

# Lasers and filters at the Image Stream

March 2021, FACS Core Facility Aarhus University

Laser	CAMERA 1						CAMERA 2					
	Ch 1	Ch 2	Ch 3	Ch 4	Ch 5	Ch 6	Ch 7	Ch 8	Ch 9	Ch 10	Ch 11	Ch 12
Default + default	435-480 nm	480-560 nm	560-595 nm	595-642 nm	642-745 nm	745-780 nm	435-505 nm	505-570 nm	570-595 nm	595-642 nm	642-745 nm	745-780 nm
561nm		480-551 nm	571-595 nm					505-551 nm	571-595 nm			
642 nm				595-625,5 nm	658-745 nm						595-625,5 nm	658-745 nm
488 nm		495-560					435-480 nm					
488 nm+561 nm		495-551	571-595 nm					505-551 nm	571-595 nm			
Brightfield Default	Acridine orange Alexa Fluor 488 <u>Alexa Fluor 514</u> <u>Calcium Green</u> DyeCycle Green DyLight 488 FITC GFP LysoTracker Green MitoTracker Green YTO RNA select green YFP	<u>Alexa Fluor 514</u> <u>Calcium Green</u> R-Phycoerythrin (PE) Alexa Fluor 546 <u>Alexa Fluor 555</u> <u>Calcium Orange</u> DII DyLight 549 eFluor 570 <u>LysoTracker Red</u> <u>MitoTracker red</u> RFP TRITC	<u>Alexa Fluor 514</u> <u>Calcium Green</u> PE-Alexa Fluor 610 PE-Texas Red (ECD) <u>Propidium Iodide</u> Alexa Fluor 546 <u>Alexa Fluor 555</u> Alexa Fluor 568 <u>Alexa Fluor 594</u> <u>Alexa Fluor 610</u> <u>Calcium Orange</u> DII DyLight 594 eFluor 570 eFluor 615 <u>LysoTracker red</u> <u>mCherry</u> <u>MitoTracker Red</u> RFP <u>Texas Red</u>	<u>7AAD</u> <u>DsRed</u> PE-Alexa Fluor 610 PE-Texas Red (ECD) <u>Propidium Iodide</u> PE-Alexa Fluor 700 Alexa Fluor 568 <u>Alexa Fluor 594</u> <u>Alexa Fluor 610</u> <u>Calcium Orange</u> DII DyLight 594 eFluor 570 eFluor 615 <u>LysoTracker red</u> <u>mCherry</u> <u>MitoTracker Red</u> RFP <u>Texas Red</u>	<u>PerCP</u> <u>PerCP-Cy5.5</u> <u>PerCP-eFluor 710</u> <u>7AAD</u> <u>PE-Cy5</u> <u>PE-Cy5.5</u> <u>Propidium Iodide</u> <u>Alexa Fluor 594</u> <u>DRAQ5</u> <u>mCherry</u> <u>Texas Red</u> <u>AlexaFluor 610</u>	PE-Cy7 PE-Vio770          Darkfield (SSC)	Alexa Fluor 405 BV421 Cascade Blue <u>*CFP</u> DAPI DyLight 405 eFluor 450 <u>eFluor 506</u> Hoechst 33258 Hoechst 33342 LIVE/DEAD Violet Marina Blue Pacific Blue	Alexa Fluor 430 BV 570 <u>* Cascade Yellow</u> <u>* CFP</u> <u>eFluor 506</u> <u>* Krome Orange</u> <u>* Lucifer Yellow</u> <u>* Pacific Orange</u> Qdot 525 Qdot 545 <u>Qdot 565</u>	<u>BV 570</u> <u>* Krome Orange</u> <u>Qdot 565</u> <u>Qdot 585</u>          Brightfield Default	BV 570 <u>BV 605</u> <u>BV 650</u> BV711 eFluor 605 Qdot 605 Qdot 625          Alexa Fluor 647 Alexa Fluor 660 Alexa Fluor 680 APC APC-Cy5.5 Cy5 Cy5.5 <u>DRAQ5</u> DyLight 649 eFluor 660 eFluor 710 MitoTracker Deep Red <u>Nile Blue</u>	BV785 Qdot800 APC-Cy7 APC-eFluor 780 APC-H7 DyLight 750          Darkfield (SSC)	
"Bandpass"	457,5/45	520/80	577,5/35	618,5/47	693,5/103	762,5/35	470/70	537,5/65	582,5/25	618,5/47	693,5/103	762,5/35
561nm		515,5/71	583/24					528/46	583/24			
642 nm				610/30,5	7101/87						610/30,5	701/86,5
488 nm		528/64,5					457,5/45					
488 nm+561 nm		528/56	583/24				457,5/45	528/46	583/24			

The numbers just below the table corresponds to bandpass filter values to be used in a spectraviewer

Excitation lasers: 405 nm laser 488 nm laser 561 nm laser 642 nm laser      Darkfield (SSC) Laser: 785 laser

488 nm and 561 nm lasers are co-linear  
405 nm and 642 nm lasers are co-linear

When ever you turn on a laser, a NOTCH filter will be applied. This will affect the area of light being detected in some channels; If you turn on the 561 nm laser, chanel 3 will change from detecting 560-596nm light to 571-595 nm  
Underlined fluorochromes can be detected in more than one channel. ' marks the best channel to use with the standard "bandpass".  
If turning on a laser affects the band width, it may also change the best chanal to detect in. Check in a spectreviewer!

Fluorochromes marked by \* have a broad emmission peak, which will may affect neighbouring channels.