CSCI FLOW

ImageStream^{X} MkII Configuration and Recommended Fluorochromes

OVERVIEW

The ImageStream captures optical signals in two individual CCD (charge coupled device) cameras. Each camera captures images in 6 dedicated channels. Camera 1 captures images in Channels 1 - 6, and Camera 2 captures images in channels (Channels 7-12).

The ImageStream is equipped with **four high power excitation lasers**: **405 nm (120 mW), 488 nm (200 mW), 561 nm (200 mW), and 642 nm (150 mW).** The lasers illuminate the stream in a partial collinear manner. The 488 and 561 nm lasers illuminate the stream at the same point, and signals from these excitation lines are measured in Camera 1. The 405 nm and 638 nm lasers illuminate the stream at a second point, and signals from these excitation lines are measured in Camera 2. This collinearity has important implications for experimental design. The ImageStream is also equipped with a fifth 785 nm laser for side scatter.

CONFIGURATION CHART

	Channel	Detection Range	Laser and Recommended Fluorochromes[1]				
			405 nm 120 mW	488 nm 200 mW	561 nm 200 mW	642 nm 150 mW	785 nm 70 mW
Camera 1 488 nm and 561 nm Lasers	1	435-480 nm (457/45)	BRIGHTFIELD				
	2	480-560 nm (528/65)		AF488			
	3	560-595 nm (577/35)		PE tdTomato	PE tdTomato		
	4	595-642 nm (610/30)		PE-Dazzle 594	PE-Dazzle 594 mCherry		
	5	642-745 nm (702/85)		PE-Cy5	PE-Cy5		
	6	745-785 nm (762/40)		PE-Cy7	PE-Cy7		Side Scatter (Option 1)
Camera 2 405 nm and 642 nm Lasers	7	435-505 nm (457/45)	BV421 DAPI				
	8	505-570 nm (537/65)	BV510				
	9	570-595 nm (582/25)	BRIGHTFIELD				
	10	595-642 nm (610/30)	BV605				
	11	642-745 nm (702/85)	No Recommended Fluorochromes			Alexa 647 DRAQ5	
	12	745-785 nm (762/40)	No Recommended Fluorochromes			APC-Cy7	Side Scatter (Option 2)

[1] Available fluorochromes on the ImageStream are not restricted to the listed colors. If the probe you need to use is not listed in the configuration chart, speak with CSCI Flow Cytometry to ensure that it is compatible with the ImageStream.

IMPORTANT NOTES

- There are two brightfield channels on the ImageStream that must both always be on. Brightfield signals are used to synchronize signal between the two cameras and to maintain autofocus. Synchronization is required to ensure that signal generated by the spatially separated cameras is properly attributed to each object. Therefore, a channel on each camera must be dedicated to a brightfield measurement. Brightfield channels are assigned to Channels 1 (Camera 1) and 9 (Camera 2).
- Due to laser collinearity, certain fluorochrome combinations that can be used together on other flow cytometers cannot be used in the same manner on the ImageStream. For example, Brilliant Violet 786 (BV786), measured from 405 nm excitation and APC-Cy7, measured from 642 nm excitation can be detected independently on most conventional flow cytometers equipped with spatially separated lasers. However, because there is only one channel on the ImageStream to measure light in BV786 and APC-Cy7 emission ranges (Channel 12), the ImageStream will not be able to differentiate between signal from these two fluorochromes. Please be aware of the limitations imposed by collinearity when designing experiments.
- Side scatter is measured using a dedicated 785 nm laser. On traditional flow cytometers, side scatter is usually measured with a 488 nm laser.
- You can measure side scatter in either Channel 6 or Channel 12. You cannot use side scatter if you need to use Channels 6 and 12 for fluorescence measurements. For example, side scatter cannot be measured if you are using both PE-Cy7 (Channel 6) and APC-Cy7 (Channel 12).
- When designing panels for colocalization experiments, it is advisable to choose fluorochromes for each colocalization probe which are detected using the same camera. While the signals are synchronized between the two cameras via the two brightfield channels as described above, synchronization is not perfectly accurate. Therefore, measuring the two probes on separate cameras may lead to variability in localization of signal and thus errors in colocalization scores during analysis.
- Certain fluorochromes do not perform well on the ImageStream. In particular, **do not use Alexa Fluor 700**. In addition, the long Stokes' shift Brilliant Violet tandem dyes **(BV711 and BV786) do not perform well and should be avoided**. Use Alexa 647 and APC-Cy7 in the respective channels that would be used for these Brilliant Violet dyes (Channel 11 and Channel 12).