

Supporting information

Live-cell fluorescence lifetime multiplexing using synthetic fluorescent probes

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* Co-corresponding

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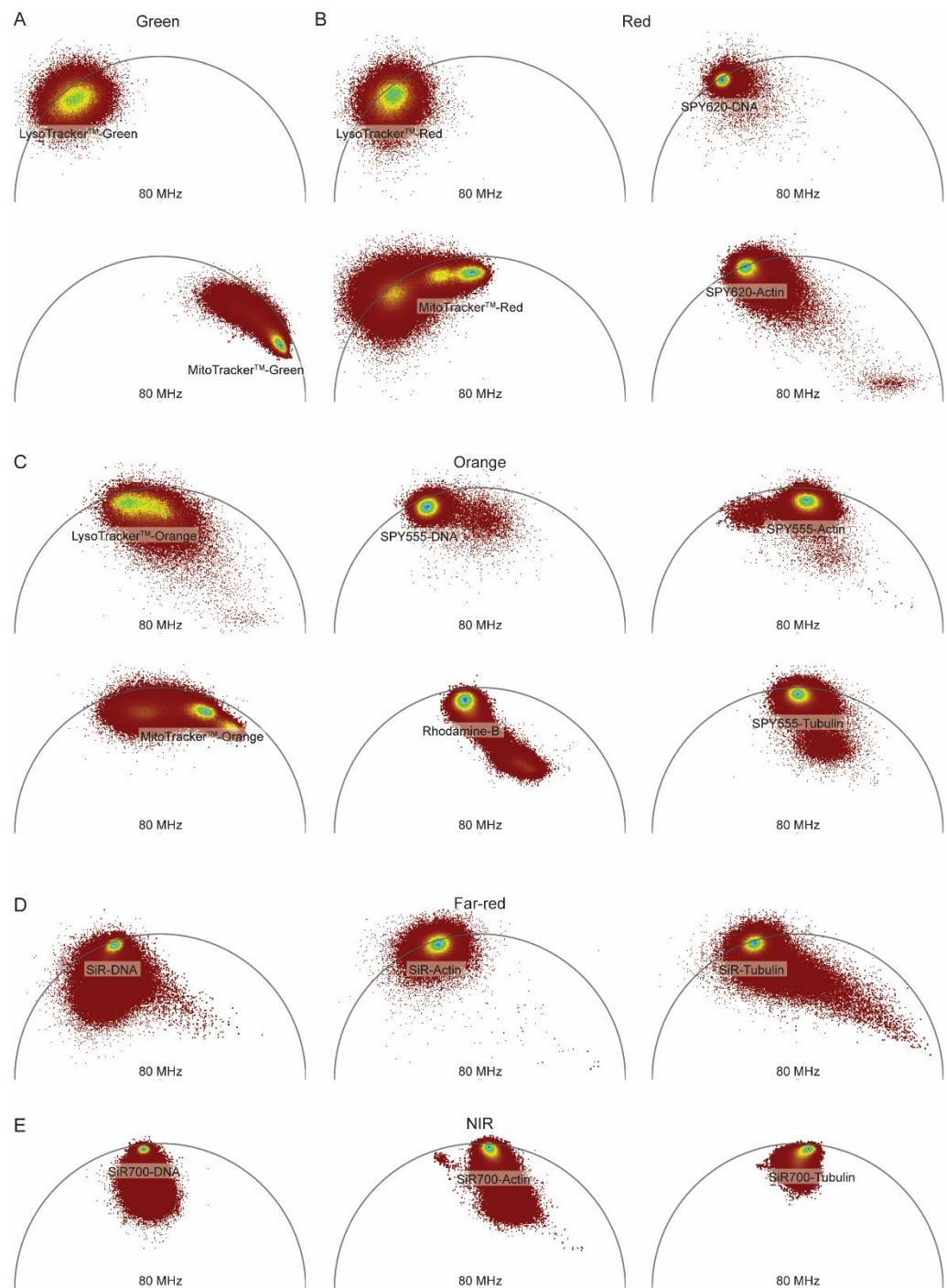
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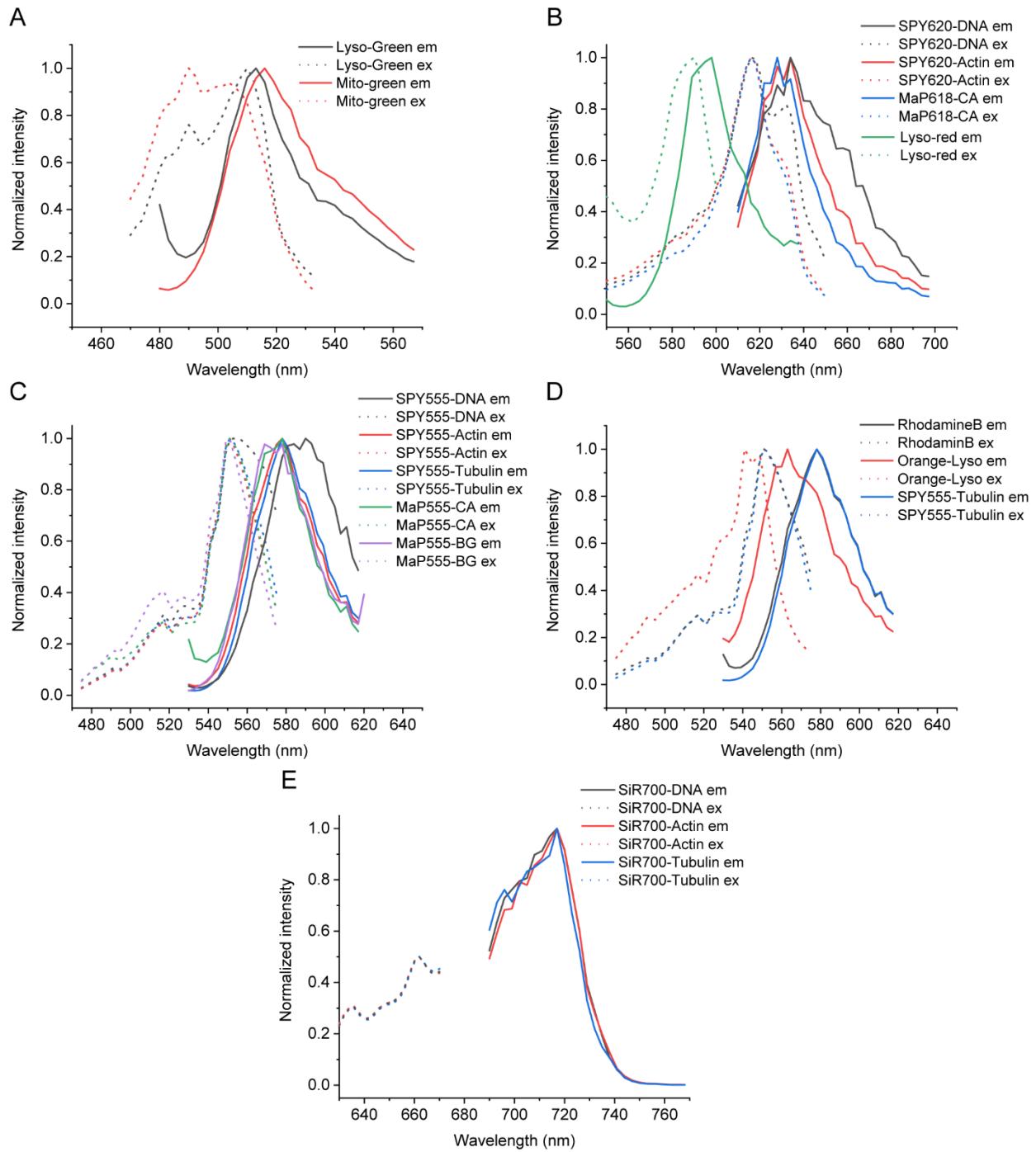
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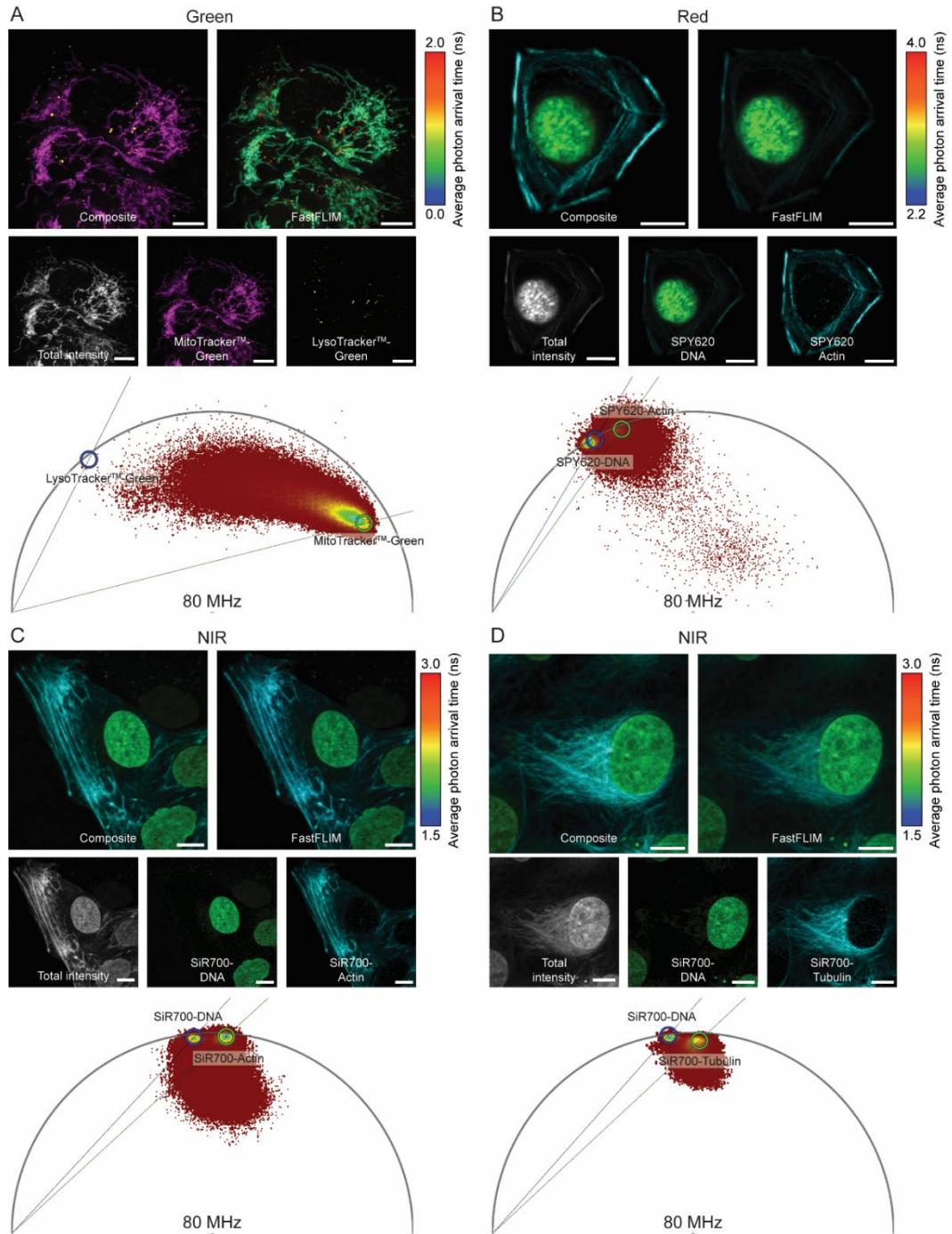
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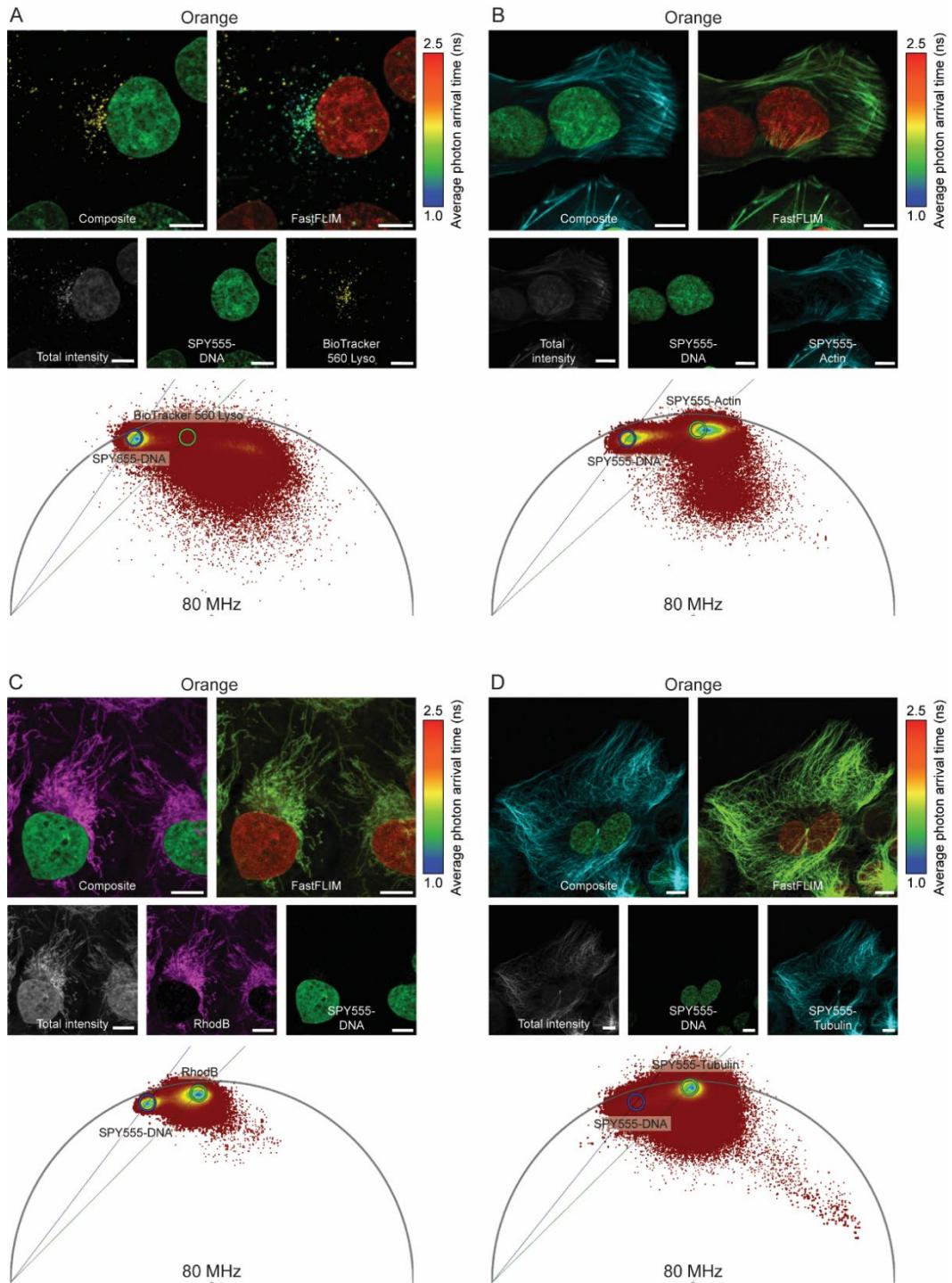
Supporting Figure S1: Phasor plots of the 18 synthetic probes tested. **A** Green spectral region. **B** Red spectral region. **C** Orange spectral region. **D** Far-red spectral region. **E** NIR spectral region.



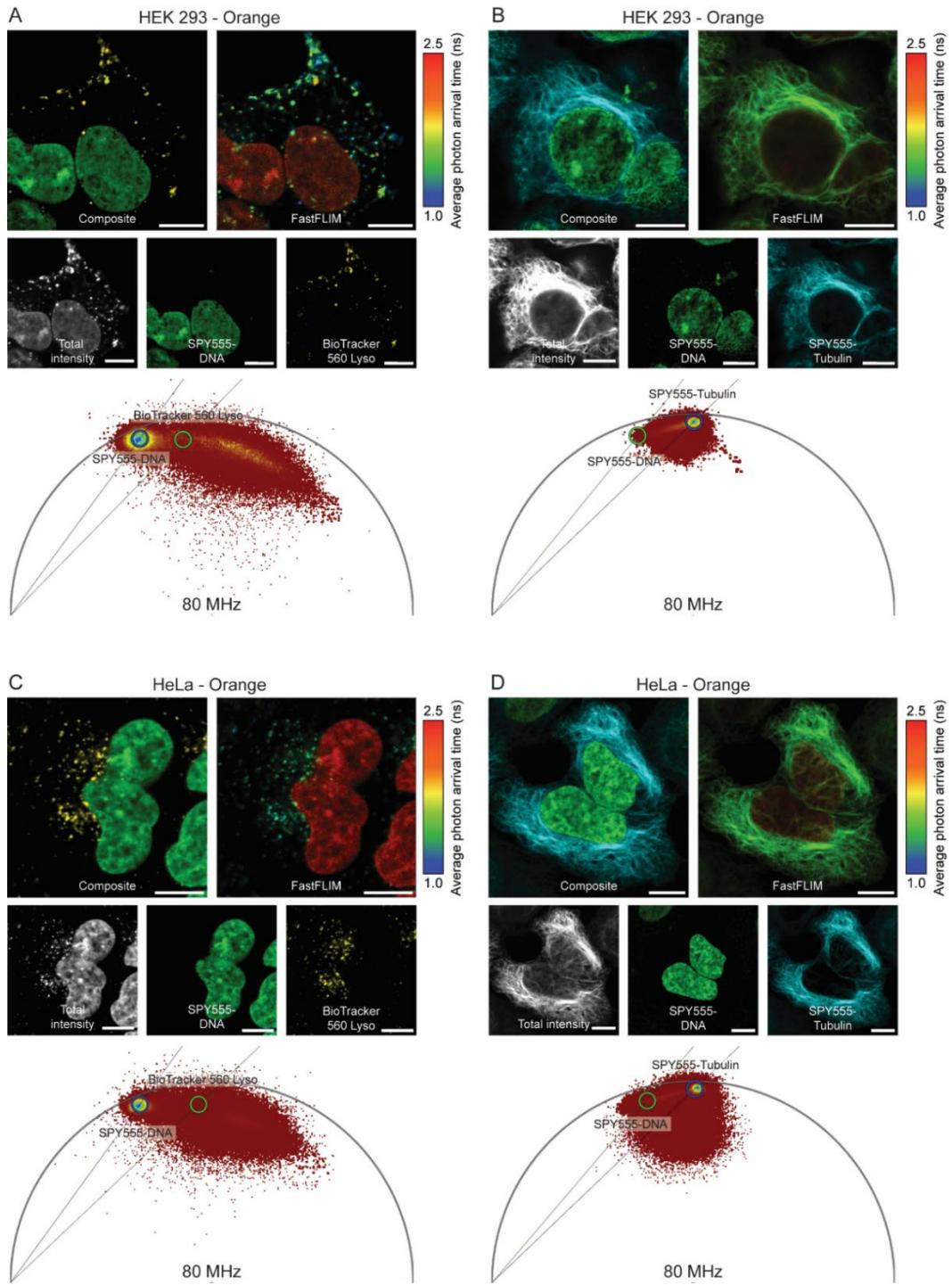
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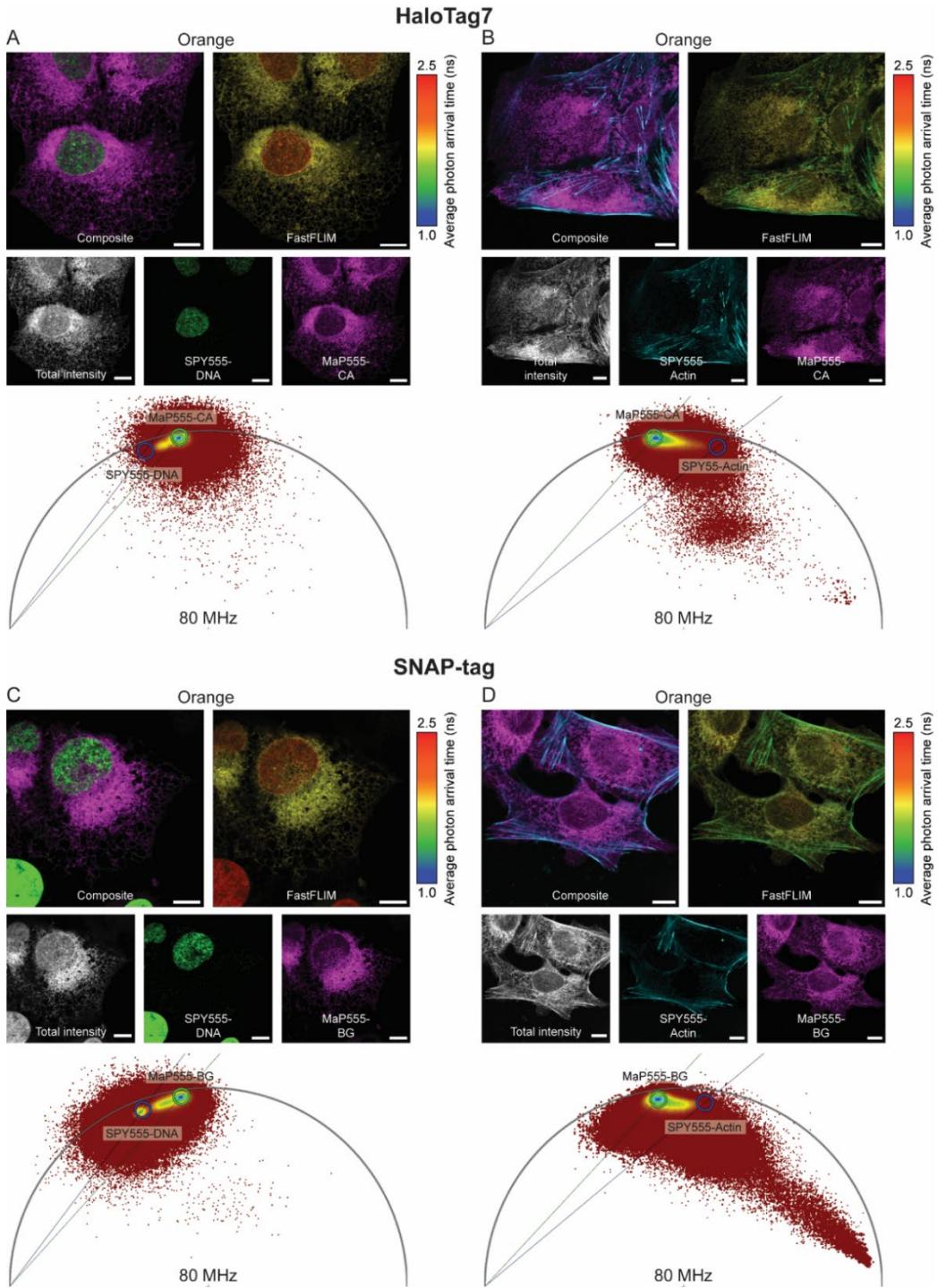
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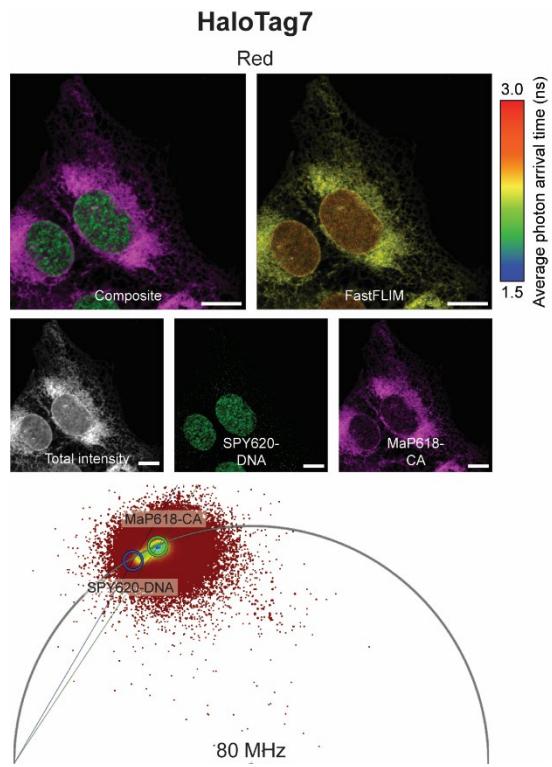
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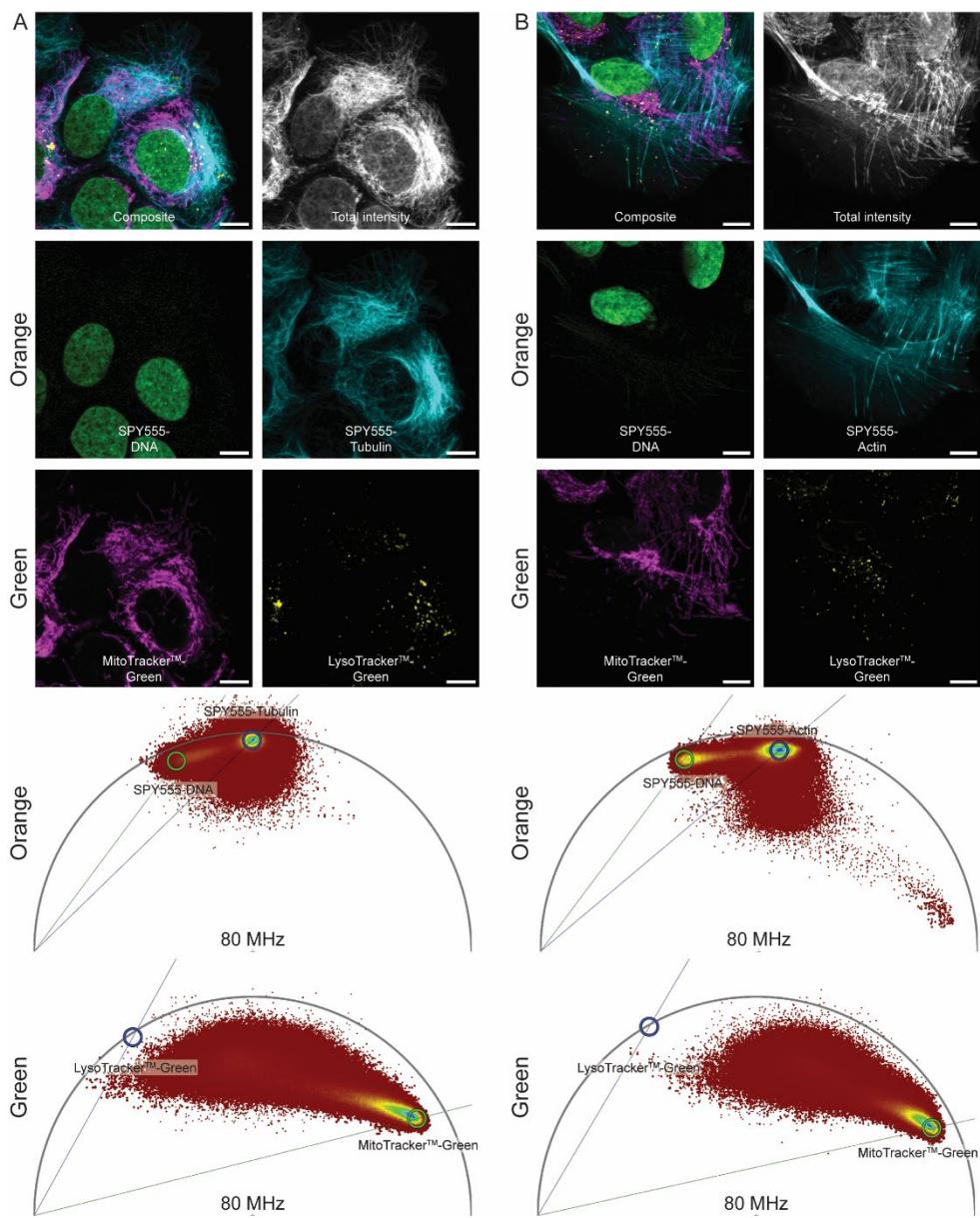
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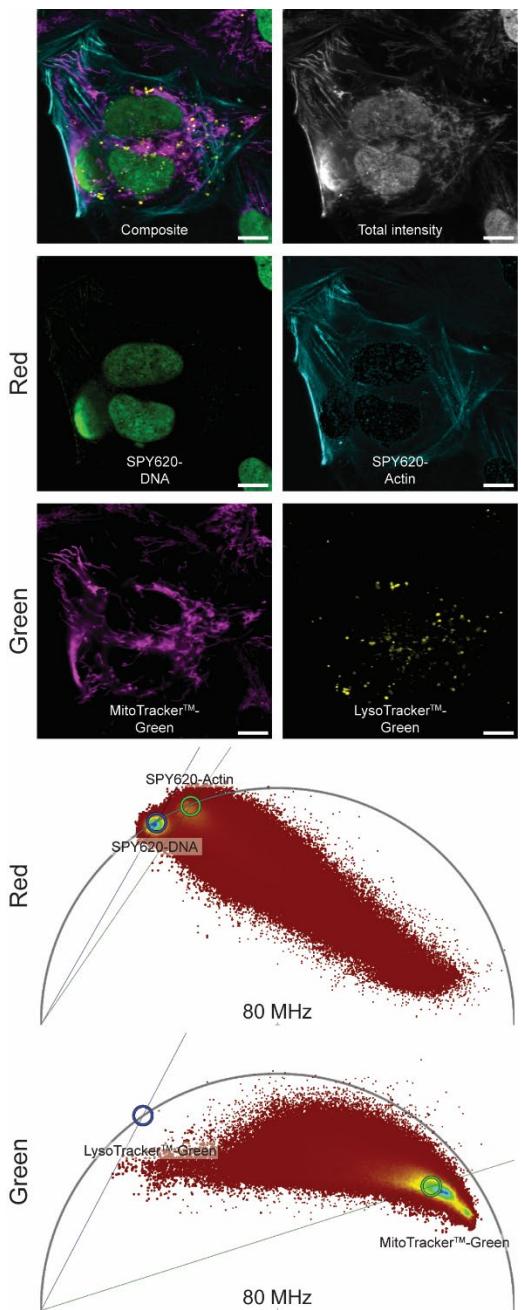
Supporting Figure S6: Multiplexing synthetic probes and self-labeling protein tags. **A-D** U-2 OS cells stably expressing the endoplasmic reticulum marker calreticulin (CalR) as a HaloTag7-SNAP-tag fusion additionally fused to a KDEL targeting peptide were labeled with MaP555-CA (**A-B**) or MaP555-BG (**C-D**) and SPY555-DNA (**A, C**) or SPY555-Actin (**B, D**). The composite, the FastFLIM image with the respective color-scale, the total fluorescence intensity, the two individual separated species as well as the corresponding wavelet-filtered phasor plot used for separation are given. Species separation was achieved using the phasor approach by positioning the cluster-circles on the phasor plot at the position of the pure species. Scale bars, 10 μ m.



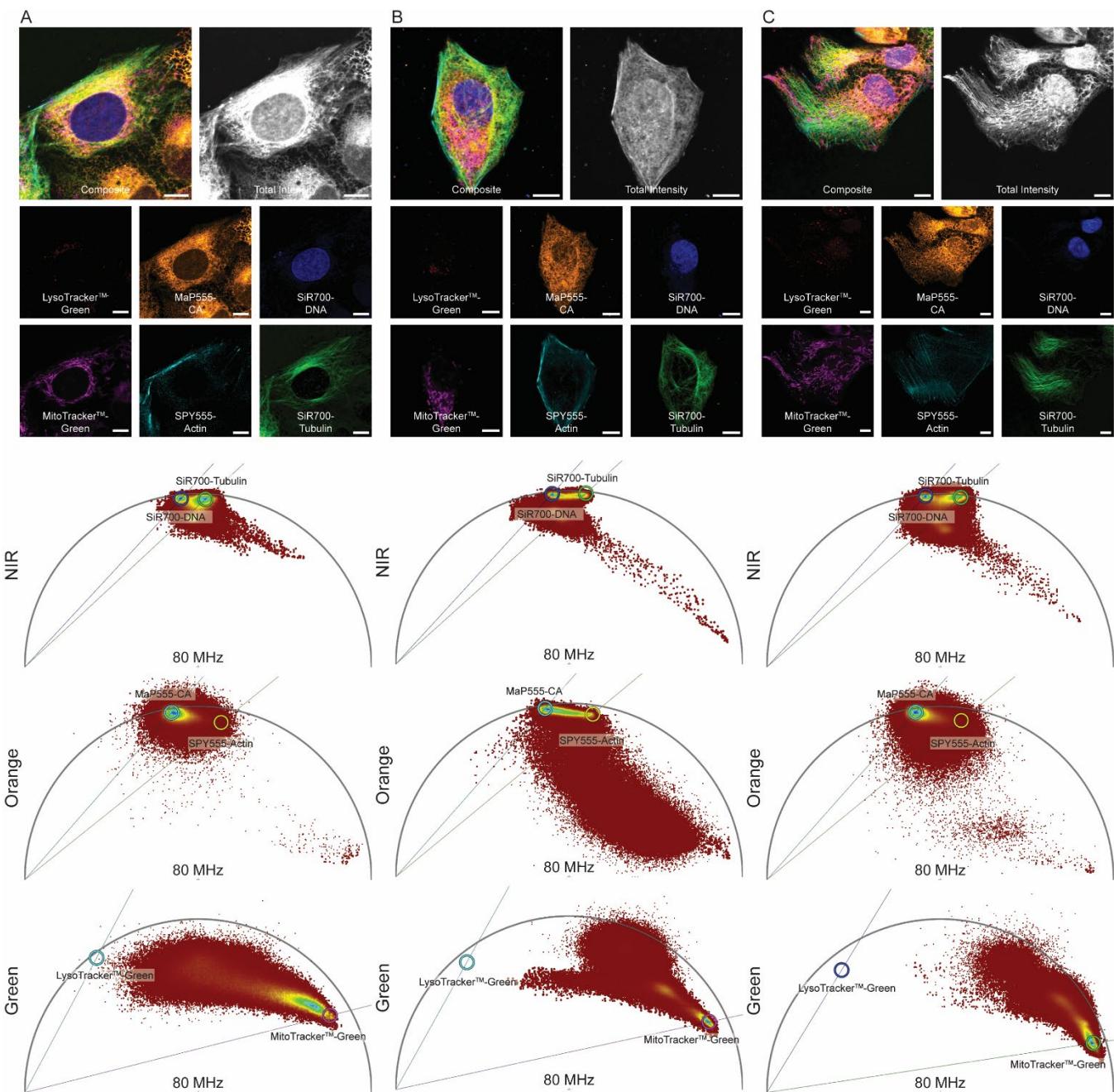
Supporting Figure S7: Multiplexing synthetic probes and HaloTag in the red channel. **A** U-2 OS cells stably expressing CalR-HaloTag7-SNAP-tag-KDEL were labeled with MaP618-CA and SPY620-DNA. The composite, the FastFLIM image with the respective color-scale, the total fluorescence intensity, the two individual separated species as well as the corresponding wavelet-filtered phasor plot used for separation are given. Species separation was achieved using the phasor approach by positioning the cluster-circles on the phasor plot at the position of the pure species. Scale bars, 10 μ m.



Supporting Figure S8: Four species images combining fluorescence lifetime multiplexing in the green and orange spectral channel. **A-B** U-2 OS cells were labeled with MitoTracker™-Green, Lysotracker™-Green, SPY555-DNA, and either SPY555-Tubulin (**A**) or SPY555-Actin (**B**) and imaged in the two spectral channels giving access to four species images. The composite, the total fluorescence intensity, the four individual separated species as well as the corresponding wavelet-filtered phasor plots used for separation are given. Species separation was achieved using the phasor approach by positioning the cluster-circles on the phasor plot at the position of the pure species. Scale bars, 10 μ m.



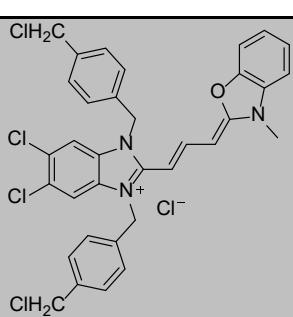
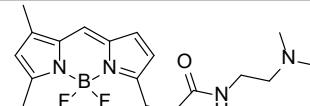
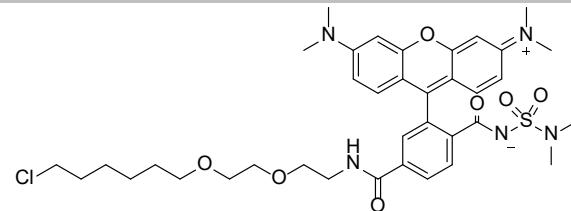
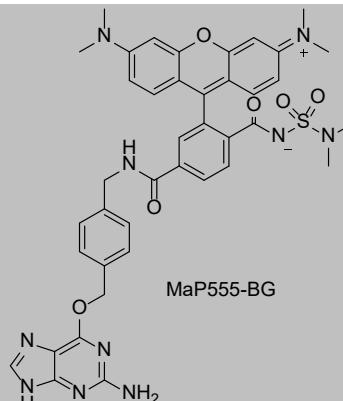
Supporting Figure S9: Four species images combining fluorescence lifetime multiplexing in the green and red spectral channel. U-2 OS cells were labeled with MitoTracker™-Green, Lysotracker™-Green, SPY620-DNA, and SPY620-Actin and imaged in the two spectral channels giving access to a four species image. The composite, the total fluorescence intensity, the four individual separated species as well as the corresponding wavelet-filtered phasor plots used for separation are given. Species separation was achieved using the phasor approach by positioning the cluster-circles on the phasor plot at the position of the pure species. Scale bars, 10 μ m.

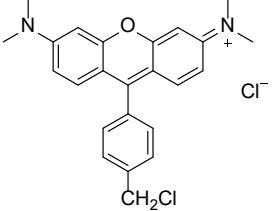
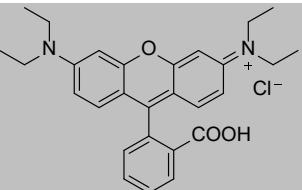
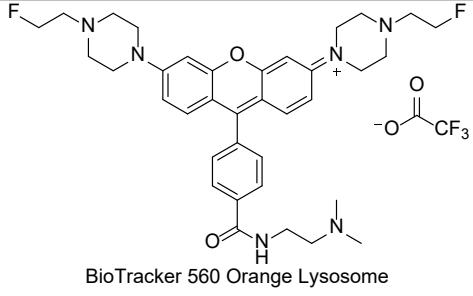
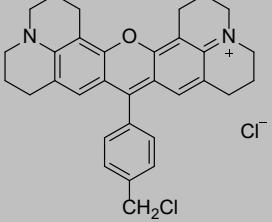
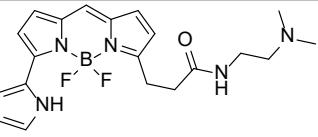
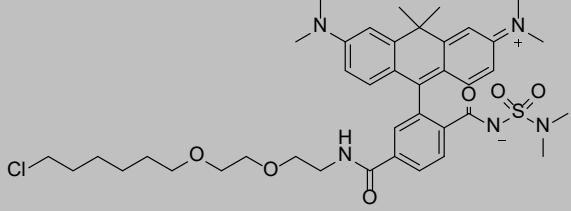


Supporting Figure S10: Six species images combining fluorescence lifetime multiplexing in the green, orange and NIR spectral channel. **A-C** U-2 OS cells stably expressing CalR-HaloTag7-SNAP-tag-KDEL were labeled with MitoTracker™-Green, Lysotracker™-Green, MaP555-CA, SPY555-Actin, SiR700-DNA and SiR700-Tubulin and imaged in the three spectral channels giving access to six species images. The composite, the total fluorescence intensity, the six individual separated species as well as the corresponding wavelet-filtered phasor plots used for separation are given. Species separation was achieved using the phasor approach by positioning the cluster-circles on the phasor plot at the position of the pure species. Scale bars, 10 µm.

Supporting Tables

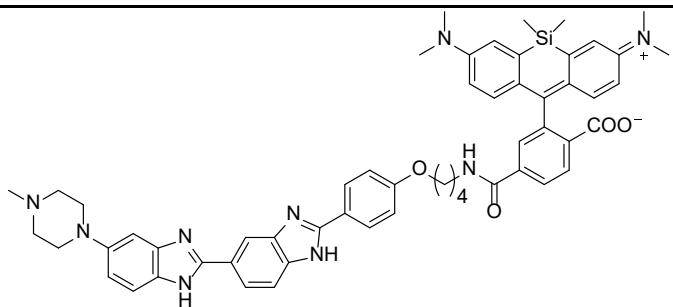
Supporting Table S1: Structures of all 18 synthetic probes tested. In addition, the vendor/reference and their excitation and emission maximum (as specified by the vendor) are given.

Name	Vendor/ Reference	Ex/Em [nm]	Structure
MitoTracker™-Green	ThermoFisher Scientific	490/516	 <p>MitoTracker™-Green</p>
LysoTracker™-Green	ThermoFisher Scientific	504/511	 <p>LysoTracker™-Green</p>
SPY555-Actin	Spirochrome	555/580	Unpublished
SPY555-DNA	Spirochrome	555/580	Unpublished
SPY555-Tubulin	Spirochrome	555/580	Unpublished
MaP555-CA	¹	558/578	 <p>MaP555-CA</p>
MaP555-BG	¹	556/576	 <p>MaP555-BG</p>

MitoTracker™-Orange	ThermoFisher Scientific	554/576	
Rhodamine B	TCI	-	
BioTracker 560 Orange Lysosomes	Merck	532/560	
MitoTracker™-Red	ThermoFisher Scientific	579/599	
LysoTracker™-Red	ThermoFisher Scientific	577/590	
SPY620-Actin	Spirochrome	619/636	Unpublished
SPY620-DNA	Spirochrome	618/636	Unpublished
MaP618-CA	¹	618/635	

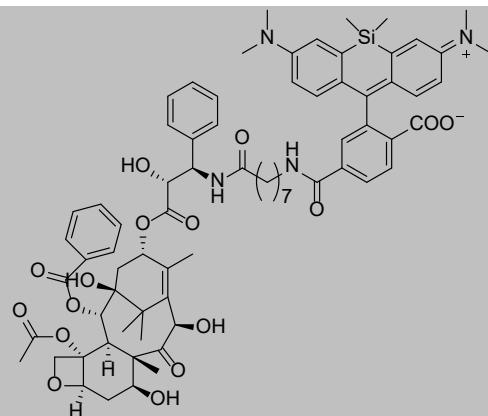
SiR-DNA

Spirochrome² 652/674



SiR-Tubulin

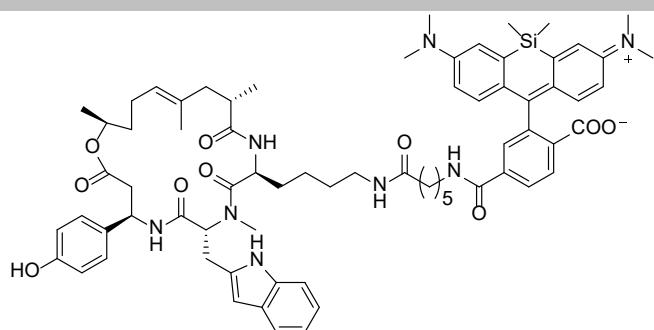
Spirochrome³ 652/674



SiR-DNA

SiR-Actin

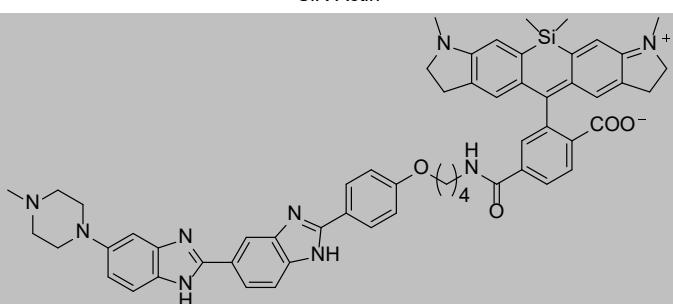
Spirochrome³ 652/674



SiR-Tubulin

SiR700-DNA

Spirochrome⁴ 689/716

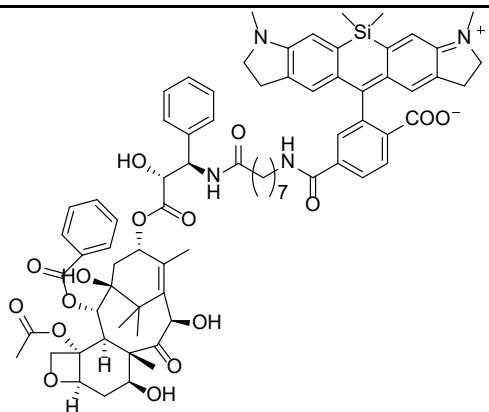


SiR-Actin

SiR700-DNA

SiR700-Tubulin

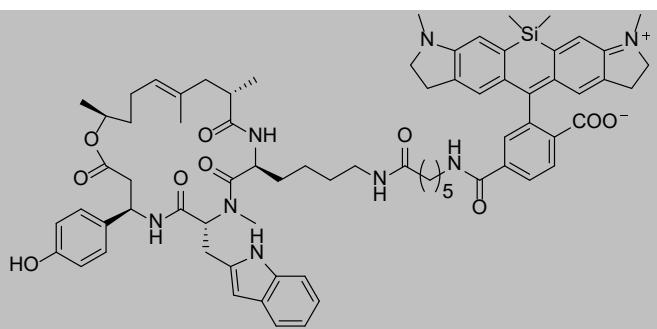
Spirochrome⁴ 689/716



SiR700-Tubulin

SiR700-Actin

Spirochrome⁴ 689/716



SiR700-Actin

Supporting Table S2: Comparison of intensity weighted fluorescence lifetimes (τ) of different synthetic probes in three different cell lines. Fluorescence lifetimes were measured by FLIM (mean \pm s.e.m., $N = 4$ field of views (FOVs) from 2 biological replicates, average $N = 12$ FOVs from 6 biological replicates).² bi-exponential fit, ³ tri-exponential fit, * tail fit (all others n-exponential deconvolution fit).

Fluorophore		U-2 OS		HeLa		HEK 293		Average	
		[ns]		[ns]		[ns]		[ns]	
		τ	s.e.m.	τ	s.e.m.	τ	s.e.m.	τ	s.e.m.
Green	MitoTracker™-Green	0.96 ^{3*}	0.03	0.88 ^{3*}	0.03	0.96 ^{3*}	0.02	0.93	0.02
	LysoTracker™-Green	3.92 ²	0.06	3.78 ²	0.05	4.24 ²	0.01	3.98	0.06
	Rhodamine B	2.26 ³	0.03	2.12 ³	0.01	2.30 ³	0.02	2.23	0.03
Orange	BioTracker Orange Lysosome	2.30 ²	0.07	1.80 ³	0.13	2.04 ²	0.05	2.05	0.08
	SPY555-Actin	1.79 ²	0.09	1.91 ²	0.02	1.88 ²	0.03	1.86	0.03
	SPY555-DNA	3.00 ²	0.02	3.01 ²	0.04	3.01 ²	0.01	3.01	0.01
	SPY555-Tubulin	2.00 ²	0.01	1.99 ²	0.01	2.02 ²	0.01	2.00	0.01
Red	LysoTracker™-Red	3.88 ²	0.07	3.57 ²	0.09	3.63 ²	0.06	3.70	0.06
	SPY620-Actin	2.93 ²	0.01	2.94 ²	0.01	2.88 ²	0.03	2.91	0.01
	SPY620-DNA	3.62 ²	0.01	3.59 ²	0.01	3.61 ²	0.01	3.60	0.01
NIR	SiR700-Actin	1.90 ²	0.01	1.88 ²	0.01	1.93 ²	0.01	1.90	0.01
	SiR700-DNA	2.28 ²	0.01	2.26 ²	0.01	2.30 ²	0.01	2.28	0.01
	SiR700-Tubulin	1.90 ³	0.01	1.90 ³	0.01	2.02 ³	0.01	1.94	0.02

Supporting Table S3: Comparison of intensity weighted fluorescence lifetimes (τ) of MaP555-BG on SNAPf-tag fused to different proteins of interest. H2B: nucleus, labeling histone 2B, LMNB1: nuclear lamina, labeling Lamin B1, Cytosol: untargeted (no fusion), NES: cytosol, fusion with a nuclear export signal, Tomm20: outer mitochondrial membrane, labeling the membrane receptor Tomm20, COX8: inner mitochondrial membrane, labeling the cytochrome c oxidase subunit 8, CalR-KDEL: endoplasmic reticulum, labeling calreticulin and additionally fused to a KDEL targeting peptide, β -4-Gal-T1: Golgi Apparatus, labeling beta-1,4-galactosidase, LAMP1: lysosomes, labeling lysosome-associated membrane glycoprotein 1, SKL: peroxisomes, targeted via a peroxisomal targeting signal, Lyn11: inner leaflet of the plasma membrane, labeling tyrosine protein kinase Lyn11, Ig- κ -PDGF: outer plasma membrane, labeling platelet-derived growth factor receptor and additionally fused to an immunoglobulin kappa light chain leader sequence, CEP41: microtubules, labeling the microtubule-binding protein CEP41, lifeact: filamentous actin (F-actin), labeling the actin-binding peptide lifeact. Fluorescence lifetime was measured by FLIM (mean \pm s.e.m., $N = 4$ FOVs from 2 biological replicates). Unless otherwise stated all mono-exponential fits.² bi-exponential. Data for HaloTag7 from reference⁵.

Fluorophore	SNAP-tag		HaloTag7	
	[ns]		[ns]	
	τ	s.e.m.	τ	s.e.m.
H2B	2.55 ²	0.03	2.41	0.01
LMNB1	2.57 ²	0.04	2.38	0.00
Cytosol	2.49 ²	0.01	2.33	0.01
NES	2.57 ²	0.02	2.41 ²	0.01
Tomm20	2.51 ²	0.02	2.38	0.00
COX8	2.46 ²	0.04	2.40 ²	0.02
CalR-KDEL	2.52 ²	0.03	2.36	0.00
β4Gal-T1	2.58 ²	0.14	2.35	0.03
LAMP1	2.62 ²	0.02	2.38	0.00
SKL	2.40 ²	0.05	2.37	0.01
Lyn11	2.37 ²	0.05	2.37 ²	0.01
Ig-κ-PDGF	2.34 ²	0.03	2.31 ²	0.01
CEP41	2.40 ²	0.06	2.51 ²	0.02
Lifeact	2.48 ²	0.03	2.36 ²	0.01
Average	2.49 ²	0.06	2.38	0.01

Supporting Table S4: Plasmids used and generated as well as the stable cell line derived thereof.

Name	Addgene#	Plasmid	Gene	Localization tag Addgene#	Stable cell lines
pCDNA5/FRT/TO_H2B-SNAPf-tag	181998	pCDNA5/FRT/TO	H2B-SNAPf-tag	135444 ⁶	-
pCDNA5/FRT/TO_SNAPf-tag-LMNB1	181999	pCDNA5/FRT/TO	SNAPf-tag-LMNB1	55069	-
pCDNA5/FRT/TO_SNAPf-tag	182000	pCDNA5/FRT/TO	SNAPf-tag	167271 ⁷	-
pCDNA5/FRT/TO_NES-SNAPf-tag	182001	pCDNA5/FRT/TO	NES-SNAPf-tag	101061 ⁸	-
pCDNA5/FRT/TO_TOMM20-SNAPf-tag	182002	pCDNA5/FRT/TO	TOMM20-SNAPf-tag	135443 ⁶	-
pCDNA5/FRT/TO_COX8-SNAPf-tag	182003	pCDNA5/FRT/TO	COX8-SNAPf-tag	113916 ⁹	-
pCDNA5/FRT/TO_CalR-SNAPf-tag -KDEL	182004	pCDNA5/FRT/TO	CalR-SNAPf-tag-KDEL	synthetic	-
pCDNA5/FRT/TO_β4Gal-T1-SNAPf-tag	182005	pCDNA5/FRT/TO	β4Gal-T1-SNAPf-tag	36205 ¹⁰	-
pCDNA5/FRT/TO_LAMP1-SNAPf-tag	182006	pCDNA5/FRT/TO	LAMP1-SNAPf-tag	34831 ¹¹	-
pCDNA5/FRT/TO_SNAPf-tag-SKL	182007	pCDNA5/FRT/TO	SNAPf-tag-SKL	synthetic	-
pCDNA5/FRT/TO_Lyn11-SNAPf-tag	182008	pCDNA5/FRT/TO	Lyn11-SNAPf-tag	synthetic	-
pCDNA5/FRT/TO_Ig-κ-SNAPf-tag-PDGFR	182009	pCDNA5/FRT/TO	Ig-κ-SNAPf-tag-PDGFR	¹²	-
pCDNA5/FRT/TO_CEP41-SNAPf	182010	pCDNA5/FRT/TO	CEP41-SNAPf	135446 ⁶	-
pCDNA5/FRT/TO_Lifeact-SNAPf-tag	182011	pCDNA5/FRT/TO	Lifeact-SNAPf-tag	synthetic	-
pCDNA5/FRT_CalR-HaloTag7-P30-SNAP-tag-KDEL	182012	pCDNA5/FRT	CalR-SNAPf-tag-KDEL	synthetic	U-2 OS Flp-In TREx

Supporting Table S5: Fluorescence microscopy data acquisition parameters. *See methods for details.

Image	Probe	Microscope	Excitation [nm]	Pixel dwell time [μs]	Pinhole [Airv Units]	Objective	Pixel size [nm]	Size [pixels]	Emission [nm]	Comment
Fig. 1C	MitoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
Fig. 1C	LysoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
Fig. 1C	RhodB	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Fig. 1C	BioTracker Orange Lysosome	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Fig. 1C	SPY555-Actin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Fig. 1C	SPY555-DNA	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Fig. 1C	SPY555-Tubulin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Fig. 1C	SPY620-Actin	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
Fig. 1C	SPY620-DNA	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
Fig. 1C	SiR700-Actin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Fig. 1C	SiR700-DNA	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Fig. 1C	SiR700-Tubulin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Fig2A	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	2.30	1	40x1.10 water	90	704x704	510-540	80 MHz 6 line accumu.
Fig2B	RhodB SPY555-DNA	SP8-FALCON	550	2.70	1	40x1.10 water	102	600x600	570-600	80 MHz 10 line accumu.
Fig2C	SPY620-DNA	SP8-FALCON	615	4.06	1	40x1.10 water	112	400x400	635-700	80 MHz

	SPY620-Actin									10 line accumu.
Fig2D	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	8.49	1	40x1.10 water	122	464x464	710-760	80 MHz 10 line accumu.
Fig3B	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	2.00	1	40x1.10 water	90	1160x1160	510-540	80 MHz 10 line accumu.
Fig3B	CalR-Halo-KDEL MaP555-CA SPY555-Actin	SP8-FALCON	550	2.00	1	40x1.10 water	90	1160x1160	570-600	80 MHz 10 line accumu.
Fig3B	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	2.00	1	40x1.10 water	90	1160x1160	710-760	80 MHz 10 line accumu.
S1A	MitoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
S1A	LysoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
S1B	MitoTracker™-Red	SP8-FALCON	575	3.16	1	40x1.10 water	142	512x512	595-625	80 MHz 500 photons
S1B	LysoTracker™-Red	SP8-FALCON	575	3.16	1	40x1.10 water	142	512x512	595-625	80 MHz 500 photons
S1B	SPY620-Actin	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
S1B	SPY620-DNA	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
S1C	RhodB	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1C	BioTracker Orange Lysosome	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1C	SPY555-Actin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1C	SPY555-DNA	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1C	SPY555-Tubulin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1C	MitoTracker™-Orange	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
S1D	SiR-DNA	SP8-FALCON	631	3.16	1	40x1.10 water	569	512x512	650-700	80 MHz 1,000 4 line accumu.

S1D	SiR-Actin	SP8-FALCON	631	3.16	1	40x1.10 water	569	512x512	650-700	80 MHz 1,000 4 line accumu.
S1D	SiR-Tubulin	SP8-FALCON	631	3.16	1	40x1.10 water	569	512x512	650-700	80 MHz 1,000 4 line accumu.
S1E	SiR700-Actin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
S1E	SiR700-DNA	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
S1E	SiR700-Tubulin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
S2A	MitoTracker™-Green or LysoTracker™-Green	SP8	*	3.16	1	40x1.10 water	142	512x512	*	Spectra
S2B	MaP618-CA or SPY620-DNA or SPY620-Actin	SP8	*	3.16	1	40x1.10 water	142	512x512	*	Spectra
S2B	LysoTracker™-Red	SP8	*	3.16	1	40x1.10 water	142	512x512	*	Spectra
S2C-D	MaP555-CA or MaP555-BG or SPY555-DNA or SPY555-Actin or SPY555-Tubulin or Rhodamine B or Bio Tracker Orange Lysosome	SP8	*	3.16	1	40x1.10 water	142	512x512	*	Spectra
S2E	SiR700-DNA or SiR700-Actin or SiR700-Tubulin	SP8	*	3.16	1	40x1.10 water	142	512x512	*	Spectra
S3A	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	2.30	1	40x1.10 water	90	704x704	510-540	80 MHz 6 line accumu.
S3B	SPY620-DNA SPY620-Actin	SP8-FALCON	615	4.06	1	40x1.10 water	112	400x400	635-700	80 MHz 10 line accumu.
S3C	SiR700-DNA SiR700-Actin	SP8-FALCON	670	6.65	1	40x1.10 water	123	592x592	710-760	80 MHz 10 line accumu.
S3D	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	8.49	1	40x1.10 water	122	464x464	710-760	80 MHz 10 line accumu.

S4A	Bio Tracker Orange Lysosome SPY555-DNA	SP8-FALCON	550	2.85	1	40x1.10 water	101	568x568	570-600	80 MHz 10 line accumu.
S4B	SPY555-Actin SPY555-DNA	SP8-FALCON	550	2.50	1	40x1.10 water	101	648x648	570-600	80 MHz 10 line accumu.
S4C	RhodB SPY555-DNA	SP8-FALCON	550	2.70	1	40x1.10 water	102	600x600	570-600	80 MHz 10 line accumu.
S4D	SPY555-Tubulin SPY555-DNA	SP8-FALCON	550	1.56	1	40x1.10 water	101	1040x1040	570-600	80 MHz 10 line accumu.
S5A	Bio Tracker Orange Lysosome SPY555-DNA	SP8-FALCON	550	3.69	1	40x1.10 water	101	440x440	570-600	80 MHz 10 line accumu.
S5B	SPY555-Tubulin SPY555-DNA	SP8-FALCON	550	9.85	1	40x1.10 water	100	400x400	570-600	80 MHz 10 line accumu.
S5C	Bio Tracker Orange Lysosome SPY555-DNA	SP8-FALCON	550	4.06	1	40x1.10 water	101	400x400	570-600	80 MHz 10 line accumu.
S5D	SPY555-Tubulin SPY555-DNA	SP8-FALCON	550	7.24	1	40x1.10 water	100	544x544	570-600	80 MHz 10 line accumu.
S6A	SPY555-DNA CalR-Halo-KDEL MaP555-CA	SP8-FALCON	550	2.25	1	40x1.10 water	101	728x728	570-600	80 MHz 10 line accumu.
S6B	SPY555-Actin CalR-Halo-KDEL MaP555-CA	SP8-FALCON	550	1.70	1	40x1.10 water	101	952x952	570-600	80 MHz 10 line accumu.
S6C	SPY555-DNA CalR-SNAP-KDEL MaP555-BG	SP8-FALCON	550	2.25	1	40x1.10 water	101	720x720	570-600	80 MHz 10 line accumu.
S6D	SPY555-Actin CalR-SNAP-KDEL MaP555-BG	SP8-FALCON	550	1.93	1	40x1.10 water	101	840x840	570-600	80 MHz 10 line accumu.
S7	SPY620-DNA CalR-Halo-KDEL MaP618-CA	SP8-FALCON	615	2.50	1	40x1.10 water	114	648x648	635-700	80 MHz 10 line accumu.
S8A	MitoTracker™-Green LyoTracker™-Green	SP8-FALCON	489	1.96	1	40x1.10 water	90	824x824	510-540	80 MHz 10 line accumu.
S8A	SPY555-DNA SPY555-Tubulin	SP8-FALCON	550	1.96		40x1.10 water	90	824x824	570-600	80 MHz 10 line accumu.

S8B	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	1.83	1	40x1.10 water	90	888x888	510-540	80 MHz 10 line accumu.
S8B	SPY555-DNA SPY555-Actin	SP8-FALCON	550	1.83	1	40x1.10 water	90	888x888	570-600	80 MHz 10 line accumu.
S9	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	1.89	1	40x1.10 water	90	856x856	510-540	80 MHz 12 line accumu.
S9	SPY620-DNA SPY620-Actin	SP8-FALCON	615	1.89	1	40x1.10 water	90	856x856	635-700	80 MHz 12 line accumu.
S10A	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	2.01	1	40x1.10 water	90	776x776	510-540	80 MHz 10 line accumu.
S10A	CalR-Halo-KDEL MaP555-CA SPY555-Actin	SP8-FALCON	550	2.01	1	40x1.10 water	90	776x776	570-600	80 MHz 10 line accumu.
S10A	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	2.01	1	40x1.10 water	90	776x776	710-760	80 MHz 10 line accumu.
S10B	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	1.46	1	40x1.10 water	90	712x712	510-540	80 MHz 10 line accumu.
S10B	CalR-Halo-KDEL MaP555-CA SPY555-Actin	SP8-FALCON	550	1.46	1	40x1.10 water	90	712x712	570-600	80 MHz 10 line accumu.
S10B	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	1.46	1	40x1.10 water	90	712x712	710-760	80 MHz 10 line accumu.
S10C	MitoTracker™-Green LysoTracker™-Green	SP8-FALCON	489	2.00	1	40x1.10 water	90	1160x1160	510-540	80 MHz 10 line accumu.
S10C	CalR-Halo-KDEL MaP555-CA SPY555-Actin	SP8-FALCON	550	2.00	1	40x1.10 water	90	1160x1160	570-600	80 MHz 10 line accumu.
S10C	SiR700-DNA SiR700-Tubulin	SP8-FALCON	670	2.00	1	40x1.10 water	90	1160x1160	710-760	80 MHz 10 line accumu.
Tab S2	MitoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
Tab S2	LysoTracker™-Green	SP8-FALCON	489	3.16	1	40x1.10 water	142	512x512	510-540	80 MHz 500 photons
Tab S2	RhodB	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S2	BioTracker Orange Lysosome	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S2	SPY555-Actin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz

										500 photons
Tab S2	SPY555-DNA	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S2	SPY555-Tubulin	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S2	LysoTracker™-Red	SP8-FALCON	575	3.16	1	40x1.10 water	142	512x512	595-625	80 MHz 500 photons
Tab S2	SPY620-Actin	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
Tab S2	SPY620-DNA	SP8-FALCON	615	3.16	1	40x1.10 water	142	512x512	635-700	80 MHz 500 photons
Tab S2	SiR700-Actin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Tab S2	SiR700-DNA	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Tab S2	SiR700-Tubulin	SP8-FALCON	670	3.16	1	40x1.10 water	142	512x512	710-760	80 MHz 500 photons
Tab S3	H2B-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	SNAP-LMNB1 MaP555-BG	SP8-FALCON	550	5.64	1	40x1.10 water	101	288x288	570-600	80 MHz 500 photons
Tab S3	SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	NES-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	Tomm20-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	COX8-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	CalR-SNAP-KDEL MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	β4Gal-T1-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	LAMP1-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	SNAP-SKL MaP555-BG	SP8-FALCON	550	7.69	1	40x1.10 water	142	512x512	570-600	80 MHz 500 photons
Tab S3	Lyn11-SNAP	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600	80 MHz

	MaP555-BG								500 photons
Tab S3	Ig-κ-SNAP-PDGFR MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600 80 MHz 500 photons
Tab S3	CEP41-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600 80 MHz 500 photons
Tab S3	Lifeact-SNAP MaP555-BG	SP8-FALCON	550	3.16	1	40x1.10 water	142	512x512	570-600 80 MHz 500 photons

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