

**Epifluorescence excitation intensity in W.cm<sup>-2</sup>** measured in the sample plane for different motorised attenuator settings (2% – 100%) and with different objectives (measured on 24/01/2020).

5x/0.16	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
20%	0.012	0.018	0.006	0.023	0.004
40%	0.025	0.033	0.012	0.041	0.007
50%	0.037	0.046	0.016	0.057	0.011
70%	0.057	0.066	0.023	0.081	0.015
100%	0.087	0.090	0.032	0.12	0.020
20x/0.5	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
2%	0.007	0.012	0.003	0.017	0.003
20%	0.17	0.27	0.09	0.37	0.06
40%	0.34	0.50	0.17	0.67	0.12
50%	0.50	0.71	0.24	0.93	0.16
70%	0.78	1.0	0.34	1.3	0.23
100%	1.2	1.4	0.47	1.7	0.33
LD-40x/0.6	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
2%	0.013	0.026	0.006	0.039	0.006
20%	0.28	0.59	0.21	0.87	0.15
40%	0.59	1.2	0.42	1.7	0.31
50%	0.89	1.7	0.60	2.5	0.46
70%	1.4	2.4	0.85	3.5	0.64
100%	2.2	3.3	1.2	4.6	0.85
LD-63x/0.75	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
2%	0.03	0.08	0.03	0.13	0.03
20%	0.70	2.0	0.70	2.8	0.52
40%	1.4	3.8	1.4	5.4	1.0
50%	2.1	5.5	2.0	7.6	1.4
70%	3.3	7.9	2.7	10.7	2.0
100%	5.2	11.1	3.8	14.5	2.6
63x/1.25	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
2%	0.03	0.08	0.03	0.13	0.03
20%	0.70	2.0	0.70	2.8	0.52
40%	1.4	3.8	1.4	5.4	1.00
50%	2.1	5.5	2.0	7.6	1.4
70%	3.3	7.9	2.7	10.7	2.0
100%	5.2	11.1	3.8	14.5	2.6
100x/1.3	49-DAPI	10-FITC	46-YFP	31-Alexa568	50-Cy5
2%	0.04	0.12	0.04	0.24	0.04
20%	1.3	2.9	1.1	4.7	0.88
40%	2.7	5.7	2.2	9.4	1.7
50%	4.1	8.1	3.1	13	2.4
70%	6.3	12	4.5	19	3.5
100%	10	16	6.0	25	4.5