

CONTROLLO QUALITA' (QC): LINEARITA' CITOFUORIMETRO GALLIOS

SPHERO™ Calibration Particles

SPHERO™ Calibration Particles are designed for routine calibration of flow cytometers. They are used extensively by many laboratories for QC and long term performance tracking of the flow cytometer. In addition, they are also used for routine alignment and calibration in fluorescence and confocal fluorescence microscopy.

SPHERO™ Rainbow Calibration Particles

- Contains multiple fluorophores incorporated in the same particle to be used in multiple channels of the flow cytometer.
- Available with different fluorescent intensities on the same size particles.
- Stable for several years when stored properly
- Withstand freeze-thaw cycles; diluted particles can be stored frozen for later use.
- Can be sanitized by treating with 70% ethanol or other antibiotic agents.

Particle Type and Surface	Size, μm	Catalog No.	Unit
Rainbow Calibration, 4 peaks, $10^7/\text{mL}$	1.8-2.2	RCP-20-5	5 mL
Rainbow Calibration, 6 peaks, $10^7/\text{mL}$	3.0-3.4	RCP-30-5	5 mL
Rainbow Calibration, 8 peaks, $10^7/\text{mL}$	3.0-3.4	RCP-30-5A	5 mL
Rainbow Calibration, 6 peaks, $10^7/\text{mL}$	3.2 (+/-0.1)	RCP-32-5	5 mL
Rainbow Calibration, 4 peaks, $10^7/\text{mL}$	3.5-4.0	RCP-35-5	5 mL
Rainbow Calibration, 6 peaks, $10^7/\text{mL}$	6.0-6.4	RCP-60-5	5 mL

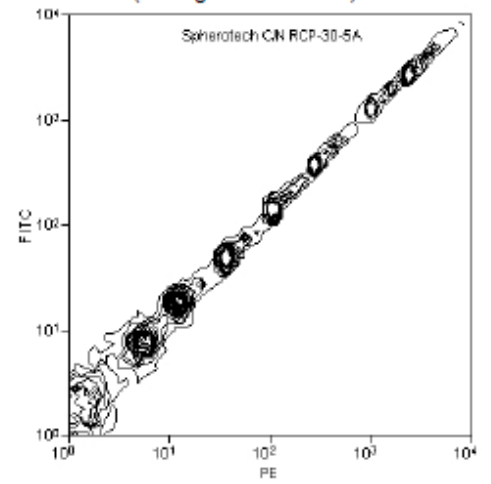
IN USO

The Rainbow Calibration Particles (RCPs) contain a mixture of several similar size particles with different fluorescence intensities. Every particle contains a mixture of fluorophores that allows excitation at any wavelength from 365 to 650 nm. As a result, most channels in the flow cytometer can be calibrated using the same set of particles.

These particles are also used to determine the relative voltage range for each flow cytometry detector. This will determine the dynamic range of specific PMT detectors*.

*Perfetto, S.P., D.Ambrozak, et al. (2006). "Quality assurance for polychromatic flow cytometry." Nat. Protocols 1(3): 1522-1530.

Figure 1 The contour plot below displays the bead distributions of the Rainbow Calibration Particles in FITC vs PE. (Catalog No. RCP-30-5A).



The RCPs provide a reliable and reproducible particle mixture for calibrating flow cytometers. They are very stable since the fluorochromes are entrapped within the particles instead of being located on the surface. In addition, Spherotech uses fluorophores that are non-spectral matching to the commonly used fluorophores such as FITC, PE or PE-Cy5. As a result, the RCPs are stable in terms of fluorescence.

The RCPs are convenient and affordable to use for long term performance tracking or routine calibration. They are packaged in a dropper bottle to facilitate the dispensing and storage. The diluted particles can be stored in the freezer for later use if desired to reduce costs. Dilution of a few drops of the particles from the dropper bottle to 1 mL of a diluent will provide adequate particle concentration for flow cytometer calibration. The diluted Rainbow Calibration Particles remain stable following repeated freezing and thawing.

QC: LINEARITA' 8 Peaks Rainbow

(#Lotto RCP-30-5A, Spherotech)

LASER BLUE

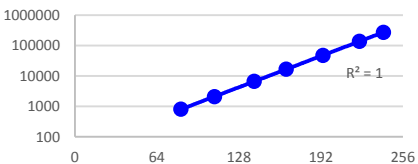
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEFL
1	23,98	
2	82,63	792
3	108,85	2079
4	139,92	6588
5	164,94	16471
6	193,56	47497
7	222,14	137049
8	240,75	271647
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEPE
1	11,27	
2	71,08	531
3	99,60	1504
4	131,08	4819
5	156,92	12506
6	185,76	36159
7	215,87	109588
8	238,47	250892
R ² =		1

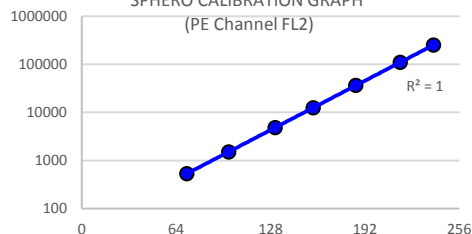
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEPTR
1	5,07	
2	67,86	233
3	95,23	669
4	126,55	2179
5	152,86	5929
6	182,39	18219
7	215,27	63944
8	243,62	188785
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MECY
1	7,51	
2	62,84	1614
3	87,37	4035
4	117,83	12025
5	144,96	31896
6	175,52	95682
7	211,83	353225
8	242,79	1077421
R ² =		1

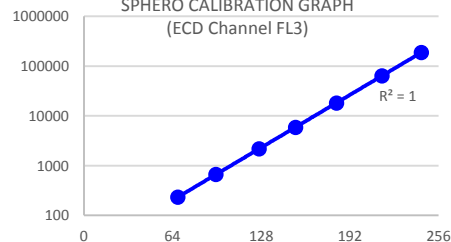
SPHERO CALIBRATION GRAPH
(FITC Channel FL1)



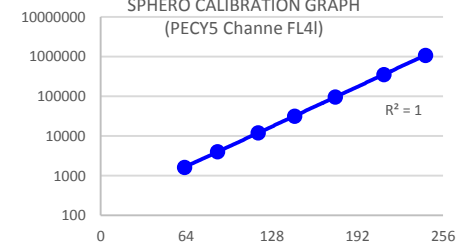
SPHERO CALIBRATION GRAPH
(PE Channel FL2)



SPHERO CALIBRATION GRAPH
(ECD Channel FL3)

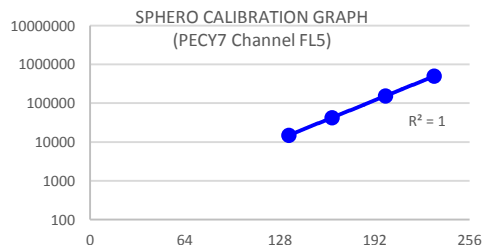


SPHERO CALIBRATION GRAPH
(PECY5 Channe FL4)



PMT LINEARITY QC RECORD

RIFERIMENTO		
PEAK #	CH #	MEPCY7
1		
2		
3		
4		
5	134,08	14916
6	163,25	42336
7	199,32	153840
8	231,96	494263
R ² =		1



QC: LINEARITA' 8 Peaks Rainbow

LASER ROSSO

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEAP
1	10,33	
2	75,49	373
3	102,13	1079
4	135,05	3633
5	161,59	9896
6	189,60	28189
7	217,33	79831
8	234,36	151008
R ² =		1

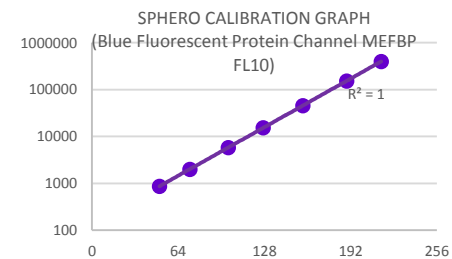
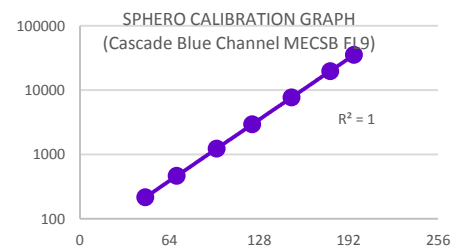
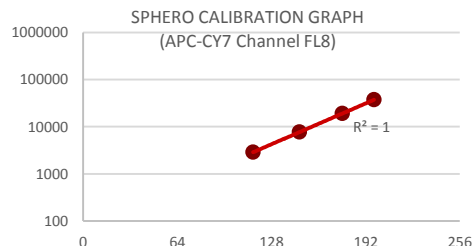
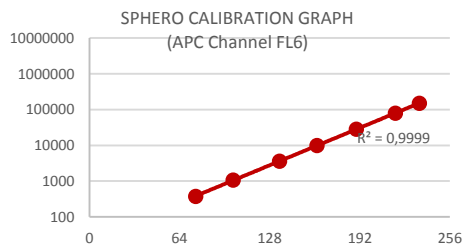
PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEAPCY7
1		
2		
3		
4		
5	115,43	2864
6	146,89	7644
7	176,21	19081
8	197,65	37258
R ² =		1

(#Lotto RCP-30-5A, Spherotech)

LASER VIOLA

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MECSB
1	11,27	
2	46,77	216
3	69,14	464
4	97,70	1232
5	123,12	2940
6	151,17	7669
7	178,92	19812
8	195,96	35474
R ² =		1

PMT LINEARITY QC RECORD		
RIFERIMENTO		
PEAK #	CH #	MEBFP
1	14,09	
2	50,03	861
3	72,61	1997
4	101,11	5776
5	127,13	15233
6	156,43	45389
7	188,97	152562
8	214,62	396759
R ² =		1

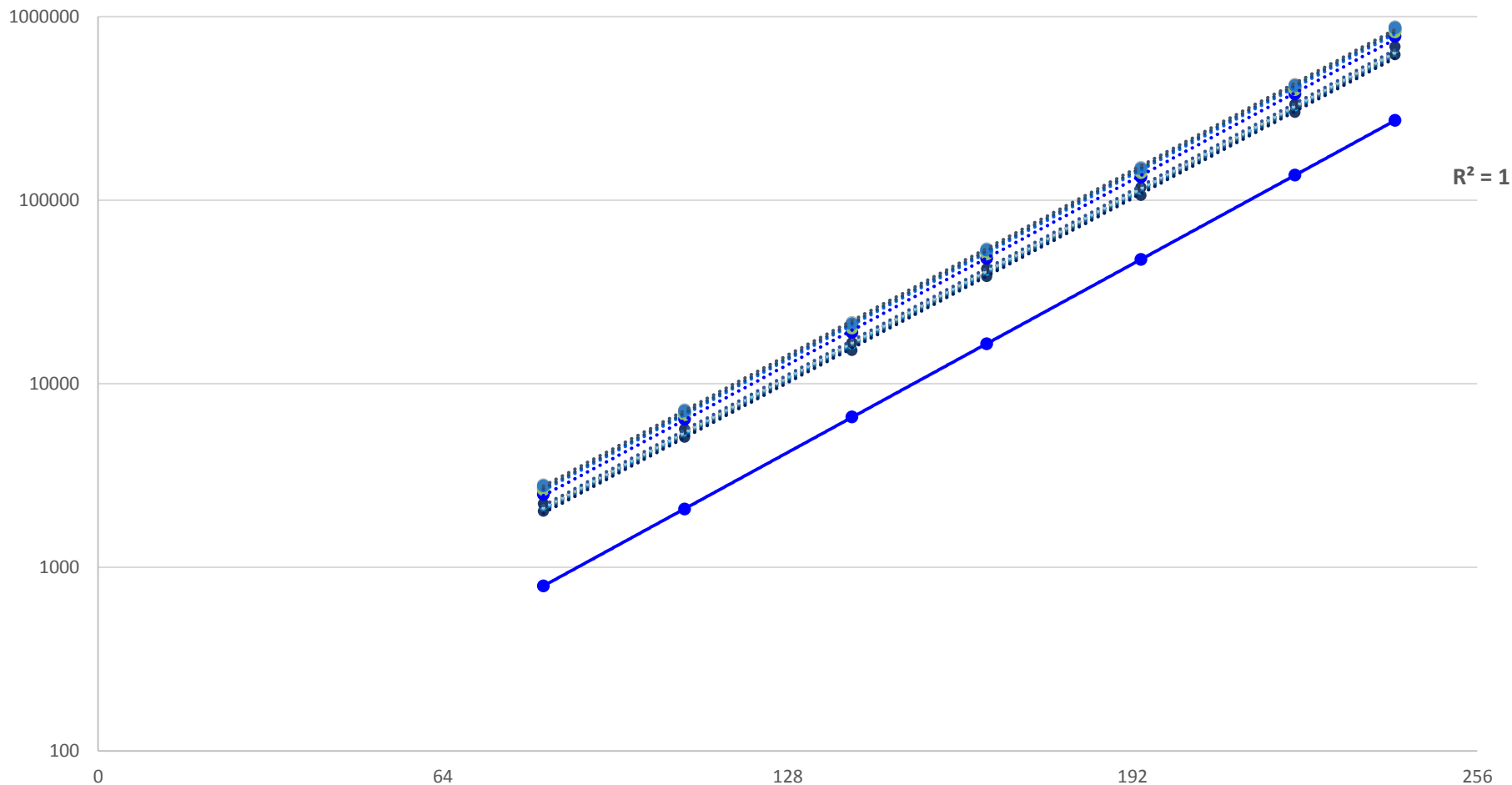


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

PEAK #	RIFERIMENTO	CH #	MEFL	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
1	23,98			314	304	315	315	311	304	301	317	326	339	351	359	367	365	356	356	348	350
2	82,63	792		2132	2092	2147	2085	2148	2065	2022	2138	2152	2221	2498	2668	2722	2817	2761	2715	2644	2776
3	108,85	2079		5398	5309	5459	5339	5439	5235	5117	5409	5486	5646	6405	6846	7014	7222	7108	6957	6779	7091
4	139,92	6588		15928	15704	16182	15777	16109	15490	15160	16065	16254	16692	19054	20408	20918	21534	21160	20686	20097	21099
5	164,94	16471		40297	39652	40920	39928	40764	39187	38308	40637	41036	42263	48227	51543	52890	54393	53549	52299	50785	53353
6	193,56	47497		111435	109799	113263	110569	112771	108400	106023	112495	113515	117176	133734	143209	146670	151075	148805	145154	140839	147988
7	222,14	137049		316755	311622	321443	313287	319909	307484	300646	319473	322230	332223	378981	405817	416080	428003	422025	412253	399617	419585
8	240,75	271647		652729	641744	661199	644585	658336	633406	619996	658863	663341	685486	779611	834622	856430	881333	870405	850862	823764	865287
	R ² =	1		0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(FITC Channel FL1)

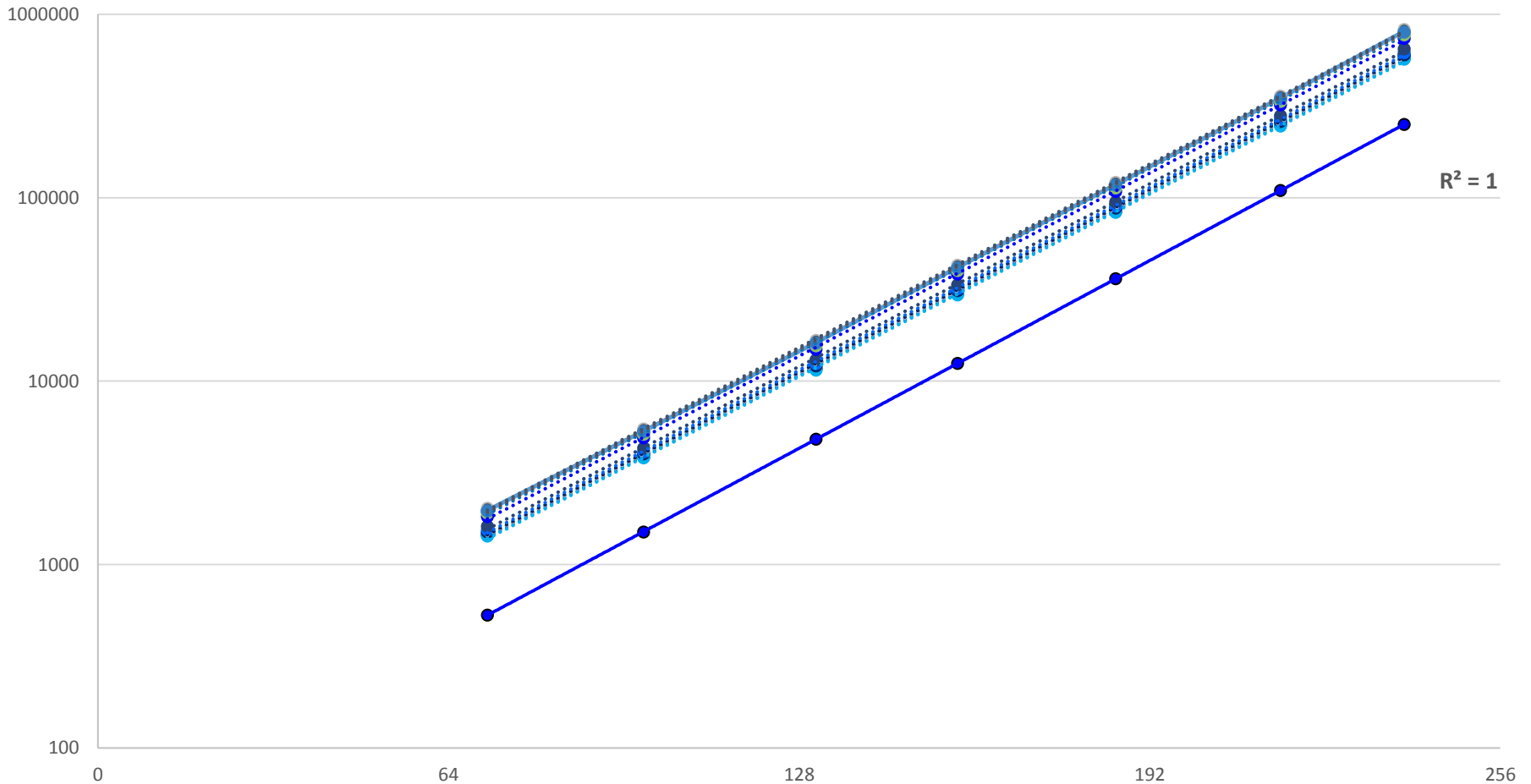


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORDO

PEAK #	RIFERIMENTO	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017	
1	11,27	301	295	301	302	301	304	302	308	314	328	344	353	360	361	350	350	344	345	
2	71,08	531	1480	1441	1480	1447	1488	1451	1428	1506	1547	1613	1828	1929	1978	2027	1973	1942	1906	1962
3	99,60	1504	3944	3852	3965	3856	3964	3872	3811	4013	4119	4319	4905	5204	5339	5483	5338	5251	5125	5313
4	131,08	4819	11928	11614	11990	11651	11972	11686	11468	12117	12444	13042	14901	15792	16228	16677	16238	15905	15579	16134
5	156,92	12506	30579	29780	30749	29834	30733	29980	29466	31109	31936	33457	38307	40589	41682	42844	41704	40887	40056	41476
6	185,76	36159	86114	84085	86818	84235	86677	84522	83049	87883	90056	94478	108150	114711	117680	121087	117868	115470	112937	117091
7	215,87	109588	255685	249300	257558	249664	257024	250673	246161	260828	267300	280079	320371	339625	348740	358632	349788	342835	334659	346996
8	238,47	250892	589370	574640	593851	576185	592391	578170	568267	601786	616574	646750	738712	782049	803480	827209	807845	792591	771586	800398
	R ² =	1	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPERO CALIBRATION GRAPH
(PE Channel FL2)

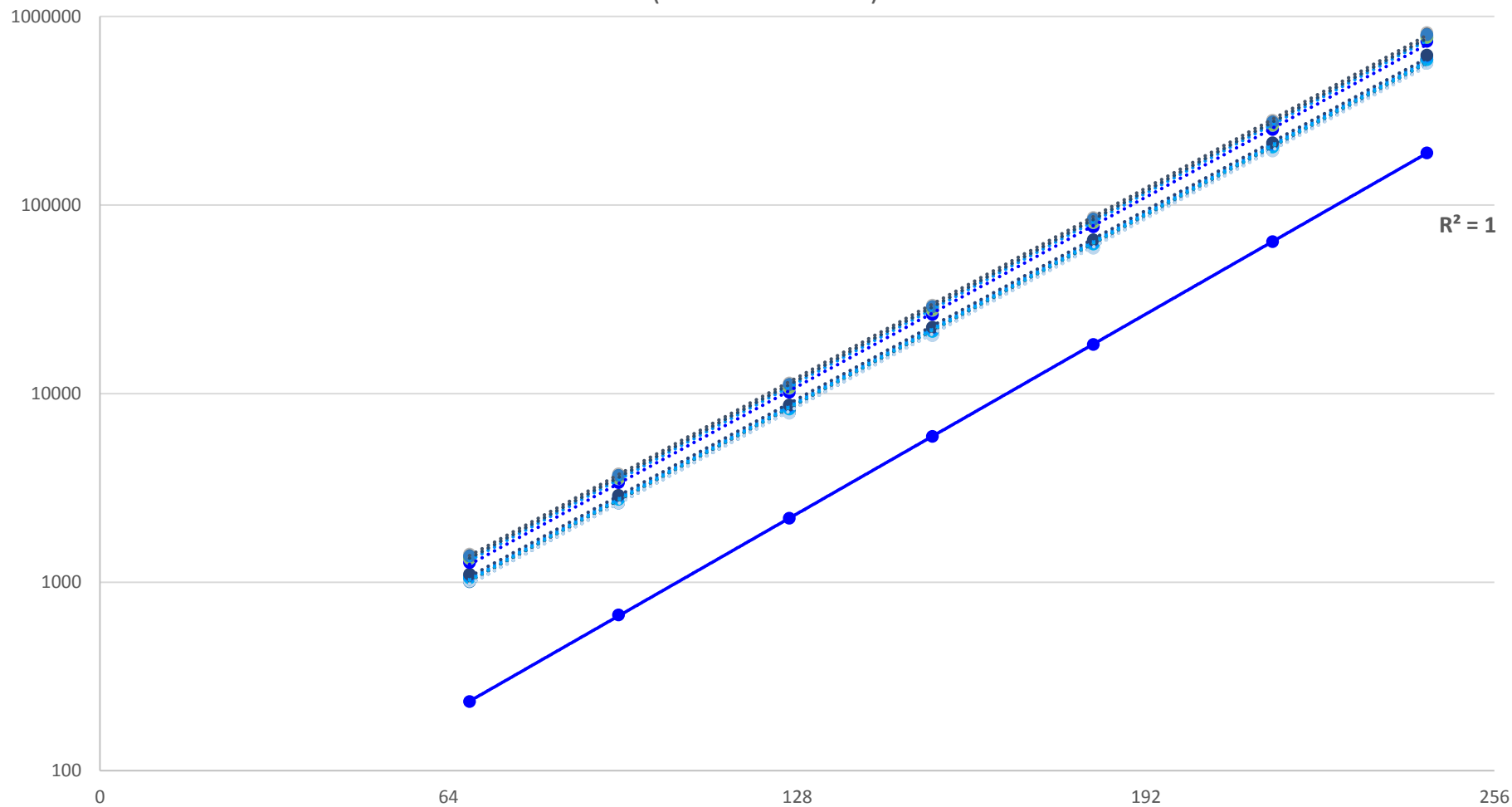


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

	RIFERIMENTO	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017	
PEAK #	CH # MEPTR																			
1	5,07	245	233	244	247	243	254	253	249	252	260	264	256	264	250	241	237	237	243	243
2	67,86 233	1044	1005	1040	1021	1041	1012	1010	1051	1070	1102	1270	1327	1372	1409	1361	1350	1340	1373	1373
3	95,23 669	2724	2627	2731	2652	2738	2645	2622	2747	2815	2882	3367	3532	3648	3752	3631	3606	3561	3680	3680
4	126,55 2179	8165	7898	8201	7960	8210	7935	7859	8266	8427	8689	10148	10662	11021	11370	10965	10898	10745	11136	11136
5	152,86 5929	21091	20359	21154	20570	21256	20485	20274	21350	21803	22463	26242	27568	28550	29413	28407	28183	27853	28828	28828
6	182,39 18219	61437	59337	61771	59924	61794	59562	58964	62051	63343	65336	76517	80485	83024	85777	82710	82140	81025	83956	83956
7	215,27 63944	201124	194051	202197	196495	202590	194976	192909	202890	207771	213548	251243	263640	272240	280864	270725	269337	265665	274753	274753
8	243,62 188785	585984	565681	591612	576622	591071	568632	562942	589881	605708	622218	735759	769830	794100	819645	789860	785278	774327	800062	800062
	R ² =	1	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(ECD Channel FL3)

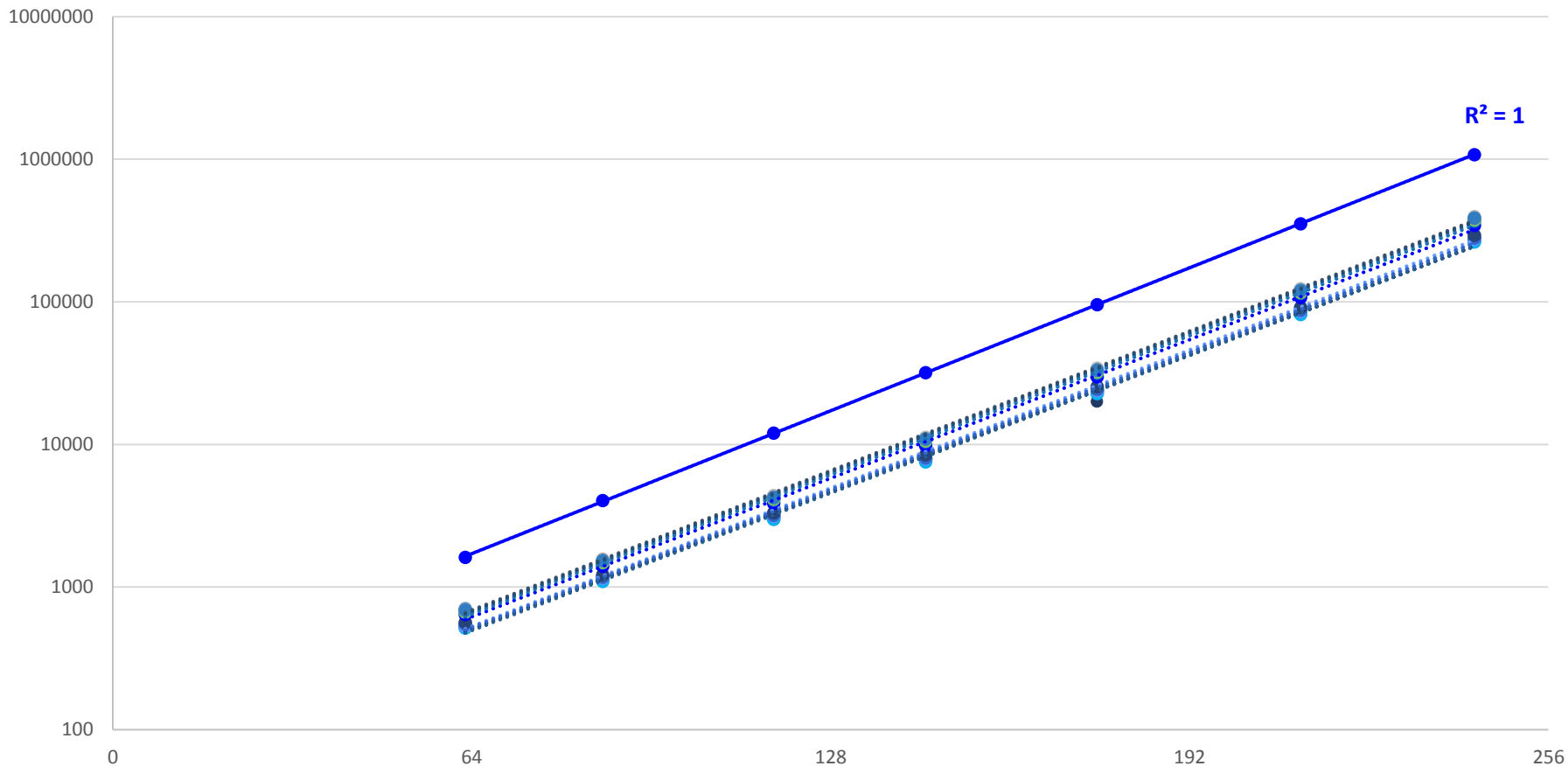


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

RIFERIMENTO			03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017	
PEAK #	CH #	MECY																			
1	7,51		180	170	184	183	170	168	170	176	183	197	194	197	199	198	189	190	185	185	
2	62,84	1614	549	535	559	538	520	515	515	530	549	567	642	665	679	711	699	690	671	689	
3	87,37	4035	1175	1156	1200	1145	1153	1123	1096	1150	1172	1216	1399	1473	1507	1570	1532	1523	1490	1533	
4	117,83	12025	3178	3121	3273	3120	3137	3000	2982	3140	3186	3321	3865	4088	4172	4380	4265	4219	4138	4238	
5	144,96	31896	8018	7901	8298	7918	7953	7678	7547	7940	8054	8440	9817	10428	10726	11244	10960	10840	10587	10879	
6	175,52	95682	24106	23945	25101	23919	20004	23011	22734	23900	24313	25433	29806	31655	32454	34204	33315	32942	32100	33045	
7	211,83	353225	86945	86511	90778	86225	86375	82701	81671	85785	87420	91245	107432	114387	117320	123485	120969	119040	116493	119632	
8	242,79	1077421	279367	279438	293303	277631	277274	265178	262810	275120	279715	292935	343654	366797	376750	396882	388479	382497	375347	385453	
	R ² =	1	0,9992	0,9992	0,9992	0,9992	0,998	0,9992	0,9992	0,9992	0,9992	0,9992	0,9994	0,9995	0,9995	0,9995	0,9995	0,9995	0,9995	0,9995	

SPHERO CALIBRATION GRAPH
(PECY5 Channel FL4)

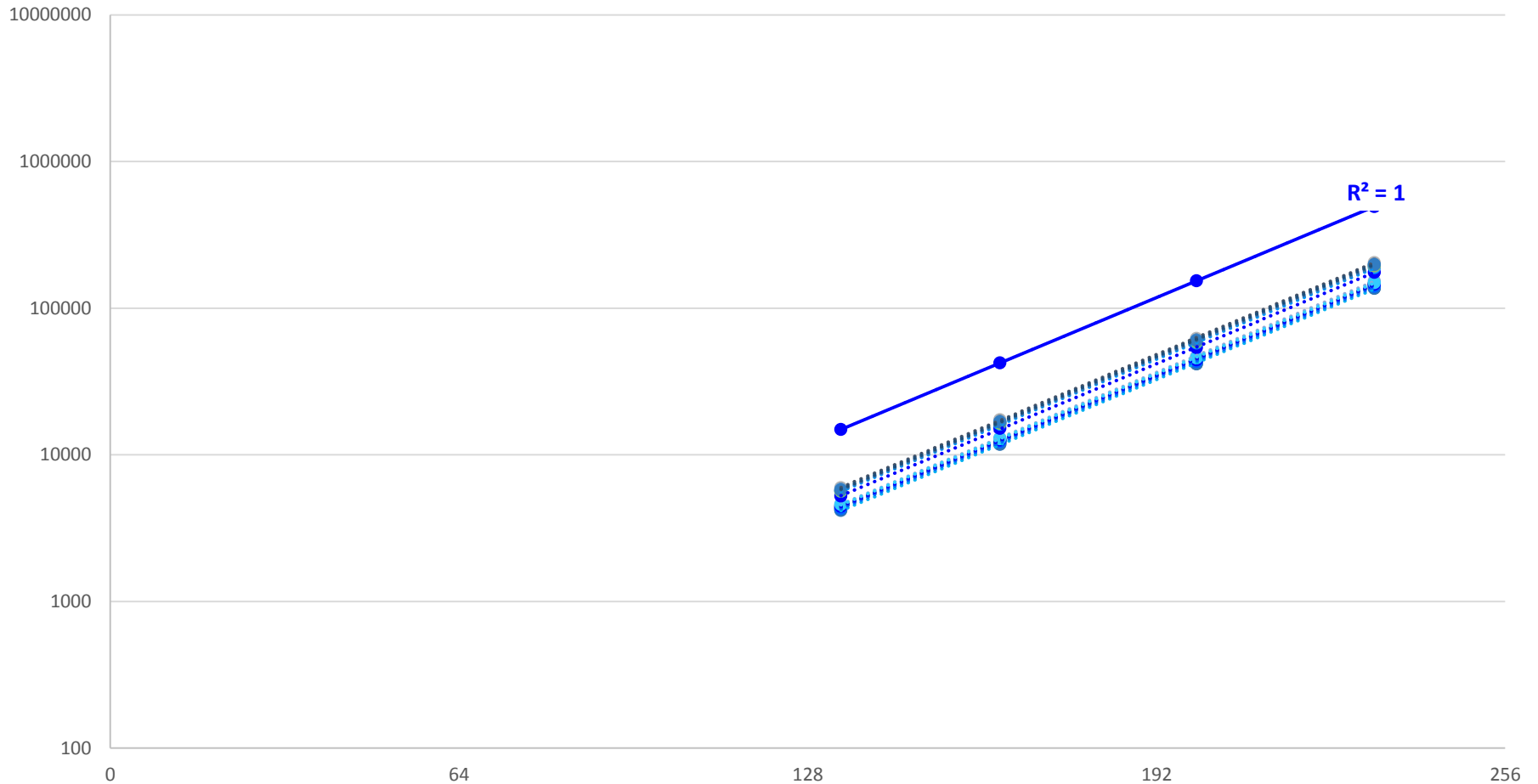


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

RIFERIMENTO			03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
PEAK #	CH #	MEPCY7																		
1			392	382	392	383	370	372	371	386	394	427	427	426	431	429	409	415	414	406
2			633	616	634	617	614	603	605	622	626	649	692	705	709	728	715	707	713	698
3			947	935	956	914	921	895	910	918	941	969	1041	1101	1115	1160	1137	1118	1099	1120
4			1974	1931	2035	1895	1940	1870	1847	1916	1968	1998	2275	2431	2461	2548	2497	2476	2445	2484
5	134,08	14916	4480	4387	4603	4281	4339	4216	4173	4307	4443	4577	5234	5624	5703	5962	5813	5731	5648	5748
6	163,25	42336	12659	12495	13040	12198	12386	11884	11785	12235	12607	12905	15129	16182	16520	17383	16820	16668	16332	16699
7	199,32	153840	44856	44551	46736	43403	43856	41882	41681	43058	44511	45849	53778	57810	59167	62307	60689	59839	58907	60026
8	231,96	494263	146790	145899	153067	141933	143587	137169	136632	140701	145467	149429	175723	188536	193300	204681	198758	196617	194483	197837
	R ² =	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

SPHERO CALIBRATION GRAPH
(PECY7 Channel FL5)

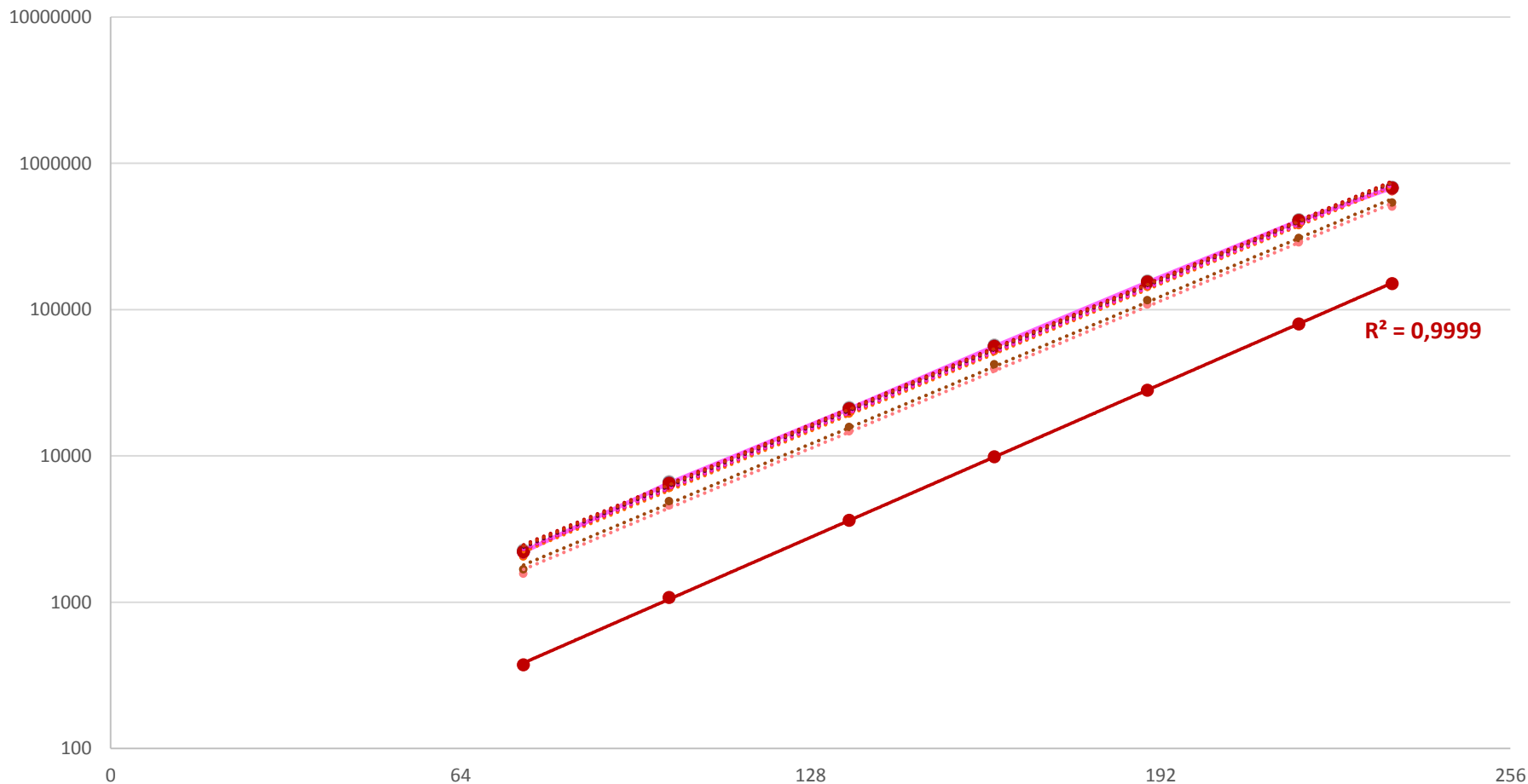


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

PEAK #	RIFERIMENTO CH # MEFL	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
1	23,98	314	304	315	315	311	304	301	317	326	339	351	359	367	365	356	356	348	350
2	82,63 792	2132	2092	2147	2085	2148	2065	2022	2138	2152	2221	2498	2668	2722	2817	2761	2715	2644	2776
3	108,85 2079	5398	5309	5459	5339	5439	5235	5117	5409	5486	5646	6405	6846	7014	7222	7108	6957	6779	7091
4	139,92 6588	15928	15704	16182	15777	16109	15490	15160	16065	16254	16692	19054	20408	20918	21534	21160	20686	20097	21099
5	164,94 16471	40297	39652	40920	39928	40764	39187	38308	40637	41036	42263	48227	51543	52890	54393	53549	52299	50785	53353
6	193,56 47497	111435	109799	113263	110569	112771	108400	106023	112495	113515	117176	133734	143209	146670	151075	148805	145154	140839	147988
7	222,14 137049	316755	311622	321443	313287	319909	307484	300646	319473	322230	332223	378981	405817	416080	428003	422025	412253	399617	419585
8	240,75 271647	652729	641744	661199	644585	658336	633406	619996	658863	663341	685486	779611	834622	856430	881333	870405	850862	823764	865287
	R ² = 1	0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(APC Channel FL6)

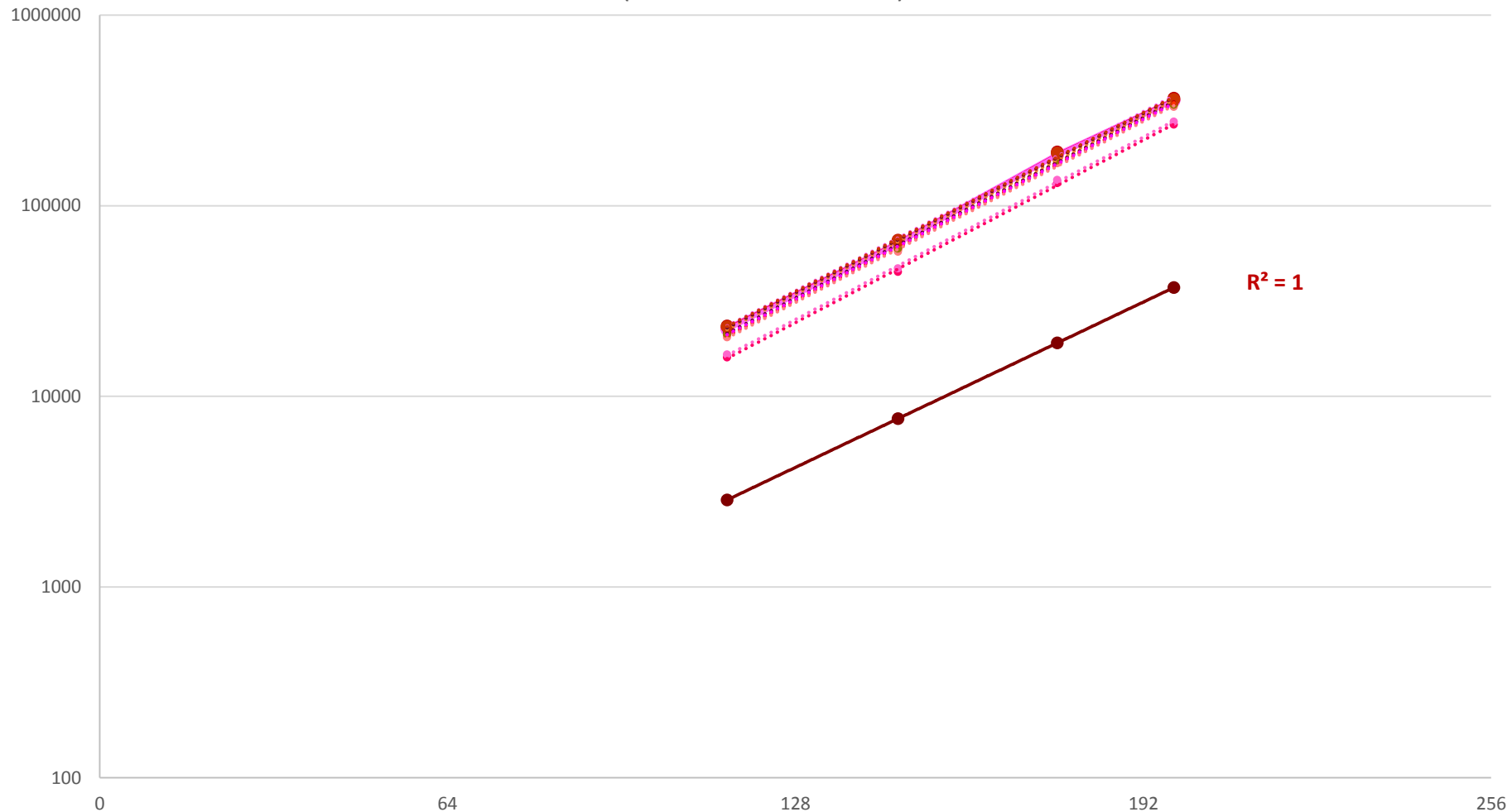


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

PEAK #	RIFERIMENTO	CH #	MEFL	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
1	23,98			314	304	315	315	311	304	301	317	326	339	351	359	367	365	356	356	348	350
2	82,63	792		2132	2092	2147	2085	2148	2065	2022	2138	2152	2221	2498	2668	2722	2817	2761	2715	2644	2776
3	108,85	2079		5398	5309	5459	5339	5439	5235	5117	5409	5486	5646	6405	6846	7014	7222	7108	6957	6779	7091
4	139,92	6588		15928	15704	16182	15777	16109	15490	15160	16065	16254	16692	19054	20408	20918	21534	21160	20686	20097	21099
5	164,94	16471		40297	39652	40920	39928	40764	39187	38308	40637	41036	42263	48227	51543	52890	54393	53549	52299	50785	53353
6	193,56	47497		111435	109799	113263	110569	112771	108400	106023	112495	113515	117176	133734	143209	146670	151075	148805	145154	140839	147988
7	222,14	137049		316755	311622	321443	313287	319909	307484	300646	319473	322230	332223	378981	405817	416080	428003	422025	412253	399617	419585
8	240,75	271647		652729	641744	661199	644585	658336	633406	619996	658863	663341	685486	779611	834622	856430	881333	870405	850862	823764	865287
	R ² =	1		0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(APC-CY7 Channel FL8)

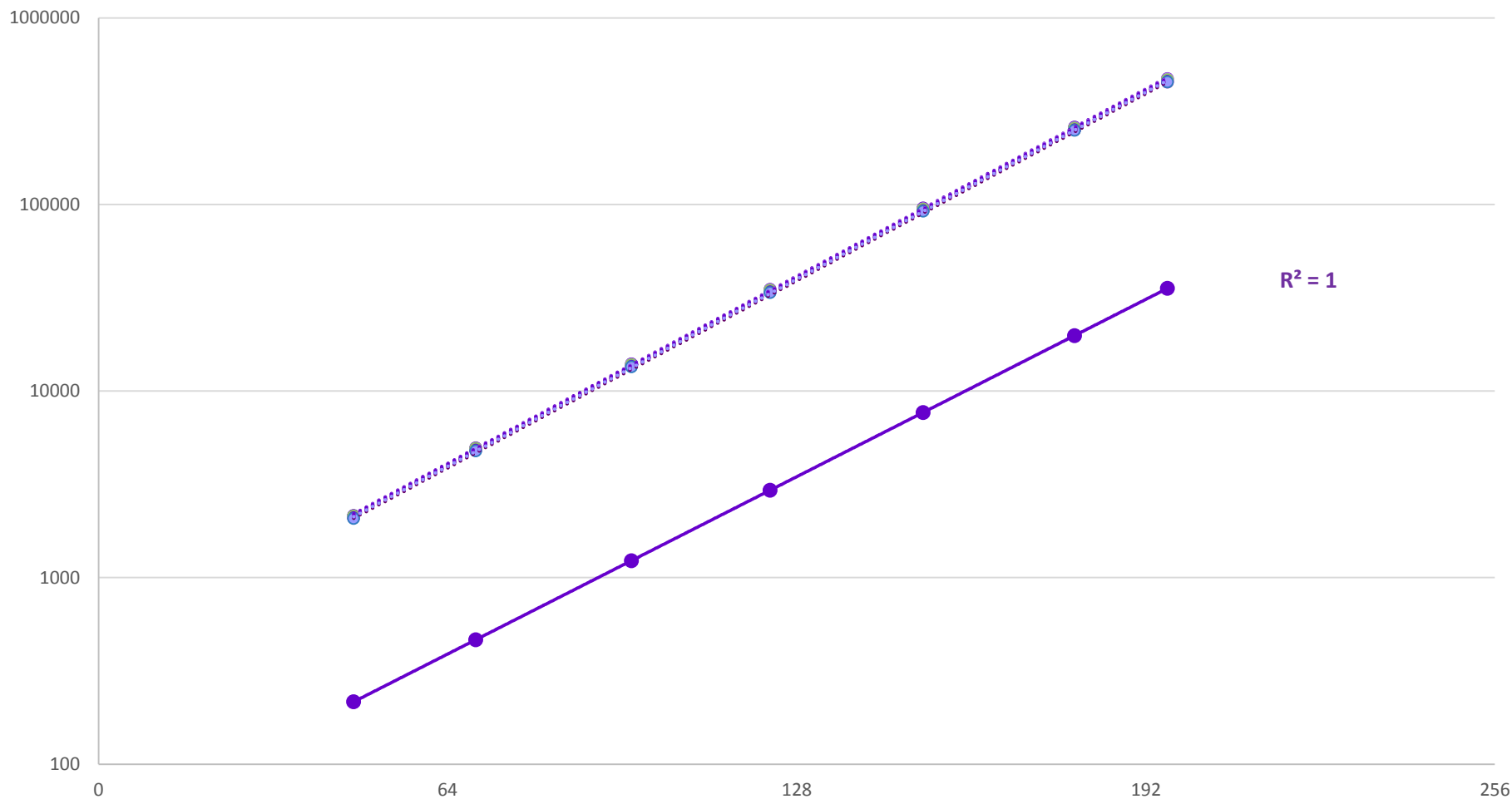


QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

PEAK #	RIFERIMENTO CH # MEFL	03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
1	23,98	314	304	315	315	311	304	301	317	326	339	351	359	367	365	356	356	348	350
2	82,63 792	2132	2092	2147	2085	2148	2065	2022	2138	2152	2221	2498	2668	2722	2817	2761	2715	2644	2776
3	108,85 2079	5398	5309	5459	5339	5439	5235	5117	5409	5486	5646	6405	6846	7014	7222	7108	6957	6779	7091
4	139,92 6588	15928	15704	16182	15777	16109	15490	15160	16065	16254	16692	19054	20408	20918	21534	21160	20686	20097	21099
5	164,94 16471	40297	39652	40920	39928	40764	39187	38308	40637	41036	42263	48227	51543	52890	54393	53549	52299	50785	53353
6	193,56 47497	111435	109799	113263	110569	112771	108400	106023	112495	113515	117176	133734	143209	146670	151075	148805	145154	140839	147988
7	222,14 137049	316755	311622	321443	313287	319909	307484	300646	319473	322230	332223	378981	405817	416080	428003	422025	412253	399617	419585
8	240,75 271647	652729	641744	661199	644585	658336	633406	619996	658863	663341	685486	779611	834622	856430	881333	870405	850862	823764	865287
	R ² = 1	0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(Cascade Blue Channel MECSB FL9)



QC: LINEARITA' APRILE – DICEMBRE 2017

PMT LINEARITY QC RECORD

	RIFERIMENTO		03/04/2017	14/04/2017	28/04/2017	16/05/2017	31/05/2017	14/06/2017	28/06/2017	17/07/2017	31/07/2017	16/08/2017	15/09/2017	25/09/2017	02/10/2017	16/10/2017	31/10/2017	15/11/2017	01/12/2017	15/12/2017
PEAK #	CH # MEFL																			
1	23,98		314	304	315	315	311	304	301	317	326	339	351	359	367	365	356	356	348	350
2	82,63 792		2132	2092	2147	2085	2148	2065	2022	2138	2152	2221	2498	2668	2722	2817	2761	2715	2644	2776
3	108,85 2079		5398	5309	5459	5339	5439	5235	5117	5409	5486	5646	6405	6846	7014	7222	7108	6957	6779	7091
4	139,92 6588		15928	15704	16182	15777	16109	15490	15160	16065	16254	16692	19054	20408	20918	21534	21160	20686	20097	21099
5	164,94 16471		40297	39652	40920	39928	40764	39187	38308	40637	41036	42263	48227	51543	52890	54393	53549	52299	50785	53353
6	193,56 47497		111435	109799	113263	110569	112771	108400	106023	112495	113515	117176	133734	143209	146670	151075	148805	145154	140839	147988
7	222,14 137049		316755	311622	321443	313287	319909	307484	300646	319473	322230	332223	378981	405817	416080	428003	422025	412253	399617	419585
8	240,75 271647		652729	641744	661199	644585	658336	633406	619996	658863	663341	685486	779611	834622	856430	881333	870405	850862	823764	865287
	R ² = 1		0,9998	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999	0,9999

SPHERO CALIBRATION GRAPH
(Blue Fluorescent Protein Channel MEFLBP FL10)

