

Standard settings for Leica SP5 confocal microscope (Used under Sequential Collection conditions)

Note: Save with your collection conditions by replacing 'Name' with your personal name

'3-color' Collection: DAPI, Alexa 488, Alexa 594							Primary fluorophore	Alternative fluorophores
	Setting Name	Laser	PMT	Emission	Color	Mirror		
a	Name SP5 3-DAPI	405	1	415-460	Blue	none	DAPI	Alexa 405
b	Name SP5 3-488	488	3	498-550	Green	none	Alexa 488	FITC/GFP
c	Name SP5 3-594	594	4	604-650	Red	none	Alexa 594	mCherry/mPlum
'4-color' Collection: DAPI, Alexa 488, Alexa 555, Alexa 633							Primary fluorophore	Alternative fluorophores
	Setting Name	Laser	PMT	Emission	Color	Mirror		
a	Name SP5 4-488	488	2	498-530	Green	none	Alexa 488	FITC/GFP
b	Name SP5 4-555	543	3	553-595	Yellow	none	Alexa 555	Alexa 546/Alexa 568/TRITC/Cy3/mOrange/PE
c	Name SP5 4-DAPI+633 (can do together)	405	1	415-460	Blue	none	DAPI/Hoechst	Alexa 405
		633	5	650-700	Magenta	none	Alexa 633	Alexa 647/ToPro3
'5-color'Collection: DAPI, Alexa 488, Alexa 555, Alexa 594, Alexa 647							Fluorophore	Alternative fluorophores
	Setting Name	Laser	PMT	Emission	Color	Mirror		
a	Name SP5 5-488	488	2	498-530	Green	none	Alexa 488	FITC/GFP
b	Name SP5 5-555	543	3	553-573	Yellow	none	Alexa 555	Alexa 546/TRITC/Cy3/mOrange
c	Name SP5 5-594	594	4	604-624	Red	none	Alexa 594	mCherry/mPlum
d	Name SP5 5-DAPI+647 (can do together)	405	1	415-460	Blue	none	DAPI/Hoechst	Alexa 405
		633	5	650-700	Magenta	none	Alexa 647	ToPro3
<p>a, to set the Emission collection, double click on the rectangle under the spectrum. Enter the beginning and ending wavelegths</p> <p>b, to activate the indicated laser, click 'active' and turn laser up to the desired % of power (note, for the 405 nm laser, a separate 'active' box must be clicked and power changed by clicking on the -- under the 'wheel diagram')</p> <p>c, to activate a specific PMT, click 'active' under that PMT. Set the color of the output to what you desire</p> <p>d, save the setting using the format 'Your Name, Setting Specific information'. See above names as examples.</p>								