Table S1. Imaging conditions and antibody dilutions

picture	pixel size	scan speed (FPS)	pixel dwell time	lasers/ Filters	laser power in the object plane in [a]	number of frames	Gain	primary AB	sec. AB	Dye	PiMP conditions
suppl. Fig. S5	60/67 nm	2 FPS (frames per second))	7.8 µS	543 nm	19.8 µW	60/80	(0-255) 65			TetraSpeck microspheres Emission 580nm	Filter 0.9/0.8 Binned to 0.05 Run Av 0.8
2A, B, C	48.48 nm	-	750 ms	488 nm	500 µW	25 / 25	non	1:300	1:600	Alexa 488 Emission 520nm	Filter 1 No binning Run Av 0.8
2D	48.48 nm	-	30 ms	561 nm	28.8 mW	20 000	max. EM Gain	1:300	1:600	Alexa 568 Emission 603nm	Not applicable
2E, F	63 nm	-	750 ms 750 ms	488 nm/ 561 nm	unknown (company test setup)	(15 per channel)	EM Gain 300 300	1:300	1:600 1:600	Alexa 488 Emission 520nm; Alexa 568 Emission 603nm	Not applicable
2H, I, J, K	48.48 nm	-	750 ms	488 nm/ 561 nm	250 µW 500 µW	50 per channel	non	1:300	1:600 1:600	Alexa 488 Emission 520nm; Alexa 568 Emission 603nm	Filter 1 / 1.2 Binned 0.05 Run Av 0.8
3A, B	67 nm	2 FPS	16.5 µS	488 nm	1.7 µW	80	(0-255) 80	1:100	1: 1000	Alexa 488 Emission 520nm	Filter 1.1 [b] Binned to 0.05 Run Av 0.8
3E, F, G	55 nm	1.4 FPS	2.8 µS	543 nm	40.5 µW	100 8 z-sections 0.3 µm steps (in total 800)	(0-1250) 700	"-"	"-"	"-"	Filter 1 Binned to 0.05 Run Av 0.8 Surface rendering details 75nm
3 I,J	69 nm	2 FPS	3.9 µS	488 nm	21.8 µW	251 / 50	(0-255) 20	-	-	GFP Emission 520nm	Filter 0.8 [b] Binned to 0.05 Run Av 0.8
3 M-V	27 nm	0.78 FPS	4.9 µS	458 nm 514 nm 543 nm 633 nm	11.6 µW 46.5 µW 14.6 µW 64.4 µW	50 per channel	(0-1250) 500 600 804 500	1:500	1: 1000	Cerulean Emission 475 nm YFP Emission 527 nm mCherry Emission 610 nm Alexa 647 Emission 668 nm	Filter 2.2 for all due to single pinhole system Binned to 0.05 Run Av 3
4 A-C	370 nm	un-delayed	800 ms	Metal Halide 645/30	-	100 images separated by 3s bleach images	-	1:200	1: 1000	Alexa 647 Emission 668 nm	Filter 0.5 Binned to 0.05 Run Av 2
4 E, F	73 nm	un-delayed	500 ms	Hg Lamp 675/55	-	116	-	1:200	1: 1000	Alexa 647 Emission 668 nm	Filter 1.0 [b] Binned to 0.1 Run Av 3
4 G, H	75 nm	1	9 µS	639 nm	2.5 µW	53	75	1:200	1: 1000	Alexa647 Emission 668 nm	Filter 1 Binned to 0.1 Run Av 3

([a]) Laser intensity was measured using an Ophir (Ophir Optronics, Jerusalem) and Nova laser power meter and Coherent (Santa Clara, CA) FieldMaxII-TO with sensor OP-2 VIS. For PiMP reconstructions, a sampling ratio (0,51emmision wavelength/NA/pixel size) of ~4.4 with a filter size of 1.1 was used in the simulations (see above). In the experiments, PiMP settings were adjusted accordingly using the emission PSF for adjusting the filter in all experiments. This is done as PiMP is an image-based technique. ([b]) Larger filter was used as the PSF is expected to be distorted due to additional blur and when measuring movements in samples and within the Drosophila embryonic tissue, respectively.