ESI: The effects of droplet stabilization by surfactants and nanoparticles on leakage, cross-talk, droplet stability, and cell adhesion

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Supplementary material

Protocols for SU8 master production

For 10 µm height (flow focusing designs 1,2 and 3: 10 µm versions) SU8 2015 was spincoated for 10 s at 500 rpm (100 rpm/s), followed by 37 s at 4000 rpm (500 rpm/s). Next a soft baking step of 3 min at 95 °C was performed and the resulting layer was exposed trough the photoplot for 140 mJ/cm^2. Next the wafer was baked for 4 min at 95 °C and developed with PGMEA for 3 min. For 15 µm height (step emulsification 1 design). SU8 2015 was spincoated for 10 s at 500 rpm (100 rpm/s), followed by 35 s at 3000 rpm (500 rpm/s). All other processing steps were similar to the 10 µm height SU8 layer. For 20 µm chips

(flow focusing designs 1,2 and 3: 20 µm versions and T-junction this process was slightly adapted. Spincoating: 10 s at 500 rpm (100 rpm/s) and 33 s 2000 rpm (500 rpm/s), soft bake: 3.5 min at 95 °C, exposure 150 mJ/cm^2, post exposure bake: 4.5 min at 95 °C, 3.5 min development. For 30 µm chips (flow focusing designs 1: 30 µm version) Spincoating: 10 s at 500 rpm (100 rpm/s) and 33 s 1250 rpm (250 rpm/s), soft bake: 4.5 min at 95 °C, exposure 160 mJ/cm^2, post exposure bake: 5.5 min at 95 °C, 4.5 min development. For an 70 µm layer (cell encapsulation experiments, SU8 2075) Spincoating: 10 s at 500 rpm (100 rpm/s) and 30 s 3500 rpm (500 rpm/s), soft bake: 3 min at 65 °C and 9 min at 95 °C, exposure 200 mJ/cm^2, post exposure bake: 2 min at 65 °C and 7 min at 95 °C, 7 min development. For an 100 µm layer (second layer step emulsification designs, SU8 2075) Spincoating: 10 s at 500 rpm (100 rpm/s) and 33 s 2000 rpm (500 rpm/s), soft bake: 5 min at 65 °C and 15 min at 95 °C, exposure 230 mJ/cm^2, post exposure bake: 5 min at 65 °C and 9 min at 95 °C, 9 min development. For a 2 µm layer (step-emulsification design 2 and combination of step-emulsification and flow focusing designs, first layer, SU8 2002) Spincoating: 10 s at 500 rpm (100 rpm/s) and 40 s 3000 rpm (250 rpm/s), soft bake: 1 min at 95 °C, exposure 80 mJ/cm^2, post exposure bake: 1.5 min at 95 °C, 1 min development. All masters were finally baked for 10 min at 120 °C.

Table S1: p-values of one-sided t-test comparing fluorescence intensity values for different dyes in the partitioned in the oil phase containing different surfactants.

Dye	RAN	Krytox	FNP
Rhod6G	1,465E-03	1,803E-12	5,544E-09
NR	1,128E-01	6,359E-01	1,000E+00
Rhod101	5,708E-01	2,479E-04	4,100E-07
RhodB	9,703E-01	3,243E-04	5,484E-07
EMA	9,872E-01	1,463E-06	6,554E-02
Cy7	2,738E-01	9,543E-04	3,683E-01
Res	9,610E-02	3,237E-03	1,972E-07
AZ	9,230E-01	6,506E-03	4,641E-01
Sulfrhod	9,288E-01	9,438E-06	4,562E-03
Fl	9,716E-01	2,782E-03	9,723E-01
Cy5	3,369E-01	7,624E-10	4,331E-01
DAPI	3,902E-01	7,419E-06	1,053E-05
AF350	3,055E-01	7,493E-06	2,651E-06
Pyr	7,723E-01	8,943E-04	9,970E-01
AF488	9,673E-01	1,904E-02	9,973E-01
AF568	2,434E-01	4,703E-05	9,437E-01

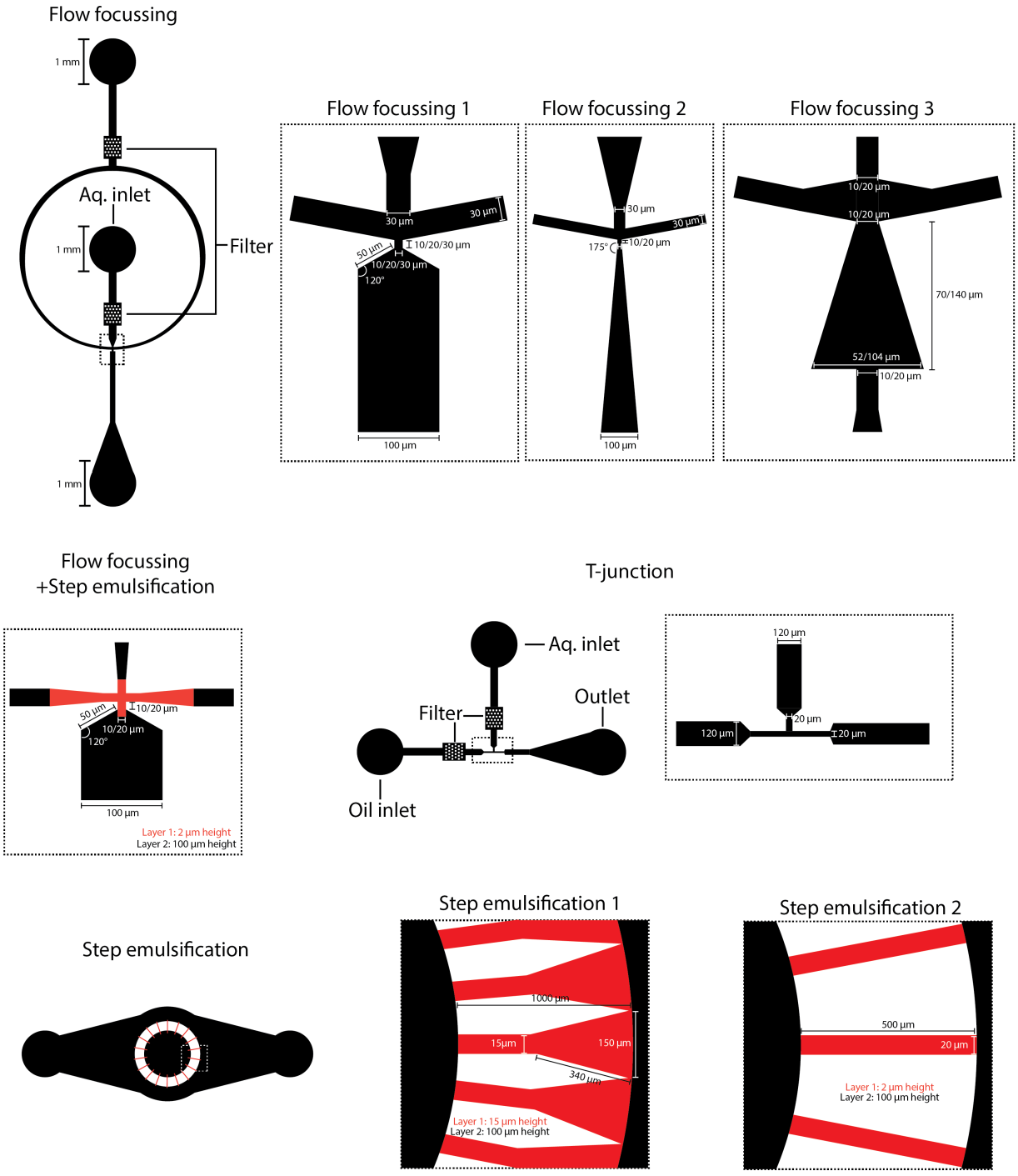

Figure S1: Different chip designs with key dimensions utilised in this paper.

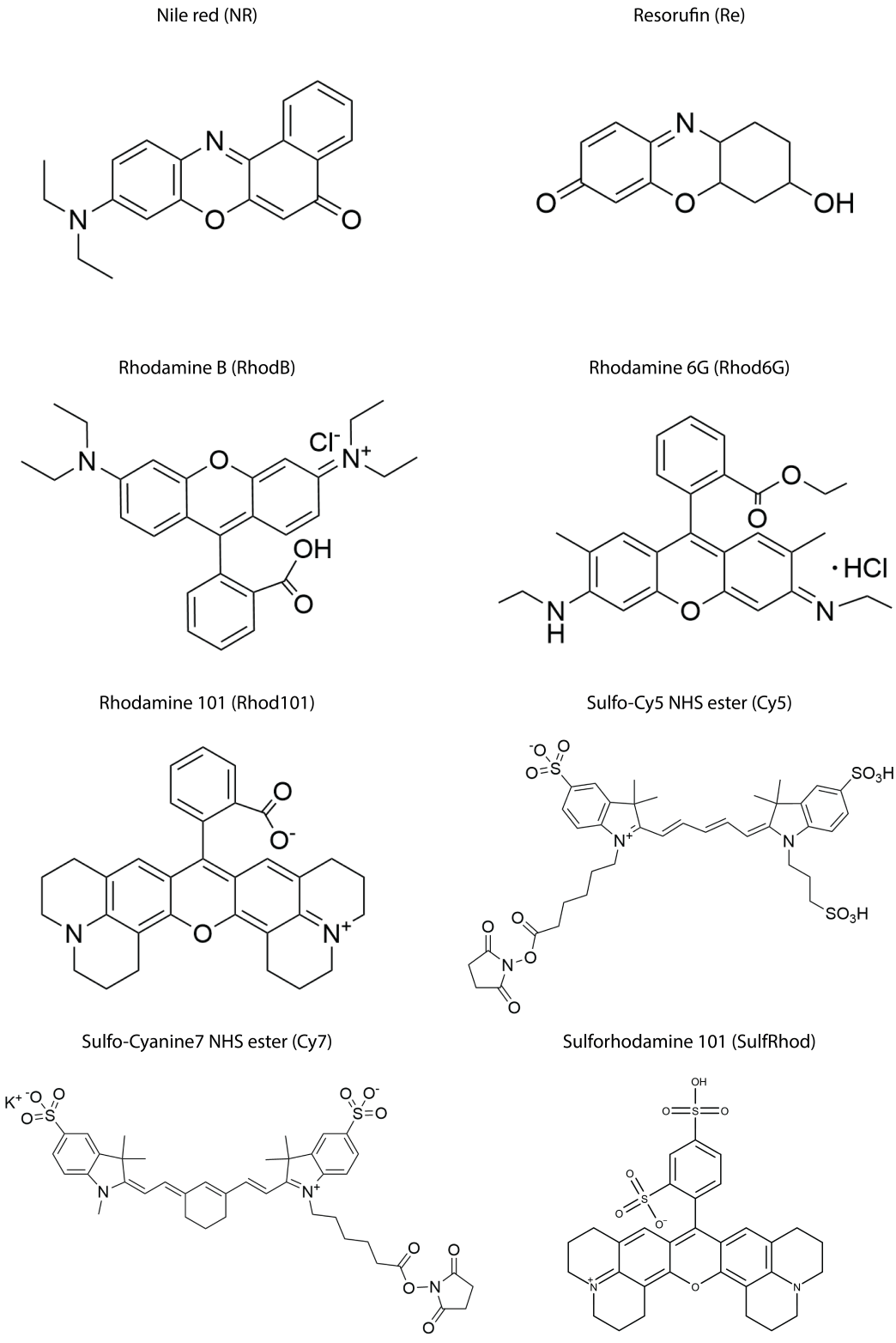

Figure S2: Chemical structure of the different dyes utilised for leakage experiments.

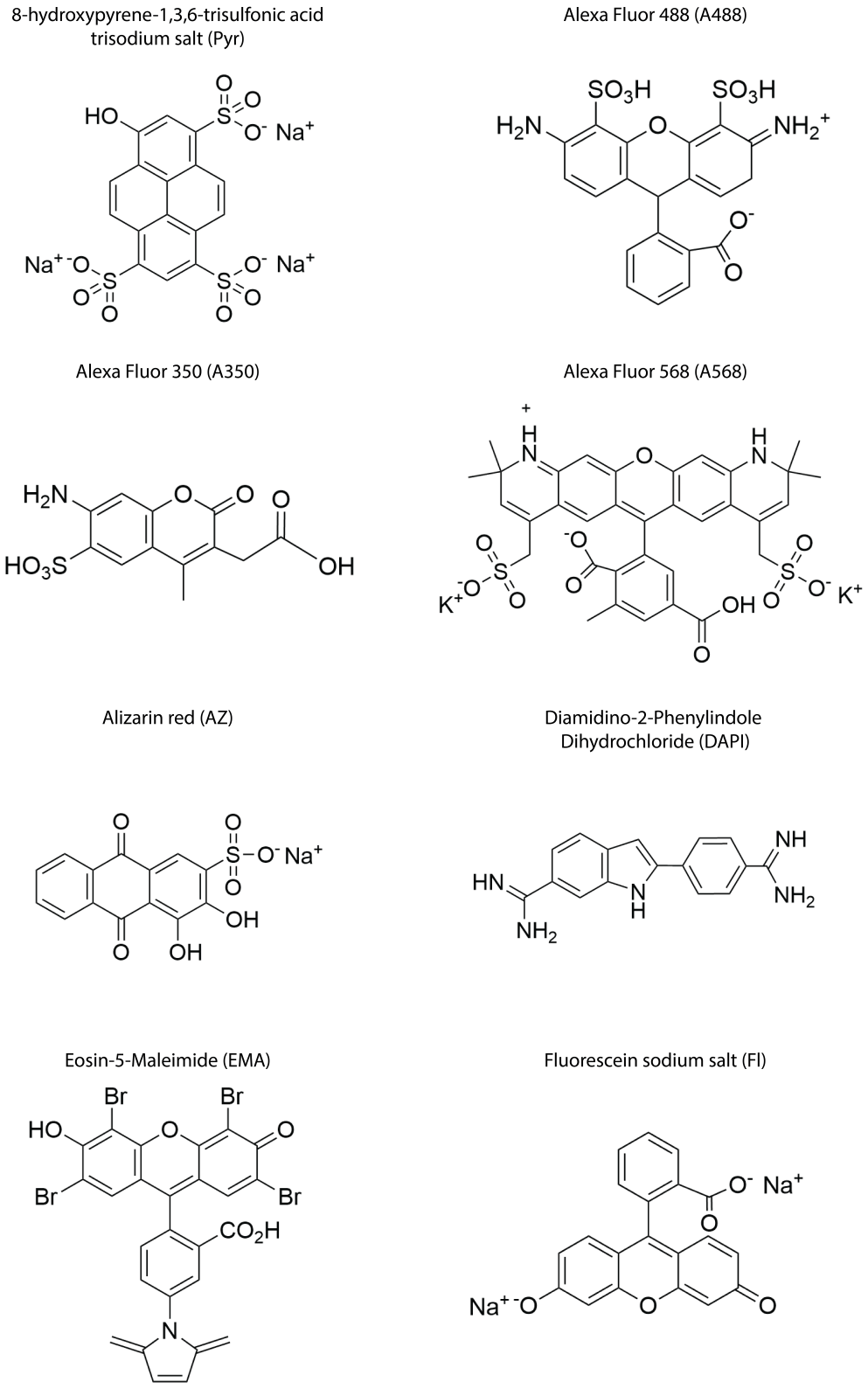

Figure S3: Chemical structure of the different dyes utilised for leakage experiments.

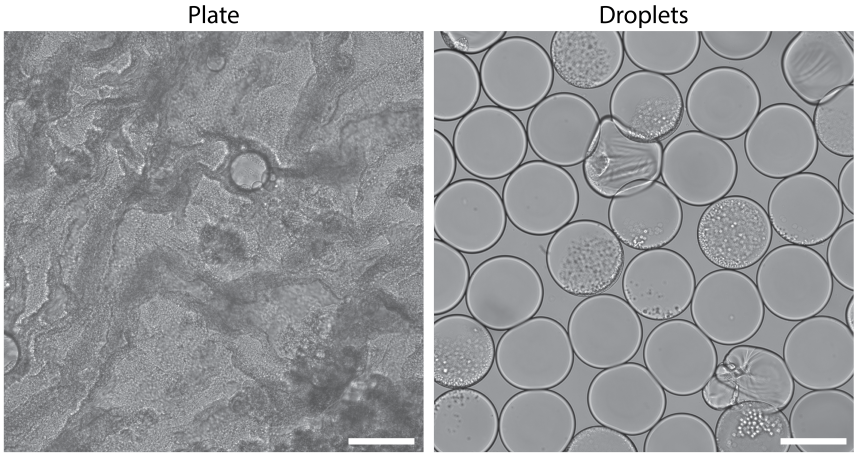

Figure S4: Cell adhesion of (MCF-7 cells) in a microwellplate plate containing a HFE 7500 layer with Krytox and in Krytox stabilised droplets. Krytox surfactant reacts with cell medium (scale bar 100 µm).

Table S2: Data calculated for the determination of the leakage categories for samples made with PBS.

Dye	Stabiliser	Day	mu1	mu2	sigma1	sigma2	p1	p2	R^2_bi	%dark	%overlap
Rhod6G	RAN	day0	15.2	45.8	5.8	24.2	0.990	0.010	0.949	95.9	20.6
Rhod6G	RAN	day1	2.0	28.5	0.7	31.0	0.973	0.027	0.992		2.8
Rhod6G	Krytox	day0	47.0	85.3	20.1	7.6	0.591	0.409	0.496	100.0	14.2
Rhod6G	Krytox	day1	26.1	61.9	13.5	8.5	0.388	0.612	0.785		10.1
Rhod6G	NP	day0	28.2	63.6	13.0	17.7	0.805	0.195	0.778	100.0	24.5
Rhod6G	NP	day1	31.1	100.0	9.6	0.3	0.999	0.001	0.889		0.0
NR	RAN	day0	13.4	37.8	6.2	18.3	0.712	0.288	0.936	83.7	24.6
NR	RAN	day1	16.5	35.1	5.0	18.0	0.966	0.034	0.974		29.6
NR	Krytox	day0	72.1	86.3	14.1	4.6	0.418	0.582	0.809	100.0	34.0
NR	Krytox	day1	44.4	86.5	22.3	6.0	0.687	0.313	0.463		10.4
NR	NP	day0	62.6	84.3	17.6	6.8	0.470	0.530	0.688	100.0	30.7
NR	NP	day1	50.9	83.9	19.1	7.9	0.621	0.379	0.589		19.3
Rhod101	RAN	day0	9.4	76.8	5.4	11.3	0.837	0.163	0.913	0.0	0.0
Rhod101	RAN	day1	23.5	64.6	9.3	12.1	0.755	0.245	0.802		5.4
Rhod101	Krytox	day0	59.1	85.4	18.1	8.6	0.641	0.359	0.725	100.0	28.6
Rhod101	Krytox	day1	38.8	69.5	14.2	9.9	0.423	0.577	0.847		19.7
Rhod101	NP	day0	32.7	50.2	18.3	19.6	0.150	0.850	0.540	100.0	63.7
Rhod101	NP	day1	30.7	62.2	13.7	12.2	0.609	0.391	0.633		22.3
RhodB	RAN	day0	25.6	60.4	11.2	9.3	0.158	0.842	0.862	0.0	8.9
RhodB	RAN	day1	40.2	66.3	18.5	6.8	0.473	0.527	0.826		25.5
RhodB	Krytox	day0	47.7	49.0	3.1	22.3	0.186	0.814	0.794	99.8	26.0
RhodB	Krytox	day1	22.0	72.3	15.0	11.5	0.412	0.588	0.483		5.7
RhodB	NP	day0	13.0	54.5	5.1	15.0	0.193	0.807	0.671	100.0	3.3
RhodB	NP	day1	31.8	51.3	13.4	19.0	0.818	0.182	0.813		52.8
EMA	RAN	day0	1.5	54.3	0.5	32.2	0.640	0.360	0.989	1.0	0.8
EMA	RAN	day1	0.7	59.5	0.4	10.3	0.698	0.302	0.964		0.0
EMA	Krytox	day0	1.5	67.1	0.7	14.6	0.576	0.424	0.986	0.3	0.0
EMA	Krytox	day1	1.4	61.6	0.6	10.3	0.595	0.405	0.993		0.0
EMA	NP	day0	1.9	78.5	0.7	12.6	0.686	0.314	0.967	6.6	0.0
EMA	NP	day1	2.4	60.3	0.8	12.0	0.635	0.365	0.984		0.0
Cy7	RAN	day0	8.7	54.9	3.1	20.2	0.469	0.531	0.874	0.0	3.0
Cy7	RAN	day1	8.2	64.6	2.9	21.8	0.765	0.235	0.861		1.4
Cy7	Krytox	day0	6.0	65.8	2.8	20.2	0.266	0.734	0.540	5.7	0.6
Cy7	Krytox	day1	9.5	64.5	2.5	18.7	0.410	0.590	0.860		0.6
Cy7	NP	day0	7.1	55.0	3.0	21.0	0.736	0.264	0.873	0.0	2.8
Cy7	NP	day1	7.0	47.6	2.5	20.8	0.535	0.465	0.840		4.3
Res	RAN	day0	1.4	48.7	0.5	15.5	0.659	0.341	0.995	0.0	0.1
Res	RAN	day1	4.2	22.3	3.0	15.1	0.548	0.452	0.744		15.4
Res	Krytox	day0	9.2	40.9	3.7	11.2	0.411	0.589	0.839	0.0	2.9
Res	Krytox	day1	8.5	23.3	3.3	12.4	0.904	0.096	0.992		23.7
Res	NP	day0	1.5	52.0	0.6	9.4	0.296	0.704	0.889	0.3	0.0
Res	NP	day1	6.1	88.5	5.5	11.8	0.635	0.365	0.423		0.0
AZ	RAN	day0	4.5	58.6	1.7	14.6	0.398	0.602	0.868	5.0	0.1
AZ	RAN	day1	7.9	52.9	2.5	26.9	0.721	0.279	0.934		5.4
AZ	Krytox	day0	4.2	66.0	1.6	9.2	0.469	0.531	0.969	0.0	0.0
AZ	Krytox	day1	12.6	66.9	4.6	8.7	0.466	0.534	0.880		0.0
AZ	NP	day0	2.8	32.4	2.0	26.0	0.778	0.222	0.672	1.4	6.4
AZ	NP	day1	5.7	31.3	4.9	21.8	0.893	0.107	0.530		17.2
SulfRhod	RAN	day0	1.5	59.9	0.6	11.8	0.518	0.482	0.981	0.0	0.0
SulfRhod	RAN	day1	0.6	47.0	0.4	10.1	0.726	0.274	0.995		0.0
SulfRhod	Krytox	day0	1.6	73.3	0.8	14.0	0.848	0.152	0.990	0.4	0.0
SulfRhod	Krytox	day1	12.7	69.2	5.8	15.3	0.911	0.089	0.926		0.7
SulfRhod	NP	day0	1.3	79.8	0.6	12.3	0.626	0.374	0.972	2.5	0.0
SulfRhod	NP	day1	1.3	74.9	0.6	11.5	0.702	0.298	0.981		0.0
Fl	RAN	day0	1.1	62.3	0.8	16.1	0.559	0.441	0.890	0.5	0.0
Fl	RAN	day1	1.1	65.0	0.7	20.6	0.594	0.406	0.981		0.1
Fl	Krytox	day0	1.6	48.3	0.6	8.8	0.436	0.564	0.994	0.0	0.0
Fl	Krytox	day1	33.4	48.7	17.1	16.0	0.110	0.890	0.856		64.1
Fl	NP	day0	1.2	51.4	0.7	9.3	0.491	0.509	0.988	2.6	0.0
Fl	NP	day1	0.9	75.4	0.6	12.0	0.800	0.200	0.985		0.0
Cy5	RAN	day0	0.5	73.9	0.4	16.7	0.755	0.245	0.997	0.0	0.0
Cy5	RAN	day1	1.0	51.9	0.5	10.6	0.522	0.478	0.932		0.0
Cy5	Krytox	day0	1.4	68.8	0.4	8.9	0.819	0.181	0.991	0.1	0.0
Cy5	Krytox	day1	0.4	55.2	0.3	8.3	0.781	0.219	0.984		0.0
Cy5	NP	day0	0.9	77.8	0.5	20.9	0.774	0.226	0.990	11.2	0.0
Cy5	NP	day1	1.0	64.0	0.5	22.5	0.698	0.302	0.985		0.1
DAPI	RAN	day0	13.4	59.7	5.3	11.6	0.242	0.758	0.788	0.3	0.6
DAPI	RAN	day1	55.1	68.8	8.0	18.3	0.575	0.425	0.834		49.3
DAPI	Krytox	day0	55.4	79.2	19.7	10.7	0.355	0.645	0.722	100.0	39.2
DAPI	Krytox	day1	51.8	68.8	17.3	13.0	0.381	0.619	0.855		56.0
DAPI	NP	day0	49.8	71.0	19.6	12.3	0.236	0.764	0.664	100.0	47.7
DAPI	NP	day1	43.4	81.2	17.2	8.9	0.851	0.149	0.635		13.8
AF350	RAN	day0	6.8	70.9	3.7	15.6	0.318	0.682	0.740	10.0	0.1
AF350	RAN	day1	3.8	49.6	0.9	18.1	0.720	0.280	0.951		0.6
AF350	Krytox	day0	5.5	66.2	1.8	15.7	0.424	0.576	0.909	3.8	0.0
AF350	Krytox	day1	4.9	54.1	0.9	15.5	0.673	0.327	0.960		0.1
AF350	NP	day0	7.3	41.9	2.5	16.6	0.619	0.381	0.952	0.0	4.3
AF350	NP	day1	5.4	45.2	1.4	18.2	0.693	0.307	0.937		1.9
Pyr	RAN	day0	12.5	38.9	7.9	18.4	0.928	0.072	0.222	0.4	26.3
Pyr	RAN	day1	0.9	25.0	0.5	13.8	0.591	0.409	0.973		0.9
Pyr	Krytox	day0	28.0	40.3	23.7	8.3	0.590	0.410	0.212	0.0	47.8
Pyr	Krytox	day1	20.9	66.0	7.4	10.7	0.448	0.552	0.805		1.2
Pyr	NP	day0	13.9	62.3	11.0	20.2	0.950	0.050	0.129	5.0	11.4
Pyr	NP	day1	10.1	59.9	10.9	24.1	0.954	0.046	0.077		13.3
AF488	RAN	day0	0.5	29.8	0.5	18.0	0.544	0.456	0.966	0.0	0.8
AF488	RAN	day1	0.8	65.1	0.5	17.2	0.682	0.318	0.995		0.0
AF488	Krytox	day0	0.6	56.4	0.5	17.1	0.875	0.125	0.999	2.4	0.0
AF488	Krytox	day1	0.5	52.3	0.4	14.5	0.787	0.213	0.991		0.0
AF488	NP	day0	0.5	59.8	0.5	26.7	0.773	0.227	0.992	0.0	0.2
AF488	NP	day1	0.8	52.7	0.5	16.4	0.830	0.170	0.994		0.0
AF568	RAN	day0	0.7	67.5	0.5	15.9	0.704	0.296	0.997	0.2	0.0
AF568	RAN	day1	1.0	55.9	0.5	16.5	0.541	0.459	0.978		0.0
AF568	Krytox	day0	0.7	67.7	0.4	10.6	0.617	0.383	0.997	1.8	0.0
AF568	Krytox	day1	0.8	64.2	0.4	11.6	0.578	0.422	0.991		0.0
AF568	NP	day0	0.9	52.0	0.4	27.7	0.708	0.292	0.996	1.8	0.4
AF568	NP	day1	0.5	43.5	0.4	25.5	0.806	0.194	0.996		0.3

Table S3: Data calculated for the determination of the leakage categories for samples made with DMEM buffer.

Dye	Stabiliser	Day	mu1	mu2	sigma1	sigma2	p1	p2	R^2_bi	%dark	%overlap p
Rhod6G	RAN	day0	6.6	63.6	2.6	15.7	0.161	0.839	0.751	100.0	0.1
Rhod6G	RAN	day1	45.0	76.0	22.0	12.7	0.507	0.493	0.413		34.3
Rhod6G	Krytox	day0	36.8	85.5	16.8	8.2	0.954	0.046	0.515	94.3	4.7
Rhod6G	Krytox	day1	35.1	74.8	17.4	10.2	0.351	0.649	0.595		14.4
Rhod6G	NP	day0	35.0	63.8	15.6	13.7	0.328	0.672	0.689	100.0	32.5
Rhod6G	NP	day1	60.0	88.8	14.7	5.9	0.859	0.141	0.753		14.1
NR	RAN	day0	0.4	17.1	0.5	19.6	0.832	0.168	0.994	0.6	1.2
NR	RAN	day1	0.5	17.1	0.4	19.8	0.775	0.225	0.996		1.2
NR	Krytox	day0	1.5	34.2	1.1	30.6	0.885	0.115	0.820	53.6	3.1
NR	Krytox	day1	1.2	30.2	0.6	30.7	0.891	0.109	0.996		2.0
NR	NP	day0	3.0	18.4	1.2	17.1	0.959	0.041	0.995	88.5	8.4
NR	NP	day1	1.5	15.5	0.7	15.4	0.886	0.114	0.985		4.5
Rhod101	RAN	day0	1.9	38.4	1.2	27.2	0.925	0.075	0.848	0.0	3.0
Rhod101	RAN	day1	7.0	39.2	3.1	21.8	0.692	0.308	0.951		9.6
Rhod101	Krytox	day0	10.2	45.4	4.9	21.2	0.496	0.504	0.849	100.0	12.0
Rhod101	Krytox	day1	41.0	71.7	15.0	9.4	0.854	0.146	0.746		20.0
Rhod101	NP	day0	43.5	52.8	17.1	17.5	0.624	0.376	0.680	100.0	78.5
Rhod101	NP	day1	39.1	71.1	13.4	12.2	0.692	0.308	0.729		21.0
RhodB	RAN	day0	32.1	43.7	12.9	4.3	0.359	0.641	0.943	0.0	37.5
RhodB	RAN	day1	48.7	78.4	18.9	7.9	0.501	0.499	0.721		23.5
RhodB	Krytox	day0	19.6	64.0	10.1	17.2	0.468	0.532	0.398	100.0	10.0
RhodB	Krytox	day1	54.1	85.3	17.2	6.3	0.876	0.124	0.821		15.7
RhodB	NP	day0	17.9	47.7	7.5	17.2	0.580	0.420	0.775	100.0	20.2
RhodB	NP	day1	44.4	77.5	15.4	9.8	0.663	0.337	0.665		18.3
EMA	RAN	day0	0.6	54.6	0.4	22.1	0.605	0.395	0.997	0.4	0.1
EMA	RAN	day1	1.0	65.0	0.5	19.5	0.725	0.275	0.995		0.0
EMA	Krytox	day0	2.2	52.5	0.8	24.7	0.450	0.550	0.966	0.4	0.9
EMA	Krytox	day1	0.7	48.8	0.5	24.8	0.741	0.259	0.990		0.4
EMA	NP	day0	0.4	47.1	0.4	31.3	0.882	0.118	0.999	8.5	0.3
EMA	NP	day1	2.2	51.6	1.0	15.8	0.198	0.802	0.821		0.1
Cy7	RAN	day0	11.2	51.4	2.4	20.8	0.691	0.309	0.975	1.0	4.6
Cy7	RAN	day1	9.2	48.9	3.3	25.2	0.679	0.321	0.968		8.3
Cy7	Krytox	day0	9.1	56.6	4.3	25.3	0.699	0.301	0.864	1.9	6.6
Cy7	Krytox	day1	9.4	61.0	3.2	23.0	0.568	0.432	0.907		3.0
Cy7	NP	day0	5.2	40.0	2.0	16.0	0.235	0.765	0.871	5.4	2.9
Cy7	NP	day1	5.5	48.8	2.3	19.6	0.361	0.639	0.857		2.6
Res	RAN	day0	0.5	42.5	0.5	18.8	0.817	0.183	0.993	5.4	0.3
Res	RAN	day1	5.7	60.9	4.4	11.5	0.555	0.445	0.660		0.0
Res	Krytox	day0	1.6	60.9	0.9	12.4	0.148	0.852	0.854	0.3	0.0
Res	Krytox	day1	2.6	59.5	2.2	20.8	0.712	0.288	0.680		0.7
Res	NP	day0	1.6	67.4	0.7	8.6	0.401	0.599	0.962	0.5	0.0
Res	NP	day1	10.7	65.3	5.7	11.4	0.743	0.257	0.907		0.1
AZ	RAN	day0	0.3	14.4	0.3	14.3	0.568	0.432	0.982	0.3	0.5
AZ	RAN	day1	1.0	15.2	0.8	13.7	0.677	0.323	0.782		4.5
AZ	Krytox	day0	1.0	23.9	0.5	23.2	0.693	0.307	0.986	7.0	1.7
AZ	Krytox	day1	1.0	18.1	0.7	20.5	0.738	0.262	0.904		3.0
AZ	NP	day0	4.2	31.0	1.8	8.8	0.352	0.648	0.866	3.8	0.9
AZ	NP	day1	24.2	41.3	18.8	6.2	0.417	0.583	0.801		37.1
SulfRhod	RAN	day0	1.8	65.2	0.9	20.4	0.670	0.330	0.927	0.0	0.1
SulfRhod	RAN	day1	1.0	62.1	0.7	29.6	0.747	0.253	0.921		0.5
SulfRhod	Krytox	day0	16.8	50.1	11.0	19.3	0.775	0.225	0.634	81.1	25.1
SulfRhod	Krytox	day1	26.5	75.8	9.5	13.1	0.966	0.034	0.665		2.9
SulfRhod	NP	day0	3.4	58.4	2.5	18.5	0.664	0.336	0.760	7.4	0.5
SulfRhod	NP	day1	6.1	54.1	2.2	15.7	0.748	0.252	0.938		0.5
FITC	RAN	day0	0.5	63.5	0.4	24.5	0.808	0.192	0.996	0.8	0.1
FITC	RAN	day1	1.4	77.0	0.7	10.6	0.590	0.410	0.993		0.0
FITC	Krytox	day0	1.4	67.7	0.8	11.1	0.335	0.665	0.939	6.5	0.0
FITC	Krytox	day1	1.3	71.0	0.5	17.9	0.537	0.463	0.987		0.0
FITC	NP	day0	0.7	76.7	0.6	17.4	0.576	0.424	0.965	5.0	0.0
FITC	NP	day1	1.4	68.8	1.0	24.7	0.690	0.310	0.935		0.2
Cy5	RAN	day0	0.3	27.9	0.4	17.9	0.645	0.355	0.995	1.4	0.4
Cy5	RAN	day1	0.7	57.1	0.4	27.4	0.636	0.364	0.996		0.2
Cy5	Krytox	day0	0.9	60.0	0.5	25.5	0.632	0.368	0.992	0.5	0.2
Cy5	Krytox	day1	0.7	54.6	0.4	28.2	0.811	0.189	0.998		0.3
Cy5	NP	day0	0.4	53.7	0.4	10.2	0.802	0.198	0.998	8.2	0.0
Cy5	NP	day1	0.7	53.4	0.5	11.8	0.663	0.337	0.996		0.0
DAPI	RAN	day0	40.2	74.9	12.1	8.7	0.802	0.198	0.785	4.7	9.4
DAPI	RAN	day1	32.3	61.9	7.7	11.9	0.613	0.387	0.869		12.7
DAPI	Krytox	day0	49.2	70.7	19.6	9.6	0.117	0.883	0.911	19.7	41.4
DAPI	Krytox	day1	18.0	67.3	11.0	12.3	0.056	0.944	0.830		3.4
DAPI	NP	day0	32.2	69.5	13.1	15.1	0.506	0.494	0.582	0.0	18.6
DAPI	NP	day1	40.1	63.6	9.9	19.6	0.541	0.459	0.706		38.9
A350	RAN	day0	6.4	57.6	1.9	17.2	0.898	0.102	0.979	4.1	0.4
A350	RAN	day1	7.2	64.8	2.5	22.3	0.765	0.235	0.924		1.2
A350	Krytox	day0	10.9	66.2	5.5	16.0	0.575	0.425	0.842	0.5	0.9
A350	Krytox	day1	8.6	65.4	2.1	21.8	0.884	0.116	0.973		1.0
A350	NP	day0	7.8	64.5	2.7	16.9	0.637	0.363	0.963	0.4	0.3
A350	NP	day1	7.8	64.1	2.5	17.9	0.447	0.553	0.876		0.4
Pyrene	RAN	day0	0.4	31.9	0.4	19.6	0.694	0.306	0.993	0.9	0.5
Pyrene	RAN	day1	0.3	31.1	0.4	19.2	0.751	0.249	0.997		0.4
Pyrene	Krytox	day0	1.0	61.2	0.6	18.7	0.667	0.333	0.965	2.4	0.0
Pyrene	Krytox	day1	1.0	49.5	0.5	12.5	0.625	0.375	0.987		0.0
Pyrene	NP	day0	2.1	58.7	1.2	9.7	0.409	0.591	0.925	0.3	0.0
Pyrene	NP	day1	18.5	57.3	26.4	7.7	0.288	0.712	0.520		20.0
A488	RAN	day0	0.7	44.6	0.5	31.5	0.642	0.358	0.971	5.8	0.7
A488	RAN	day1	0.9	54.9	0.4	20.6	0.650	0.350	0.980		0.1
A488	Krytox	day0	0.5	29.3	0.4	28.3	0.910	0.090	0.998	10.6	0.7
A488	Krytox	day1	0.6	36.6	0.4	28.4	0.836	0.164	0.995		0.5
A488	NP	day0	0.6	33.4	0.6	17.5	0.521	0.479	0.881	1.1	0.8
A488	NP	day1	2.0	48.1	1.1	18.8	0.488	0.512	0.921		0.6
A568	Krytox	day0	1.3	75.4	0.5	11.1	0.410	0.590	0.990	1.4	0.0
A568	Krytox	day1	1.0	67.9	0.5	9.5	0.693	0.307	0.996		0.0
A568	RAN	day0	0.5	53.8	0.4	25.3	0.845	0.155	0.988	0.5	0.1
A568	RAN	day1	1.3	59.6	0.5	13.0	0.542	0.458	0.974		0.0
A568	NP	day0	0.7	69.6	0.4	11.5	0.755	0.245	0.995	1.1	0.0
A568	NP	day1	0.7	76.5	0.4	19.6	0.679	0.321	0.990		0.0

Table S4: Data calculated for the determination of the leakage categories for samples made with Tris buffer.

Dye	Stabiliser	Day	mu1	mu2	sigma1	sigma2	p1	p2	R^2_bi	%dark	%overlap
Rhod6G	RAN	day0	46.7	50.4	17.3	7.1	0.553	0.447	0.930	91.4	58.7
Rhod6G	RAN	day1	36.9	67.1	11.4	8.5	0.637	0.363	0.828		12.7
Rhod6G	Krytox	day0	16.0	46.5	8.0	20.1	0.418	0.582	0.725	100.0	23.2
Rhod6G	Krytox	day1	53.9	78.9	19.4	9.0	0.353	0.647	0.809		33.4
Rhod6G	NP	day0	18.5	57.4	11.9	21.7	0.459	0.541	0.209	98.2	22.9
Rhod6G	NP	day1	45.5	78.4	18.5	7.8	0.622	0.378	0.596		18.7
NR	RAN	day0	1.3	15.0	0.9	16.2	0.749	0.251	0.892	44.6	5.3
NR	RAN	day1	1.3	18.2	0.7	20.6	0.813	0.187	0.989		3.4
NR	Krytox	day0	1.8	100.0	0.9	0.3	0.999	0.001	0.968	98.7	0.0
NR	Krytox	day1	1.6	100.0	0.6	0.3	0.999	0.001	0.995		0.0
NR	NP	day0	1.8	45.7	1.1	39.5	0.978	0.022	0.977	97.8	2.4
NR	NP	day1	2.2	20.6	0.9	26.0	0.981	0.019	0.985		4.7
Rhod101	RAN	day0	26.4	42.2	21.1	4.8	0.245	0.755	0.920	0.0	31.0
Rhod101	RAN	day1	4.4	48.3	2.7	28.0	0.752	0.248	0.887		5.2
Rhod101	Krytox	day0	18.3	49.6	7.1	16.8	0.470	0.530	0.780	93.2	16.9
Rhod101	Krytox	day1	27.9	68.5	11.6	15.2	0.113	0.887	0.708		12.9
Rhod101	NP	day0	44.8	87.4	14.8	7.1	0.898	0.102	0.687	100.0	4.9
Rhod101	NP	day1	39.5	51.6	16.4	30.1	0.994	0.006	0.503		65.6
RhodB	RAN	day0	41.5	55.5	16.7	8.3	0.684	0.316	0.868	0.0	50.5
RhodB	RAN	day1	24.4	79.0	7.9	14.6	0.955	0.045	0.846		1.5
RhodB	Krytox	day0	50.1	73.6	17.2	7.7	0.607	0.393	0.792	100.0	30.8
RhodB	Krytox	day1	32.3	60.1	14.4	17.5	0.397	0.603	0.715		38.1
RhodB	NP	day0	19.5	53.3	10.2	20.8	0.345	0.655	0.435	100.0	24.7
RhodB	NP	day1	40.4	74.8	16.2	8.6	0.273	0.727	0.916		15.6
EMA	RAN	day0	2.0	58.4	2.1	7.4	0.405	0.595	0.479	0.7	0.0
EMA	RAN	day1	0.8	58.8	0.5	14.1	0.721	0.279	0.989		0.0
EMA	Krytox	day0	3.3	57.2	2.3	17.3	0.338	0.662	0.814	6.4	0.4
EMA	Krytox	day1	1.9	55.6	0.7	13.0	0.866	0.134	0.984		0.0
EMA	NP	day0	0.6	22.9	0.4	5.4	0.459	0.541	0.949	1.9	0.0
EMA	NP	day1	1.3	63.1	0.6	10.8	0.559	0.441	0.994		0.0
Cy7	RAN	day0	11.7	50.9	2.9	17.4	0.297	0.703	0.764	9.8	3.7
Cy7	RAN	day1	7.0	51.4	2.4	18.5	0.423	0.577	0.906		2.0
Cy7	Krytox	day0	5.5	40.6	2.4	19.1	0.357	0.643	0.769	8.8	5.2
Cy7	Krytox	day1	10.6	48.9	5.8	17.3	0.781	0.219	0.820		8.0
Cy7	NP	day0	5.8	58.1	2.5	22.0	0.326	0.674	0.725	4.8	1.8
Cy7	NP	day1	4.2	48.6	2.0	19.3	0.718	0.282	0.943		1.8
Res	RAN	day0	14.4	21.2	14.0	2.9	0.395	0.605	0.892	0.0	33.3
Res	RAN	day1	2.2	22.6	1.9	17.2	0.475	0.525	0.630		8.4
Res	Krytox	day0	18.9	26.1	15.3	3.0	0.349	0.651	0.904	0.0	31.9
Res	Krytox	day1	10.9	40.0	3.9	23.5	0.972	0.028	0.980		15.6
Res	NP	day0	2.6	61.4	1.4	11.2	0.258	0.742	0.828	0.5	0.0
Res	NP	day1	1.7	15.3	1.1	10.4	0.696	0.304	0.893		7.0
AZ	RAN	day0	0.9	15.1	0.6	8.3	0.431	0.569	0.952	0.0	2.4
AZ	RAN	day1	0.8	46.2	0.5	16.9	0.693	0.307	0.976		0.1
AZ	Krytox	day0	5.5	36.9	2.4	5.8	0.345	0.655	0.877	0.2	0.0
AZ	Krytox	day1	0.9	65.0	0.5	9.5	0.693	0.307	0.985		0.0
AZ	NP	day0	5.5	17.7	3.1	14.2	0.903	0.097	0.632	1.7	23.7
AZ	NP	day1	0.9	74.5	0.5	19.7	0.757	0.243	0.989		0.0
SulfRhod	RAN	day0	1.1	67.4	0.7	12.0	0.384	0.616	0.970	2.4	0.0
SulfRhod	RAN	day1	0.5	46.7	0.4	8.6	0.700	0.300	0.988		0.0
SulfRhod	Krytox	day0	34.8	76.3	15.9	8.4	0.807	0.193	0.651	33.2	8.2
SulfRhod	Krytox	day1	38.5	49.7	15.5	6.8	0.659	0.341	0.908		50.4
SulfRhod	NP	day0	2.8	82.2	1.1	9.3	0.265	0.735	0.937	1.1	0.0
SulfRhod	NP	day1	1.9	73.5	0.8	11.7	0.560	0.440	0.983		0.0
Fl	RAN	day0	0.8	67.4	0.4	15.2	0.511	0.489	0.971	0.3	0.0
Fl	RAN	day1	0.6	58.5	0.4	10.5	0.649	0.351	0.982		0.0
Fl	Krytox	day0	2.1	73.8	0.9	7.4	0.614	0.386	0.986	0.5	0.0
Fl	Krytox	day1	6.7	36.2	3.9	18.9	0.437	0.563	0.841		11.7
Fl	NP	day0	0.9	70.8	0.6	11.1	0.548	0.452	0.815	0.0	0.0
Fl	NP	day1	0.8	82.7	0.5	9.3	0.820	0.180	0.993		0.0
Cy5	RAN	day0	1.3	57.4	0.7	7.2	0.121	0.879	0.957	8.2	0.0
Cy5	RAN	day1	0.8	54.8	0.5	17.5	0.639	0.361	0.989		0.1
Cy5	Krytox	day0	1.8	45.6	0.7	15.9	0.210	0.790	0.789	0.6	0.2
Cy5	Krytox	day1	0.9	42.7	0.4	16.1	0.775	0.225	0.990		0.1
Cy5	NP	day0	1.5	76.7	0.8	10.8	0.574	0.426	0.969	0.2	0.0
Cy5	NP	day1	0.7	71.3	0.5	15.7	0.685	0.315	0.996		0.0
DAPI	RAN	day0	32.5	82.0	6.7	16.1	0.998	0.002	0.829	7.2	2.7
DAPI	RAN	day1	9.2	100.0	3.0	0.3	0.999	0.001	0.681		0.0
DAPI	Krytox	day0	53.3	76.6	10.1	7.1	0.573	0.427	0.865	100.0	17.1
DAPI	Krytox	day1	49.9	75.8	20.5	11.4	0.328	0.672	0.845		38.4
DAPI	NP	day0	27.9	71.3	5.5	10.7	0.658	0.342	0.849	1.3	0.7
DAPI	NP	day1	48.4	53.3	8.3	19.3	0.526	0.474	0.784		59.8
AF350	RAN	day0	12.2	59.1	1.1	18.3	0.513	0.487	0.946	4.5	0.7
AF350	RAN	day1	11.6	45.4	1.8	25.2	0.731	0.269	0.977		6.9
AF350	Krytox	day0	11.6	76.6	2.9	10.5	0.318	0.682	0.853	6.7	0.0
AF350	Krytox	day1	11.1	73.1	2.5	9.7	0.614	0.386	0.895		0.0
AF350	NP	day0	14.4	76.9	4.4	9.8	0.267	0.733	0.837	1.0	0.0
AF350	NP	day1	11.3	77.9	2.4	9.9	0.666	0.334	0.966		0.0
Pyr	RAN	day0	0.5	19.9	0.4	9.7	0.477	0.523	0.958	9.5	0.5
Pyr	RAN	day1	0.3	20.7	0.3	10.5	0.562	0.438	0.976		0.3
Pyr	Krytox	day0	1.1	51.6	0.4	8.8	0.267	0.733	0.963	0.0	0.0
Pyr	Krytox	day1	6.6	72.4	3.2	11.5	0.568	0.432	0.908		0.0
Pyr	NP	day0	1.2	63.2	0.6	8.2	0.691	0.309	0.970	2.2	0.0
Pyr	NP	day1	1.5	67.8	0.6	9.6	0.571	0.429	0.996		0.0
AF488	RAN	day0	0.8	33.1	0.4	11.8	0.365	0.635	0.935	0.6	0.1
AF488	RAN	day1	0.6	44.1	0.4	9.5	0.564	0.436	0.983		0.0
AF488	Krytox	day0	1.8	70.9	0.7	9.6	0.468	0.532	0.990	1.0	0.0
AF488	Krytox	day1	2.0	66.4	0.8	8.5	0.708	0.292	0.997		0.0
AF488	NP	day0	2.3	63.5	1.7	11.9	0.578	0.422	0.642	2.3	0.0
AF488	NP	day1	1.5	48.2	0.9	11.6	0.570	0.430	0.935		0.0
AF568	RAN	day0	1.4	66.4	0.5	15.7	0.375	0.625	0.960	0.0	0.0
AF568	RAN	day1	0.8	46.2	0.5	16.9	0.693	0.307	0.976		0.1
AF568	Krytox	day0	1.5	78.0	0.6	10.5	0.526	0.474	0.992	0.1	0.0
AF568	Krytox	day1	0.9	65.0	0.5	9.5	0.693	0.307	0.985		0.0
AF568	NP	day0	0.6	77.9	0.5	13.8	0.703	0.297	0.998	0.0	0.0
AF568	NP	day1	0.9	74.5	0.5	19.7	0.757	0.243	0.989		0.0

Table S5: Data calculated for the determination of the leakage categories for samples made with MiliQ.

Dye	Stabiliser	Day	mu1	mu2	sigma1	sigma2	p1	p2	R^2.bi	%dark	%overlap
Rhod6G	RAN	day0	18.9	58.3	9.4	15.1	0.287	0.713	0.667	100.0	10.4
Rhod6G	RAN	day1	14.7	58.6	5.0	31.4	0.997	0.003	0.965		12.8
Rhod6G	Krytox	day0	49.3	68.2	17.0	9.4	0.478	0.522	0.914	99.9	44.3
Rhod6G	Krytox	day1	48.1	66.7	16.6	7.2	0.564	0.436	0.821		37.7
Rhod6G	NP	day0	38.4	62.7	14.9	11.3	0.307	0.693	0.839	100.0	34.7
Rhod6G	NP	day1	28.0	35.9	6.6	13.1	0.143	0.857	0.886		58.8
NR	RAN	day0	13.0	32.2	4.7	20.2	0.944	0.056	0.879	93.4	27.0
NR	RAN	day1	3.0	100	1.2	0.3	0.995	0.005	0.931		0.0
NR	Krytox	day0	15.2	21.8	9.1	1.8	0.620	0.380	0.954	99.4	28.4
NR	Krytox	day1	6.7	31.3	3.3	27.6	0.984	0.016	0.919		14.1
NR	NP	day0	3.8	100	1.7	0.3	0.996	0.004	0.872	99.4	0.0
NR	NP	day1	2.9	74.5	1.1	30.4	0.974	0.026	0.960		0.5
Rhod101	RAN	day0	7.4	73.6	3.5	9.8	0.633	0.367	0.935	0.0	0.0
Rhod101	RAN	day1	12.0	36.7	5.4	15.3	0.259	0.741	0.849		18.9
Rhod101	Krytox	day0	47.5	70.1	17.2	4.7	0.489	0.511	0.886	100.0	22.4
Rhod101	Krytox	day1	31.6	41.5	14.0	14.7	0.577	0.423	0.778		72.6
Rhod101	NP	day0	25.3	51.2	10.5	15.3	0.466	0.534	0.547	100.0	30.8
Rhod101	NP	day1	30.6	50.1	16.6	16.0	0.412	0.588	0.658		54.7
RhodB	RAN	day0	17.4	34.9	7.7	12.9	0.457	0.543	0.852	0.0	37.3
RhodB	RAN	day1	28.6	33.2	24.3	4.4	0.131	0.869	0.947		32.0
RhodB	Krytox	day0	29.3	39.6	14.4	6.3	0.560	0.440	0.927	100.0	50.5
RhodB	Krytox	day1	2.9	26.1	2.1	17.0	0.320	0.680	0.743		8.1
RhodB	NP	day0	40.6	54.6	16.2	5.6	0.888	0.112	0.778	100.0	39.5
RhodB	NP	day1	31.8	93.7	13.8	6.6	0.989	0.011	0.779		0.2
EMA	RAN	day0	1.2	53.2	0.5	14.5	0.478	0.522	0.977	0.0	0.0
EMA	RAN	day1	0.6	38.6	0.4	6.7	0.541	0.459	0.982		0.0
EMA	Krytox	day0	1.0	66.5	0.6	8.9	0.538	0.462	0.979	0.0	0.0
EMA	Krytox	day1	4.3	65.4	1.5	12.3	0.760	0.240	0.935		0.0
EMA	NP	day0	1.1	58.3	0.7	22.2	0.718	0.282	0.986	2.0	0.2
EMA	NP	day1	2.5	100	6.6	0.3	0.999	0.001	0.085		0.0
Cy7	RAN	day0	31.7	68.6	20.5	8.0	0.652	0.348	0.296	5.0	17.2
Cy7	RAN	day1	6.1	59.9	1.6	15.3	0.654	0.346	0.956		0.1
Cy7	Krytox	day0	8.0	58.9	2.7	19.5	0.337	0.663	0.660	7.9	1.4
Cy7	Krytox	day1	6.4	46.3	2.6	16.9	0.695	0.305	0.948		2.5
Cy7	NP	day0	31.7	66.4	17.1	10.9	0.546	0.454	0.495	1.5	20.9
Cy7	NP	day1	3.6	63.6	1.8	22.7	0.613	0.387	0.908		0.6
Res	RAN	day0	7.8	28.8	6.2	17.4	0.944	0.056	0.263	0.0	26.2
Res	RAN	day1	14.9	32.1	8.6	15.9	0.805	0.195	0.859		42.1
Res	Krytox	day0	5.7	46.5	5.3	29.5	0.831	0.169	0.199	0.0	11.4
Res	Krytox	day1	2.2	24.8	1.1	20.2	0.825	0.175	0.994		4.8
Res	NP	day0	0.5	27.8	0.4	20.1	0.829	0.171	0.999	0.8	0.7
Res	NP	day1	0.7	24.6	0.5	19.5	0.793	0.207	0.989		1.4
AZ	RAN	day0	5.4	32.1	4.3	20.7	0.856	0.144	0.375	0.0	14.7
AZ	RAN	day1	0.9	11.9	1.0	14.6	0.925	0.075	0.690		5.8
AZ	Krytox	day0	16.6	69.5	11.4	18.5	0.979	0.021	0.321	0.0	7.4
AZ	Krytox	day1	1.0	100	1.6	0.3	0.999	0.001	0.615		0.0
AZ	NP	day0	0.2	6.4	0.3	14.2	0.862	0.138	0.958	0.8	0.7
AZ	NP	day1	0.3	11.1	0.4	20.7	0.888	0.112	0.992		0.9
SulfRhod	RAN	day0	0.6	58.3	0.5	27.8	0.769	0.231	0.994	0.1	0.2
SulfRhod	RAN	day1	0.5	57.6	0.4	24.9	0.805	0.195	0.996		0.2
SulfRhod	Krytox	day0	0.5	41.5	0.4	7.0	0.287	0.713	0.971	3.1	0.0
SulfRhod	Krytox	day1	2.4	59.5	0.9	11.7	0.639	0.361	0.976		0.0
SulfRhod	NP	day0	2.6	61.6	1.0	12.2	0.246	0.754	0.895	2.1	0.0
SulfRhod	NP	day1	1.3	71.2	0.8	10.0	0.462	0.538	0.960		0.0
Fl	RAN	day0	0.3	41.2	0.4	12.5	0.809	0.191	0.992	8.4	0.0
Fl	RAN	day1	0.7	46.3	0.6	13.8	0.701	0.299	0.941		0.0
Fl	Krytox	day0	0.5	55.1	0.4	13.8	0.573	0.427	0.995	2.1	0.0
Fl	Krytox	day1	0.9	22.9	0.6	16.7	0.238	0.762	0.830		2.1
Fl	NP	day0	1.6	72.7	0.7	8.6	0.625	0.375	0.993	1.1	0.0
Fl	NP	day1	0.3	79.7	0.4	9.0	0.957	0.043	0.993		0.0
Cy5	RAN	day0	26.2	32.3	20.2	5.1	0.365	0.635	0.688	0.2	40.9
Cy5	RAN	day1	0.8	44.2	0.5	12.0	0.511	0.489	0.985		0.0
Cy5	Krytox	day0	28.6	32.2	25.8	3.9	0.525	0.475	0.464	0.1	28.3
Cy5	Krytox	day1	0.4	29.3	0.4	17.8	0.659	0.341	0.981		0.5
Cy5	NP	day0	1.4	63.4	0.8	10.5	0.240	0.760	0.955	8.8	0.0
Cy5	NP	day1	1.2	67.4	0.6	11.7	0.706	0.294	0.995		0.0
DAPI	RAN	day0	25.8	67.5	7.0	10.0	0.285	0.715	0.849	6.4	1.4
DAPI	RAN	day1	16.3	67.4	5.1	11.2	0.731	0.269	0.906		0.2
DAPI	Krytox	day0	55.2	58.1	19.6	10.9	0.231	0.769	0.939	41.2	71.8
DAPI	Krytox	day1	37.4	64.7	13.9	11.7	0.589	0.411	0.727		28.7
DAPI	NP	day0	28.8	53.1	13.7	12.9	0.414	0.586	0.731	97.9	36.1
DAPI	NP	day1	48.4	55.5	18.7	9.8	0.721	0.279	0.795		65.7
AF350	RAN	day0	9.5	63.6	2.3	10.9	0.446	0.554	0.891	7.0	0.0
AF350	RAN	day1	5.3	58.4	1.7	15.5	0.634	0.366	0.943		0.1
AF350	Krytox	day0	12.9	61.4	2.1	13.6	0.336	0.664	0.760	3.9	0.1
AF350	Krytox	day1	6.2	69.4	2.2	15.5	0.789	0.211	0.957		0.0
AF350	NP	day0	11.8	71.0	3.7	12.3	0.369	0.631	0.802	9.6	0.0
AF350	NP	day1	5.2	57.7	2.0	12.7	0.839	0.161	0.974		0.0
Pyr	RAN	day0	1.1	63.3	0.5	11.7	0.444	0.556	0.974	2.3	0.0
Pyr	RAN	day1	0.5	47.4	0.4	14.2	0.629	0.371	0.992		0.0
Pyr	Krytox	day0	0.7	47.3	0.4	12.9	0.478	0.522	0.960	1.7	0.0
Pyr	Krytox	day1	1.6	31.8	1.2	18.7	0.632	0.368	0.863		2.8
Pyr	NP	day0	1.1	64.1	0.6	16.1	0.692	0.308	0.971	4.6	0.0
Pyr	NP	day1	0.4	72.6	0.4	12.6	0.924	0.076	0.998		0.0
AF488	RAN	day0	1.6	72.6	0.6	14.2	0.381	0.619	0.975	0.3	0.0
AF488	RAN	day1	0.2	20.4	0.3	11.0	0.694	0.306	0.984		0.2
AF488	Krytox	day0	1.8	59.3	0.7	18.2	0.544	0.456	0.988	4.4	0.1
AF488	Krytox	day1	0.4	43.2	0.4	13.0	0.786	0.214	0.987		0.0
AF488	NP	day0	0.8	67.4	0.5	22.8	0.867	0.133	0.993	4.8	0.0
AF488	NP	day1	0.4	42.1	0.4	13.9	0.709	0.291	0.991		0.0
AF568	RAN	day0	1.0	61.9	0.7	14.1	0.357	0.643	0.959	0.0	0.0
AF568	RAN	day1	1.0	47.5	0.6	11.1	0.503	0.497	0.968		0.0
AF568	Krytox	day0	1.2	51.2	0.8	8.0	0.397	0.603	0.908	0.9	0.0
AF568	Krytox	day1	0.6	51.2	0.4	15.2	0.632	0.368	0.990		0.0
AF568	NP	day0	2.9	79.9	0.8	8.6	0.153	0.847	0.914	0.4	0.0
AF568	NP	day1	0.6	71.1	0.5	10.9	0.333	0.667	0.979		0.0