

	High sensitivity setup (Figure 1)		Screening setup (Figures 2, 3, 4, 5)				
Name of reagent	bDNA smFISH	o-nuc smFISH	bDNA smFISH	DAPI	Mitotracker	Succinidmidyl ester	staining
Product number	Panomics, custom version of QVP0201	Stellaris SMF-2013-1	Panomics, custom version of QVP0201	Invitrogen D1306	Invitrogen M-512	Invitrogen A-20006	
Simultaneous acquisition	no	no	no	with succinimidylester	no	with DAPI	
imaging medium	PBS	Glox Buffer (see Stellaris manual)	PBS	PBS	PBS	PBS	
fluorochromes	Alexa 546	Quasar 570	Alexa 488	DAPI	MitoTracker Red CMXRos: Ex 579 nm ; Em 599 nm	Alexa 647	
imaging temperature	room temperature	room temperature	room temperature	room temperature	room temperature	room temperature	
microscope	custom built by VisitronSystems	custom built by VisitronSystems	Yokogawa CellVoyager 7000-ET01	Yokogawa CellVoyager 7000-ET01	Yokogawa CellVoyager 7000-ET01	Yokogawa CellVoyager 7000-ET01	microscope
Microscope batch number	71171/0110-TIRF	71171/0110-TIRF	KZL900005	KZL900005	KZL900005	KZL900005	
Excitation Source	HXP 120X illumination system: 120 W mercury short arc lamp	HXP 120X illumination system: 120 W mercury short arc lamp	Sapphire 488 LP (Coherent Inc.) 100mW Laser	CUBE 405-100C (Coherent Inc.) 100mW Laser	Sapphire 561 LP (Coherent Inc.) 200mW Laser	CUBE 640-100C (Coherent Inc.) 100mW Laser	
immersion	oil	oil	air	air	air	air	
Numerical aperture	1.49	1.49	0.95	0.95	0.95	0.95	
space resolution x,y,z	not determined (experimentally); may approximate by theoretical resolution obtainable with numerical aperture and refraction index						
Pixel size	0.13 um	0.13 um	162.5 nm	162.5 nm	162.5 nm	162.5 nm	
beam splitter	n/a	n/a	DM488 (ASAHI Spectra)	DM488 (ASAHI Spectra)	DM488 (ASAHI Spectra)	DM488 (ASAHI Spectra)	
acquisition software	VisiView	VisiView	CellVoyager Measurement System	CellVoyager Measurement System	CellVoyager Measurement System	CellVoyager Measurement System	
objective lens	Apo TIRF 100x objective (Nikon)	Apo TIRF 100x objective (Nikon)	UPLASAPO 40X (Olympus)	UPLASAPO 40X (Olympus)	UPLASAPO 40X (Olympus)	UPLASAPO 40X (Olympus)	
cameras	ImageEM 1K C9100-14, Hamamatsu).	ImageEM 1K C9100-14, Hamamatsu).	Neo (Andor)	Neo (Andor)	Neo (Andor)	Neo (Andor)	
excitation filter / mirror	Chroma ET Filter-mCherry	Chroma ET Filter-mCherry	JDSU CSUDM405/488/561	JDSU CSUDM405/488/640	JDSU CSUDM405/488/561	JDSU CSUDM405/488/640	
emission filter	Chroma ET Filter-mCherry	Chroma ET Filter-mCherry	Semrock BP525/50 (FF01-525/50-35 custom order)	Semrock BP445/45 (FF01-445/45-35 custom order)	Semrock BP620/70 (FF01-617/73-35 custom order)	Semrock BP675/30 (FF01-676/29-35)	
Excitation strength	maximum	maximum	30	30	30	30	acquisition settings
exposure time	see experimental comparison of varying exposure time	see experimental comparison of varying exposure time	400 ms	400 ms	550 ms	400 ms	
acquired planes	33	33	10	8	7	8	
z-step	0.3 um	0.3 um	1 um	1 um	1 um	1 um	
image bit depth	16	16	16	16	16	16	
gain	see experimental comparison of varying gain	see experimental comparison of varying gain	1,3	1,3	1,3	1,3	

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