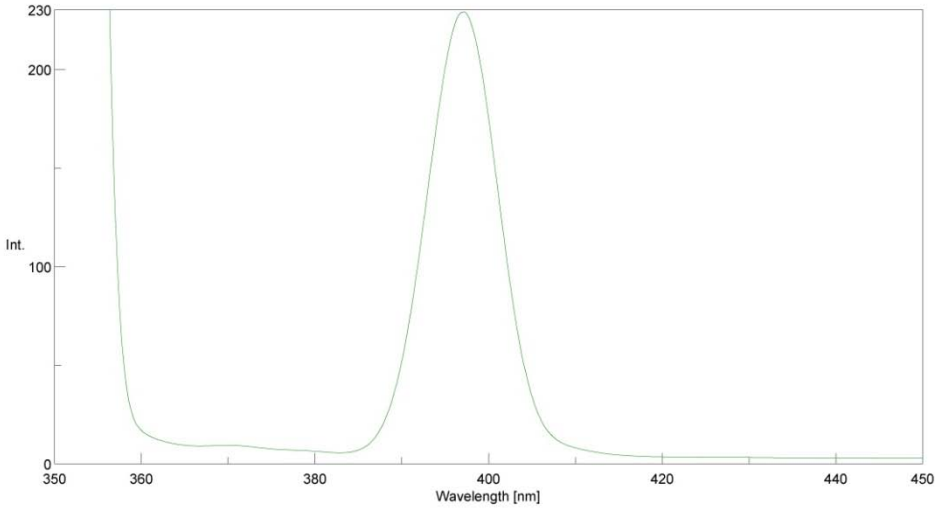
Distilled water

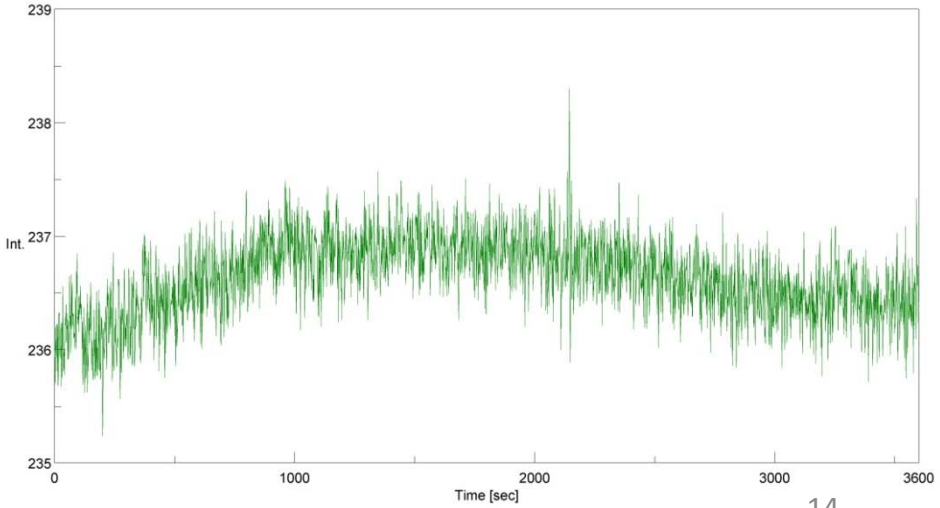
1.6 Photometric Stability

INSPECTION SHEET

Model: FP-6500
Serial No.: C089160822
Date: 30 / Mar / 2014 Room temp.: 24.6 C
Humidity: 41 %
Operator: Ankit

Photometric stability	Overall Pass/Fail: Pass
Acceptance criterion	2 %/hr(Ex 350 nm, Em 397.1 nm, 60 min)
Result(s)	0.00915567 %/hr



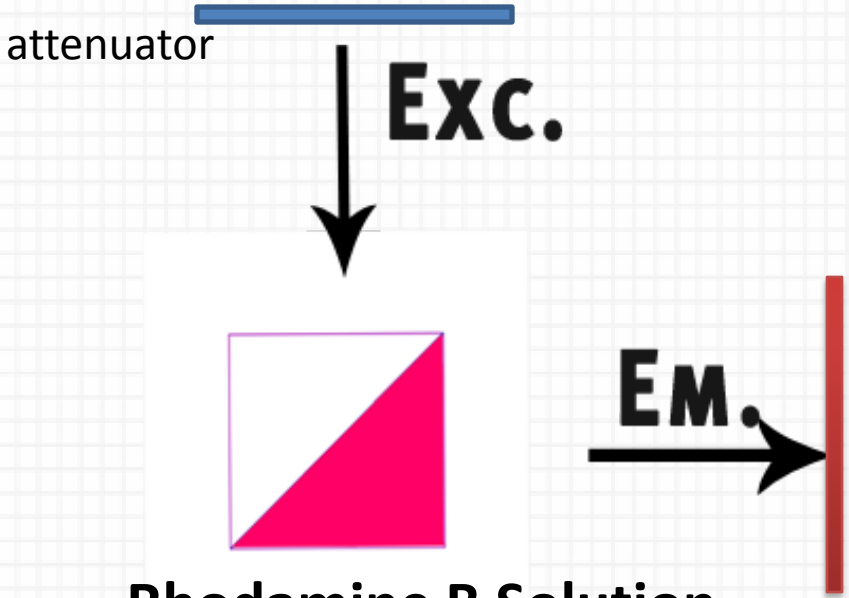


1. Correction
(by inbuilt software and given accessories)

- We can correct the Excitation intensity, Emission intensity, over the range 200-600nm using Rhodamine B solution.
  For full spectral range correction we require Standard light source.

•Correction using Rhodamine B solution.

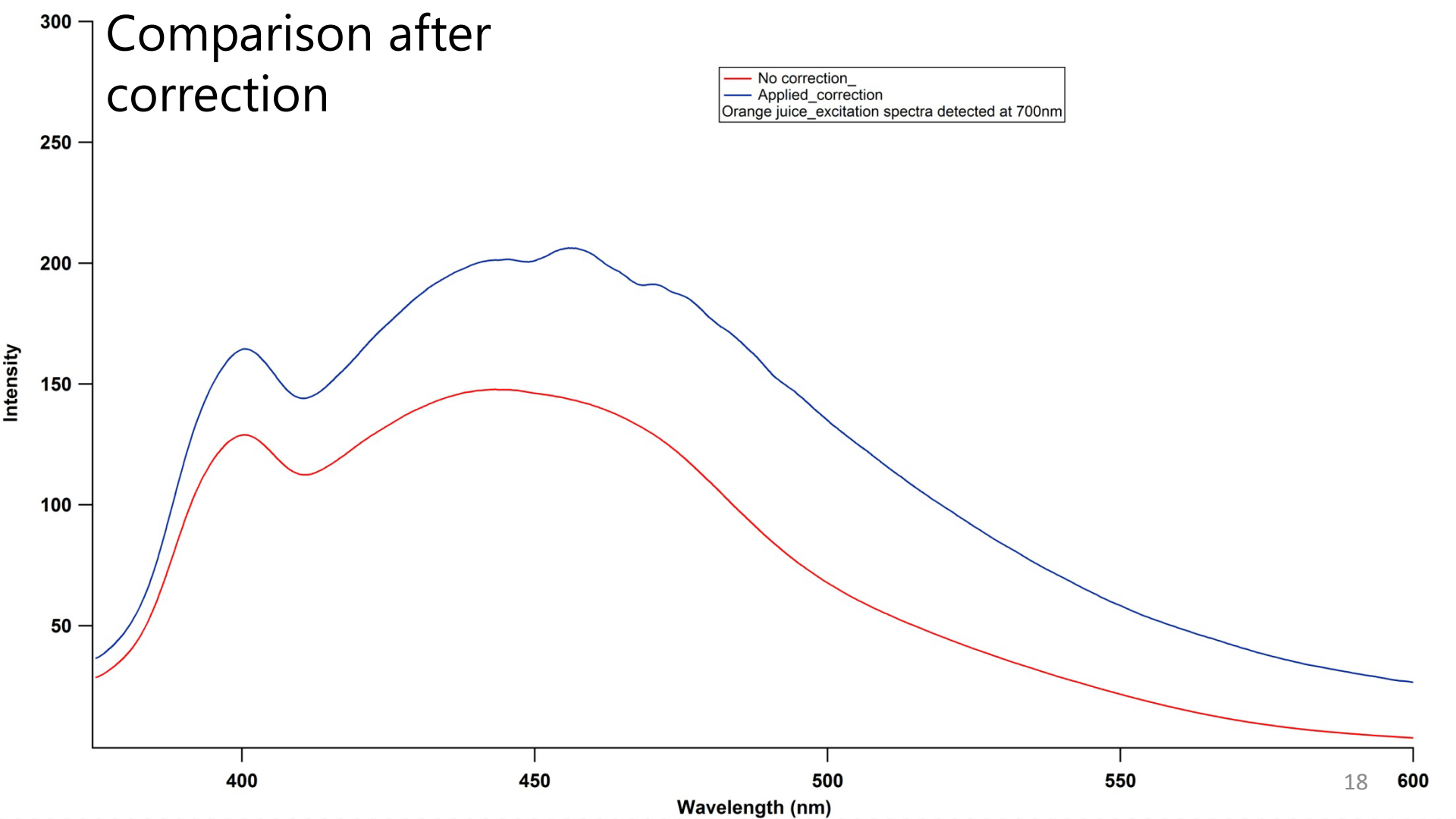
We measure the excitation of Rhodamine B over the range of 200-600nm; emission wavelength is set at 640nm.
The sensitivity of the PMT is adjusted so that the peak value lies in between 500-800. Then we normalize at a particular wavelength.



- For our case:

  We normalized at 400nm for both Excitation and Emission.
  This was the first correction data produced.
  And then we applied the correction data to compare with previous results.

Comparison after correction



I acknowledge Sudhakar Bhaiya for his kind help and giving time on Sunday!