

OptoScan Monochromator

Slit width calibration for manual input and exit unit

Why change bandwidth?

Most commercial monochromators include the facility to change bandwidth between experiments using a manual control. This is sufficient for many purposes, but is not ideal for complex protocols especially those involving multiple fluorescence markers. Only by controlling bandwidth in real-time during experiments can each excitation wavelength be optimized independently.

This means that where Stoke's shifts are large or where fluorescence intensities are weak then a relatively large bandwidth can be selected to maximize signal-to-noise. Conversely if there is a small Stoke's shift or if the fluorescence intensity is high, then the slit width can be minimized to reduce bleed through and optimize dynamic range.

Setting manual input and exit slits

Unfortunately adding galvanometer drives to the input and exit slit of a monochromator does have cost implications. For this reason, we also have versions with manual (micrometer controlled) slits on either the input slit, the exit slit, or both. In this instance the micrometers need to be set to give the appropriate bandwidth.



For precise control of this, then please run the OSDAC.EXE which allows any wavelength and bandwidth combination to be selected and will indicate the corresponding physical settings in microns. If running multiwavelength protocols, then it is recommended to choose a center-wavelength that is the average of the center-wavelengths being used when selecting the slits. This will give the best approximation of the true bandwidth, which does in reality have a small wavelength interdependence.

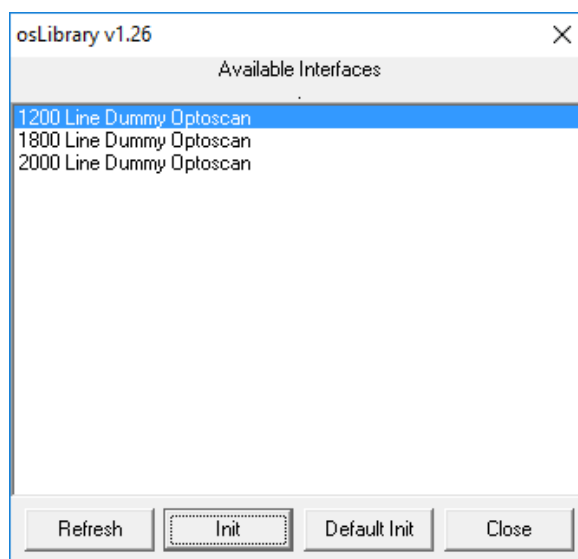
For most purposes the table below will give sufficiently accurate values for Fura 2 / Indo 1 (centered at 360nm) and Fluo 3 / GFP / Calcium Green (centered at 480nm). Bandwidths are specified as the point where the signal is 50% of peak e.g. 10nm half bandwidth at 360nm would give a half maximum signal at 355nm and 365nm. This table is based on the standard 1200-line grating monochromator; for other variations, please contact us.

Half Bandwidth	CWL = 360nm		CWL = 480nm	
	Input slit / μm	Exit slit / μm	Input slit / μm	Exit slit / μm
2nm	130.7	141.5	131.5	146.5
5nm	326.7	353.7	328.9	366.3
10nm	653.5	707.5	657.7	732.7
15nm	980.3	1061.2	986.6	1099
20nm	1307	1414.9	1315.4	1465.4
25nm	1633.8	1768.6	1644.3	1831.7
30nm	1960.5	2122.4	1973.1	2198

Worked example

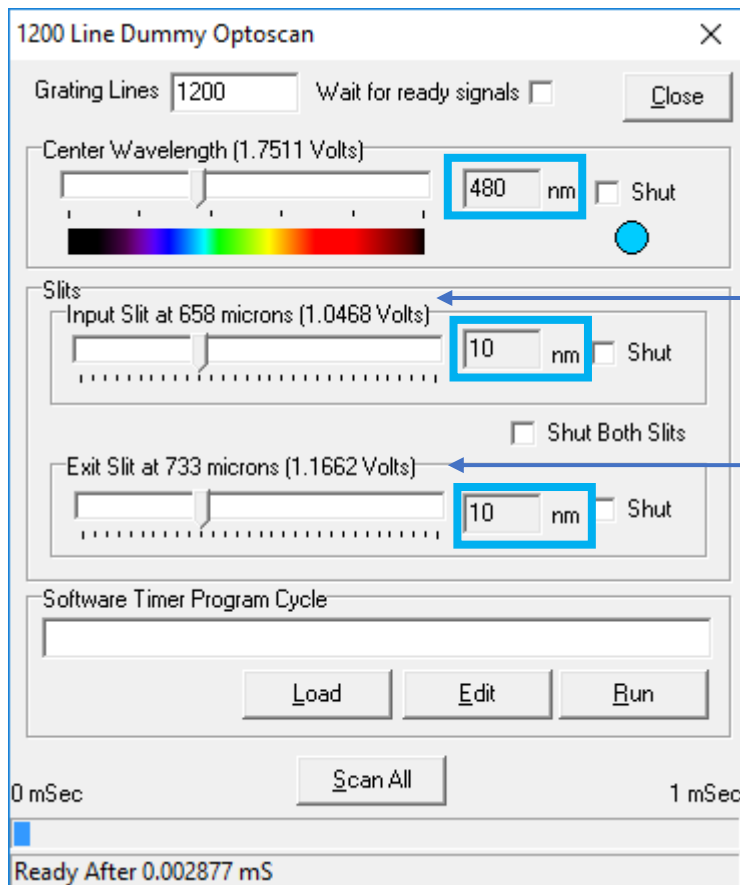
480nm peak excitation with a 10nm bandwidth on both the input and exit slit.

1. Install osDAC (normally included on a USB stick with your order, or alternatively this can be downloaded on the Software page of our website)
 - www.cairn-research.co.uk > Support > Software > OptoScan 64-bit test program
2. Select 1200 Line Dummy Optoscan



3. Click 'Init'

4. Enter the appropriate center wavelength and bandwidth for both the input and exit slits.



Note the corresponding micron values for both the input and exit slits

5. Adjust each micrometer accordingly:

Input slit (658 μ M)

- Zero = closed
- One full turn = 500 μ M
- Therefore 658 μ M = 1 full turn, followed by 15.8 on the outer micrometer dial

Exit slit (733 μ M)

- One full turn, followed by 23.3 on the outer micrometer dial

Please note, the maximum bandwidth of 30nm is 2198 μ M, i.e. < 2.5 full turns, therefore please do not over adjust the micrometers as this may cause damage to the unit. Similarly, do not adjust to values < zero.

Please do not hesitate to contact us if you have any questions during setup (tech@cairn-research.co.uk)