

Fluorescence Based Quench Correction of Scintillation Proximity Assay (SPA)*

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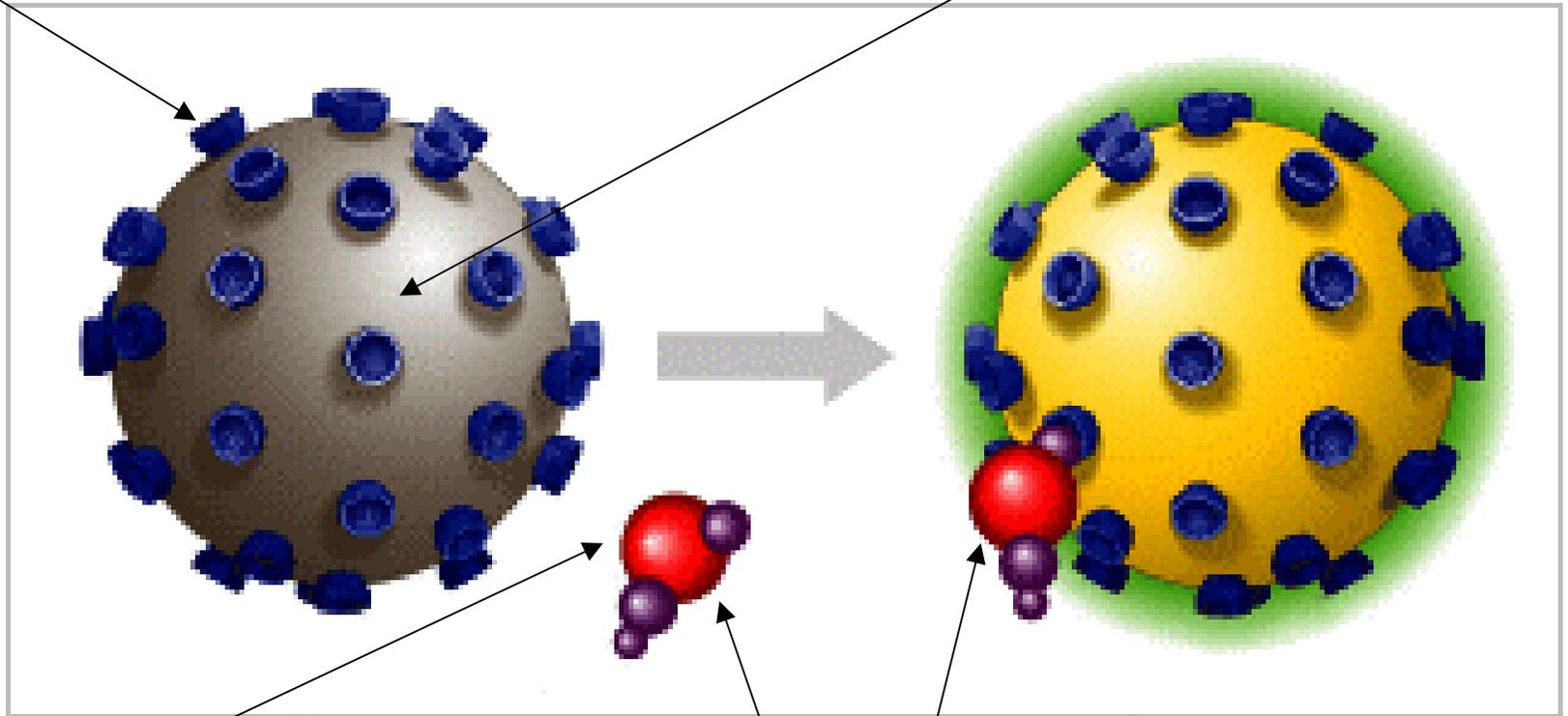
Phil Harrison

*GE Healthcare, formerly
Amersham Biosciences

SPA Theory

Attached biological capture molecule

5 micron PVT Bead with added scintillators



Energy absorbed by medium no light generated

**Bound labelled target molecule
Bead stimulated to emit light**

Free labelled target molecule (^3H , ^{125}I , ^{35}S)

Scintillation Proximity Assay (SPA)

Why?

- Homogenous:
No separation or washing steps required. Simply mix and measure.
- Reduced assay costs:
Eliminates requirements for harvesting, filters and scintillation cocktails.
- Reduced disposal costs:
No radioactive liquid waste from harvesting filtration steps.

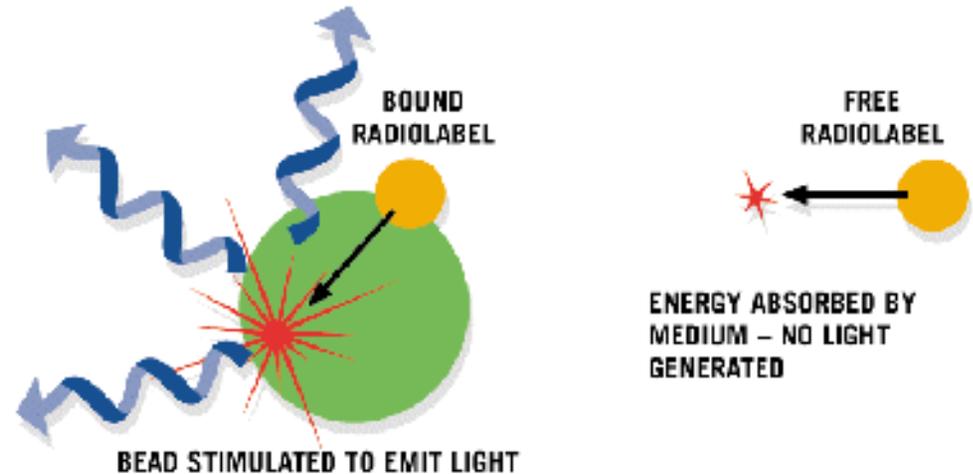


Plate CHAMELEON V



45% unquenched ^3H counting efficiency
in 96-well plate using LSC cocktails.

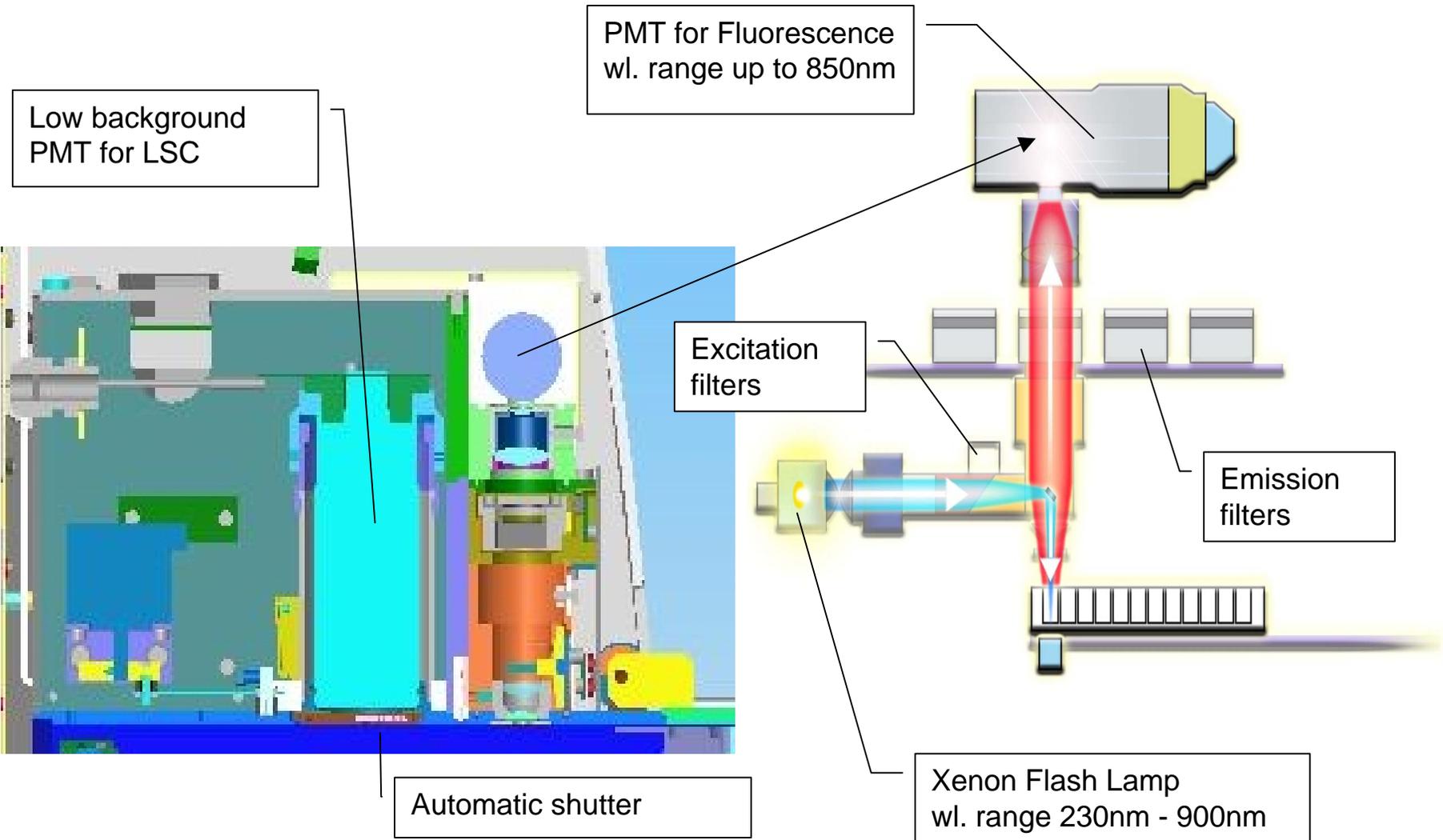
90% unquenched ^{14}C counting efficiency
in 96-well plate using LSC cocktails.



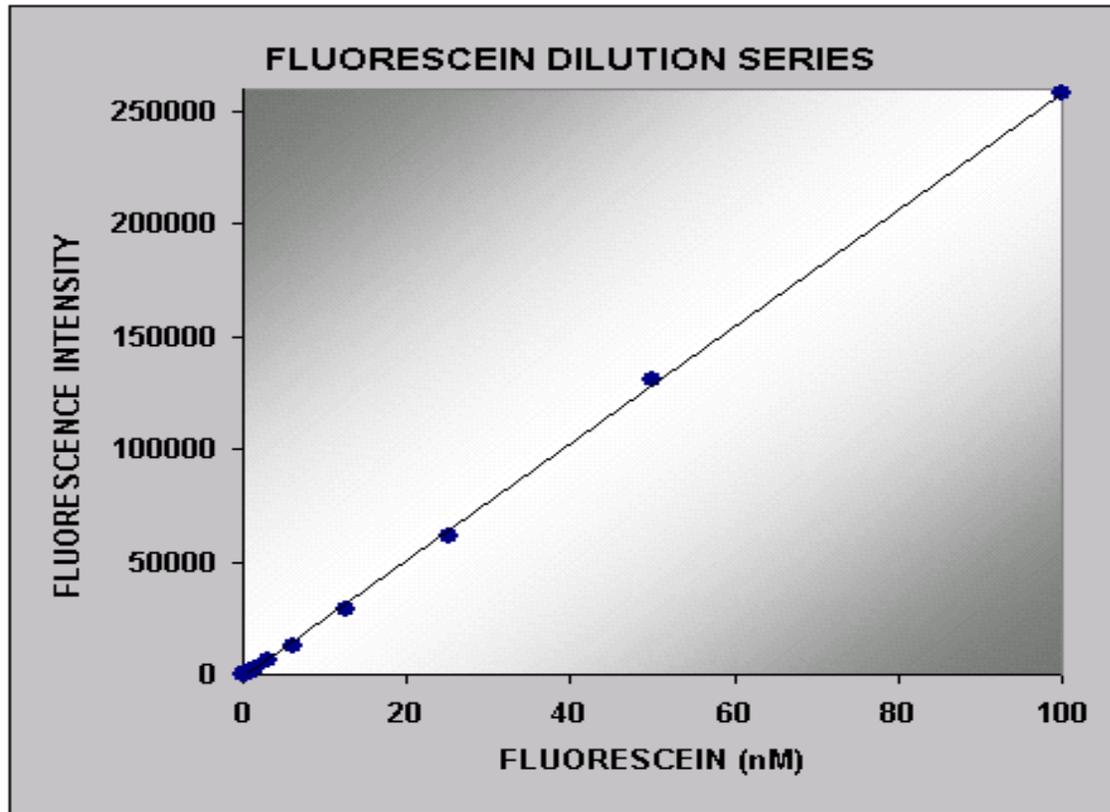
Multi Modality, Dual Detector, Two Measurements

LSC Mode

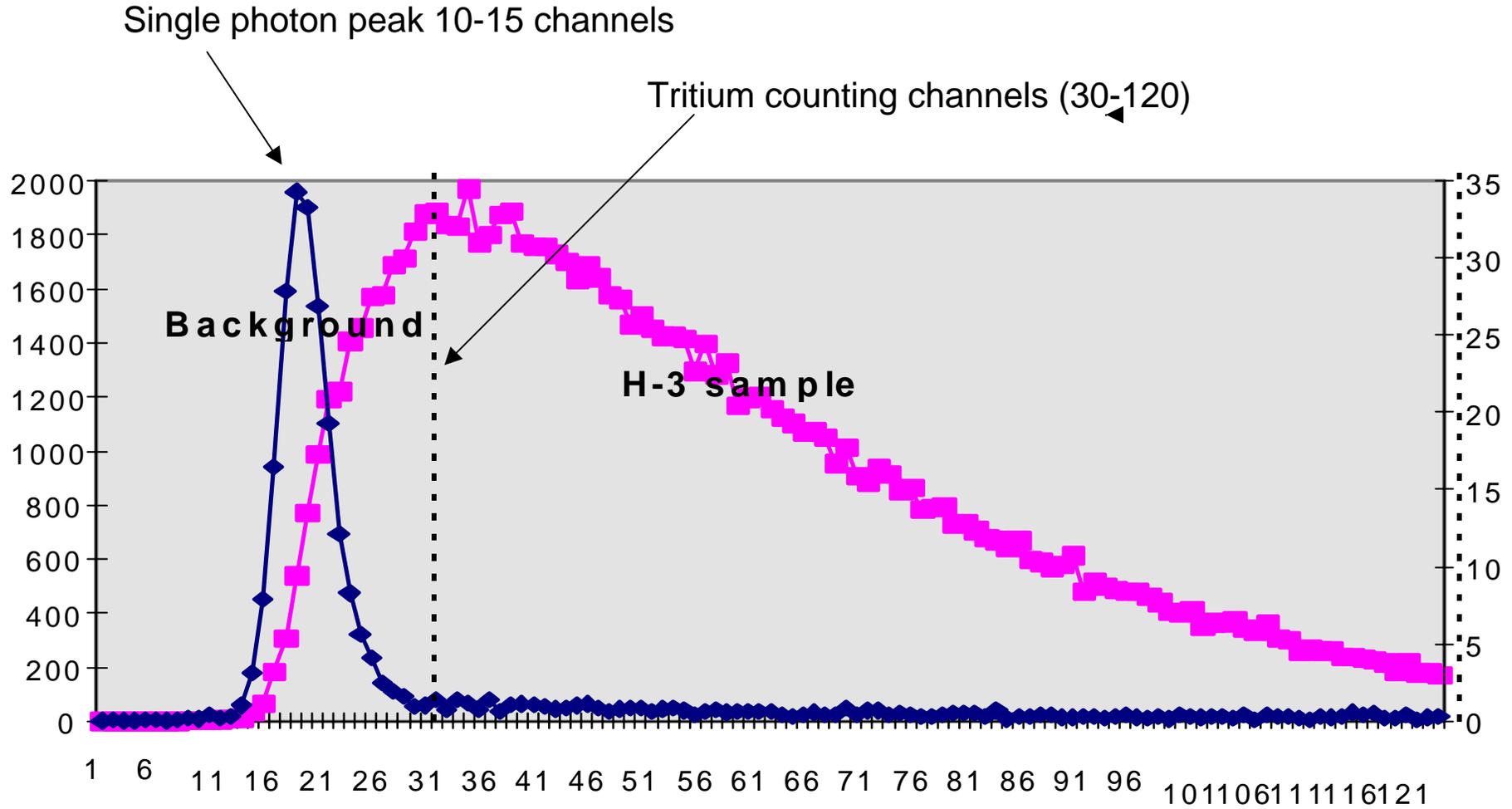
Fluorescence Mode



Fluorescein Dilution Series Fluorescence Mode



LSC Mode



TRKQ 7080 kit

exc 355 nm, em 460 nm

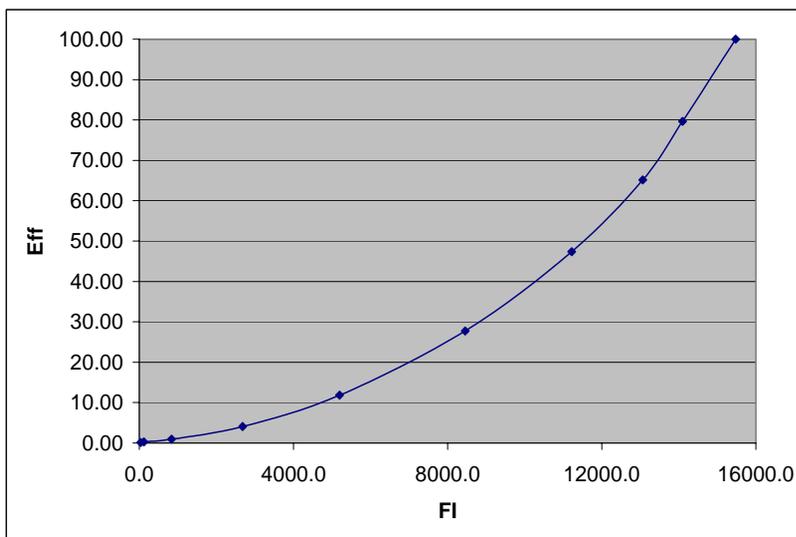
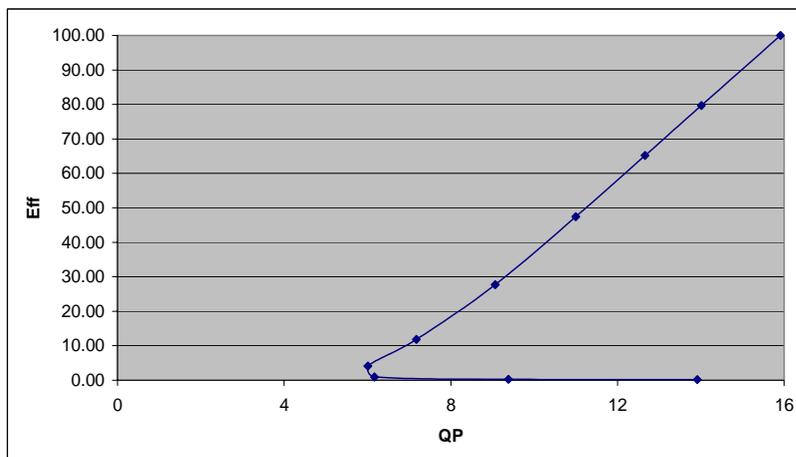
tartrazine mg/ml	CPM	Eff (%)	QP	FI
0	77956	100.00	15.907	15476.5
0.002	62119	79.68	14.008	14094.9
0.004	50814	65.18	12.661	13063.3
0.008	36950	47.40	10.997	11218.4
0.016	21624	27.74	9.064	8456.3
0.031	9226	11.83	7.173	5199.1
0.063	3202	4.11	6.007	2682.4
0.125	765	0.98	6.165	842.5
0.250	218	0.28	9.381	122.6
0.500	119	0.15	13.912	34.6

**³H SPA Colour Quench Kit
(code TRKQ7080):**

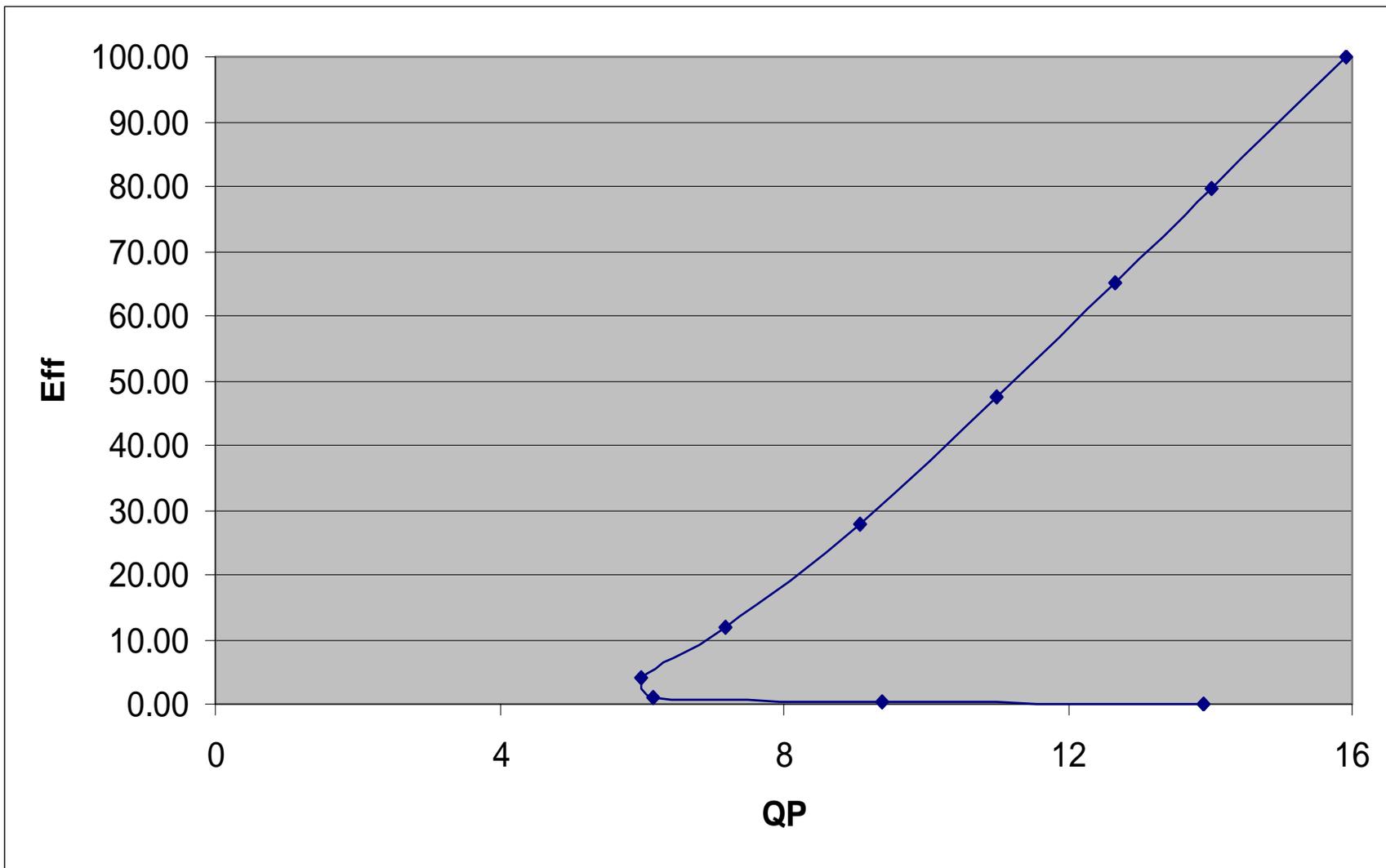
- 100 ul of buffer.
- 50 ul of bead suspension
- 50 ul of tartrazine solution.

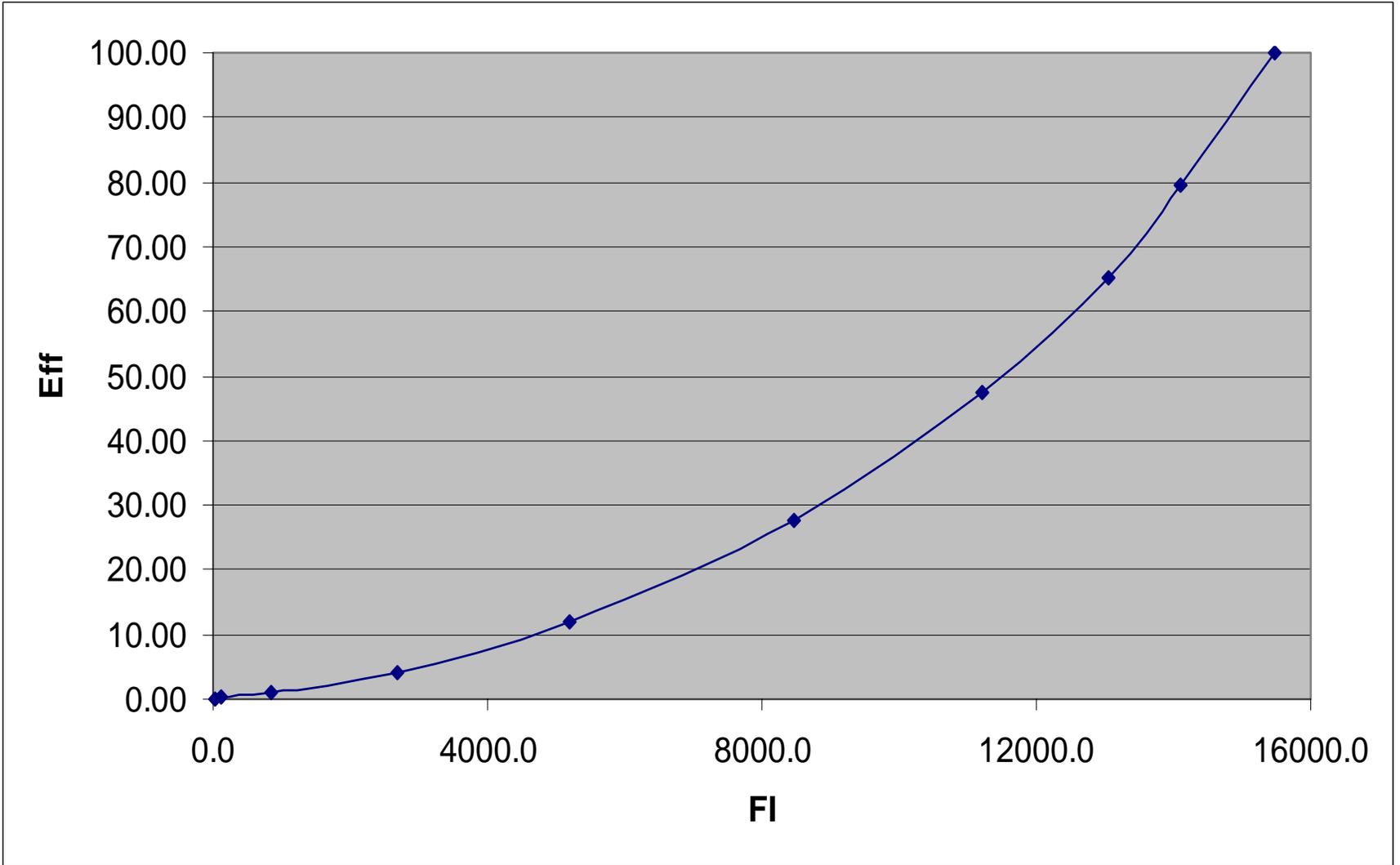
Total volume/well is 200 ul.
The beads were allowed to settle over night.

Calculated 400 000 DPM ³H
per 50 ul of bead
suspension, i.e. 400 000
DPM per well (approx).

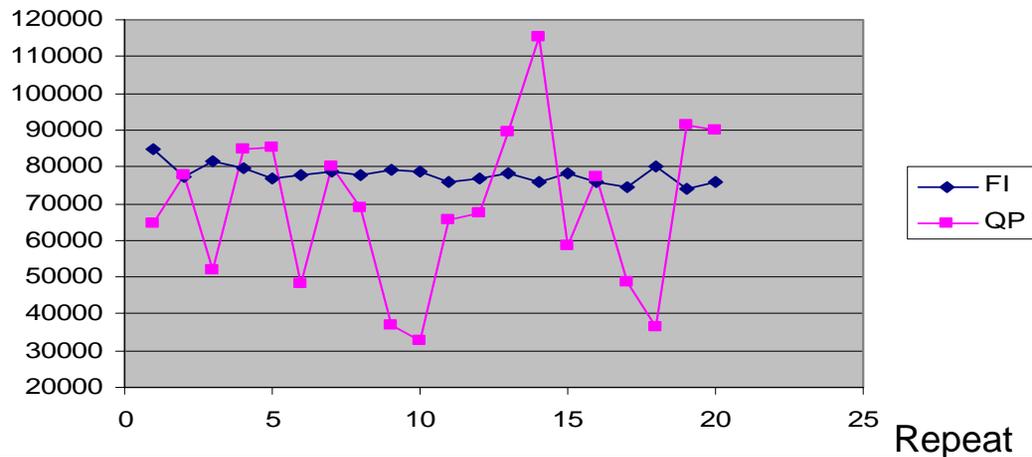


QP is SQP(I) (Spectral
Quench Parameter Isotope).

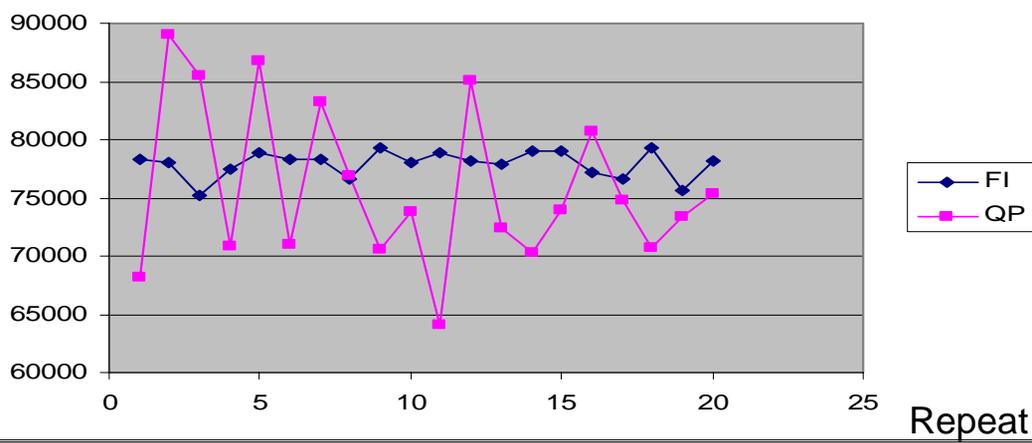




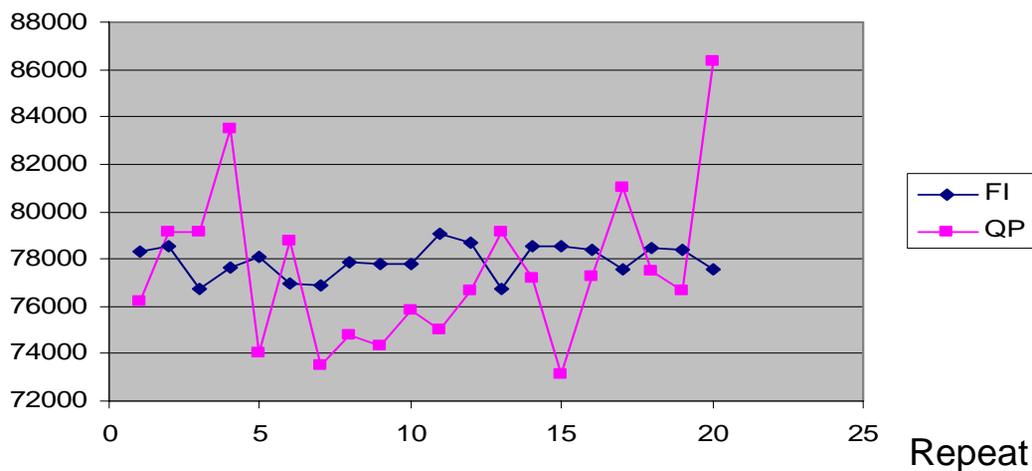
Calculated DPM



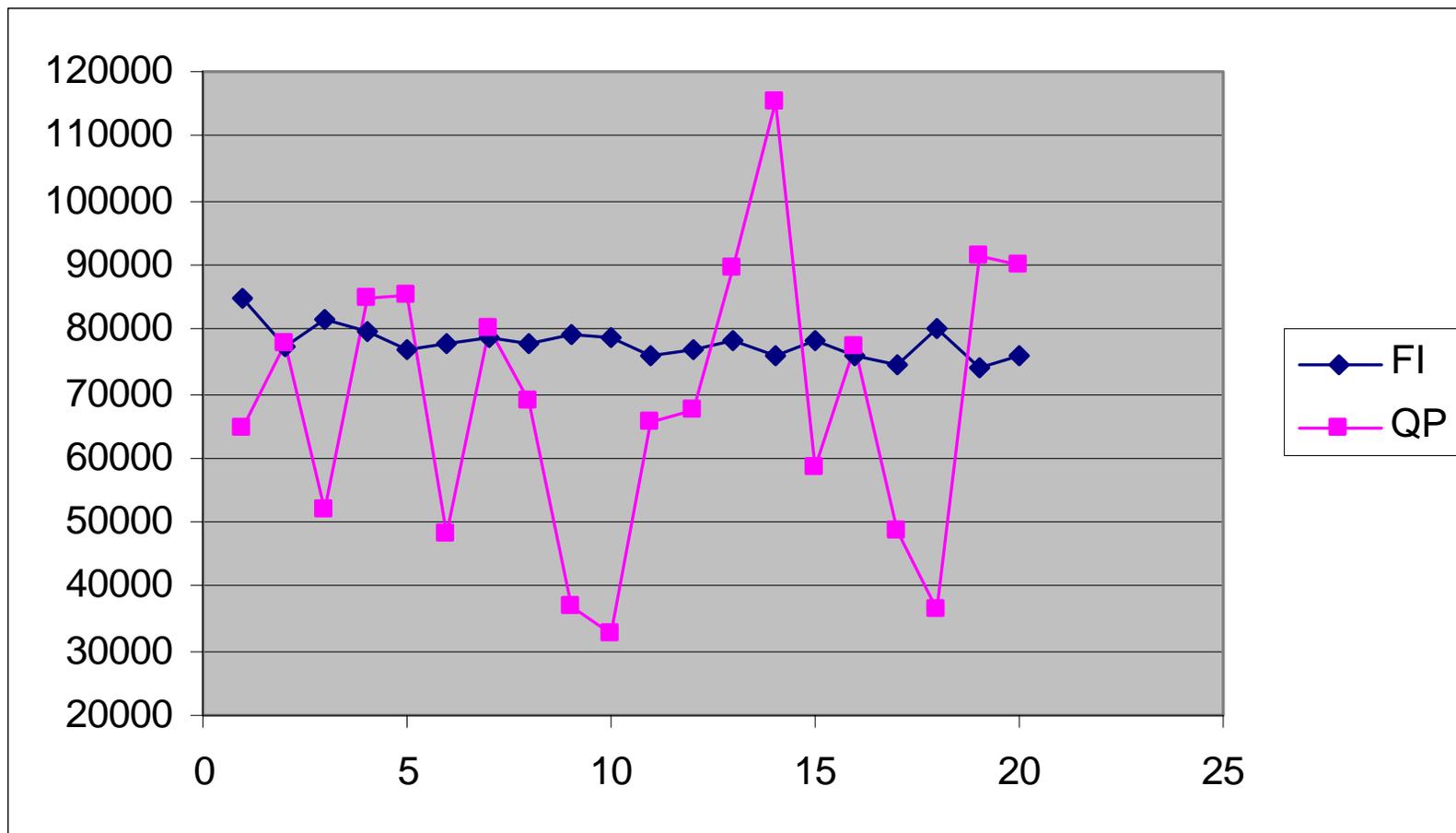
Calculated DPM



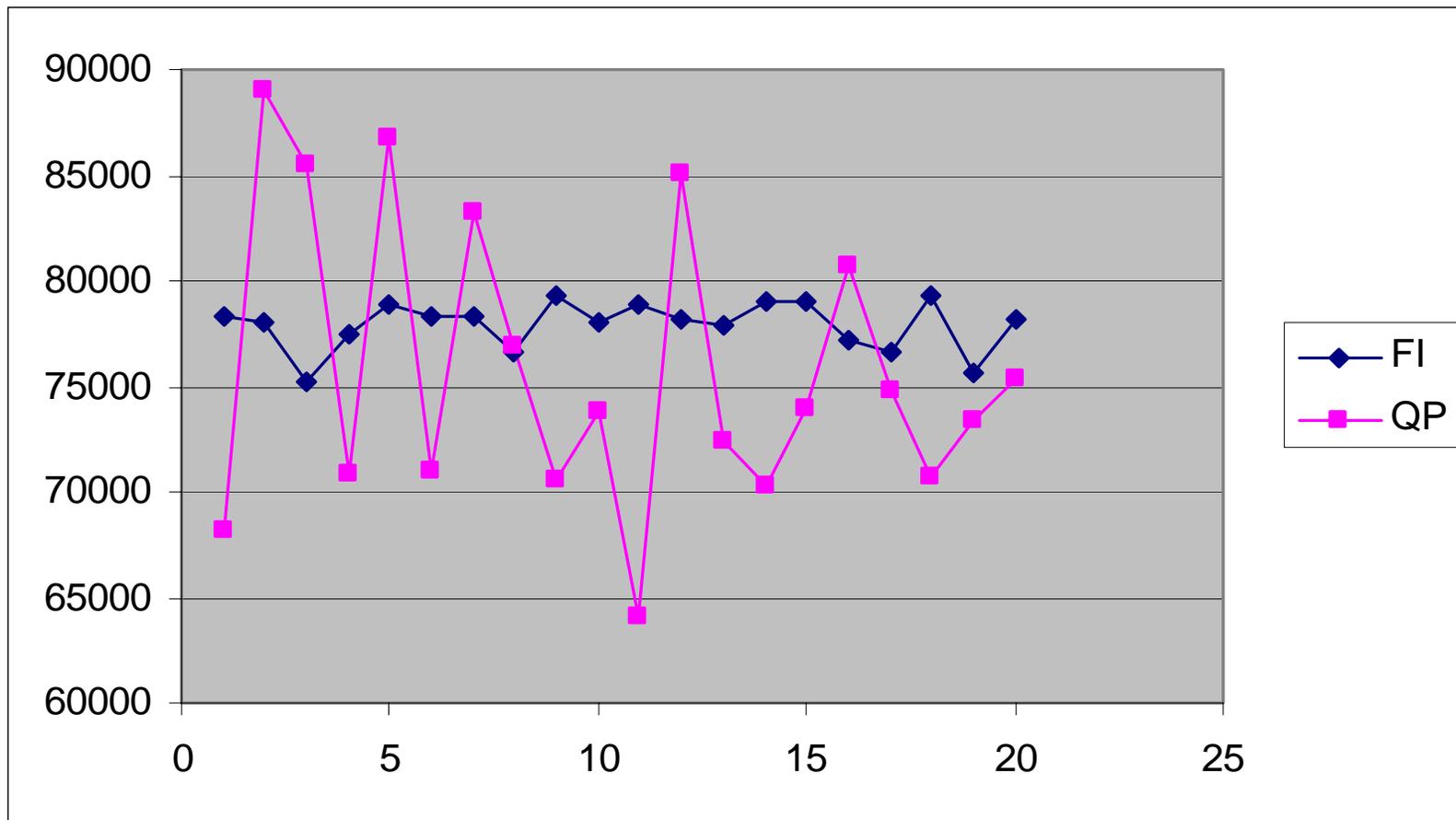
Calculated DPM



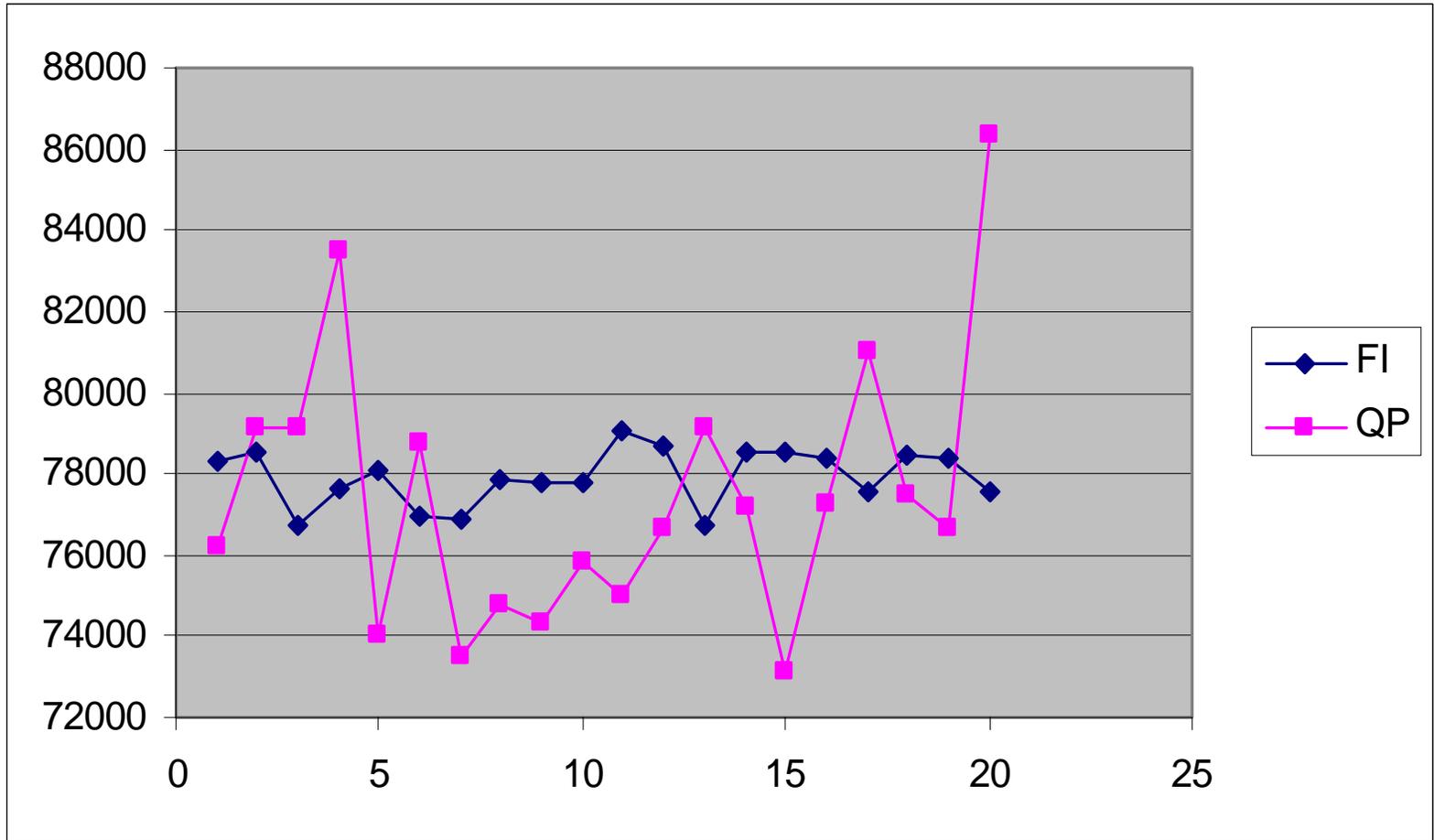
0.125 mg/ml tartrazine



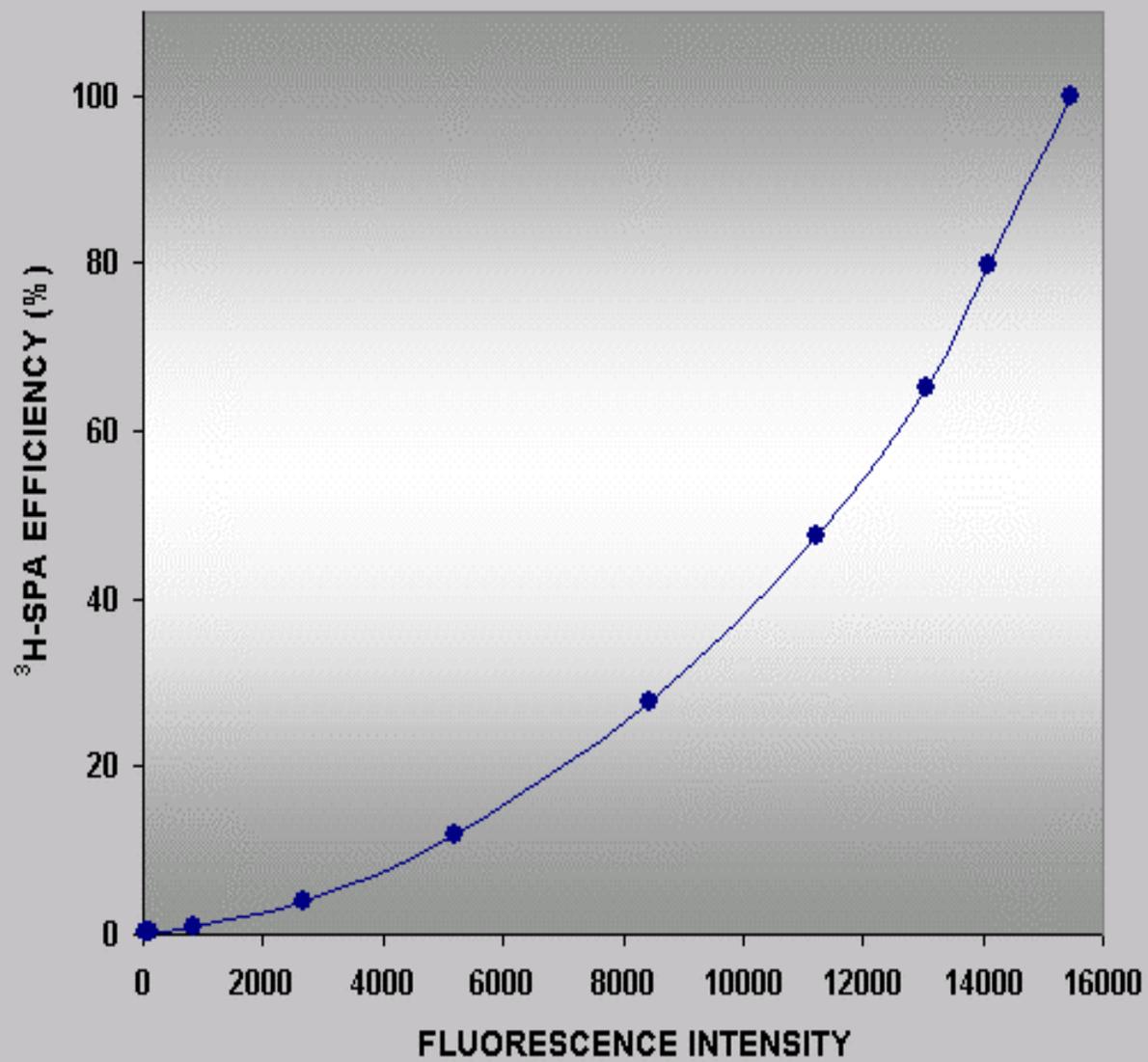
0.063 mg/ml tartrazine



0.031 mg/ml tartrazine



^3H -SPA WITH FLUORESCENCE CORRECTION



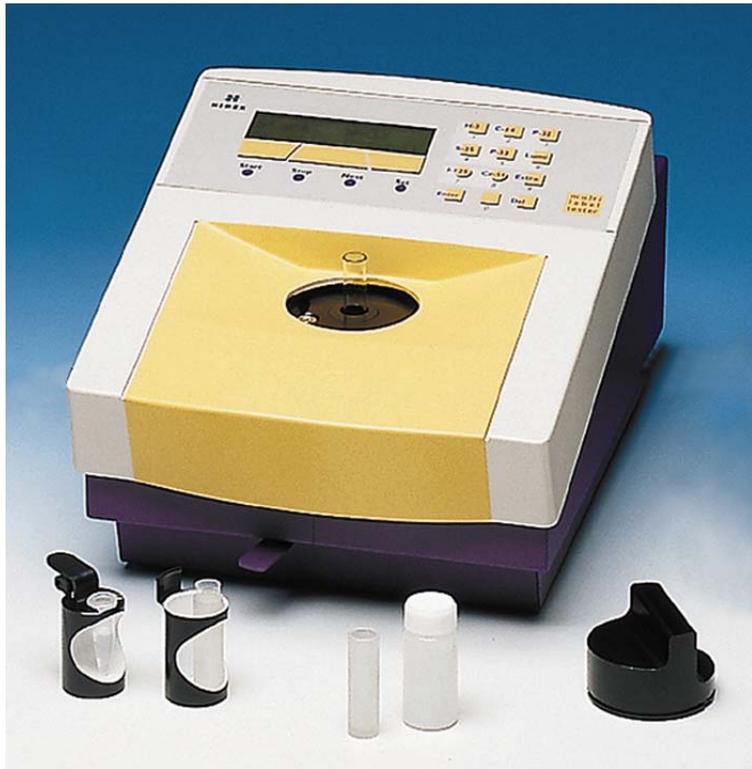
TRIATHLER

LIQUID SCINTILLATION COUNTER

GAMMA COUNTER

LUMINOMETER

Portable



- 9 Kg.
- Field Operation (12v power supply)
- Dual MCA
- Beta (2000 KeV), Gamma (1000 KeV).
- Alpha-Beta Separation

Triathler Flexibility



- Different Vial Types.
- 3 Technologies.
- Possibility for External Detector.
- Stand-alone or PC control.

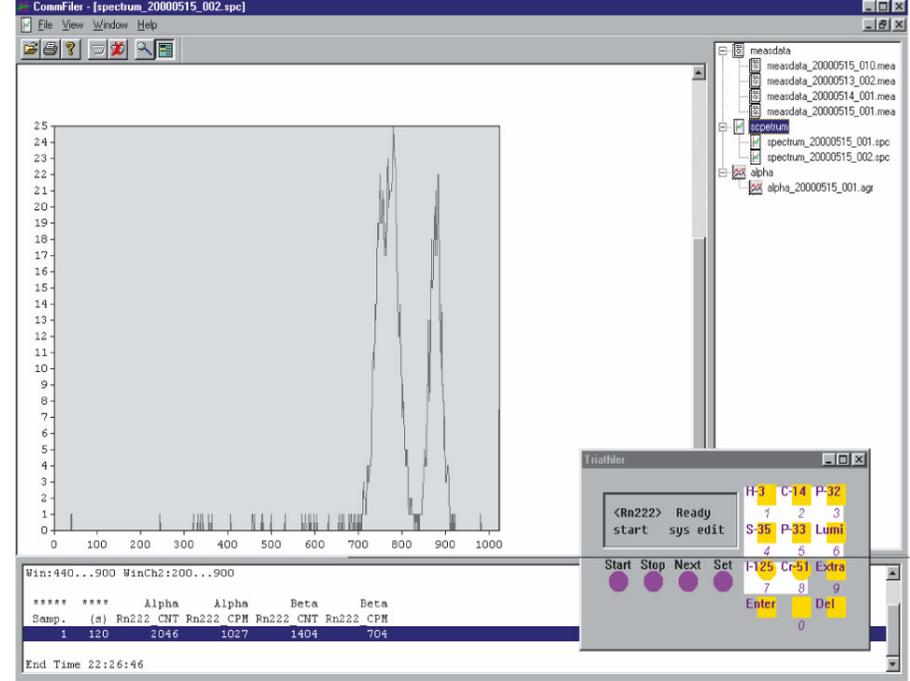
Triathler Applications.

- Wipe tests for isotope contamination



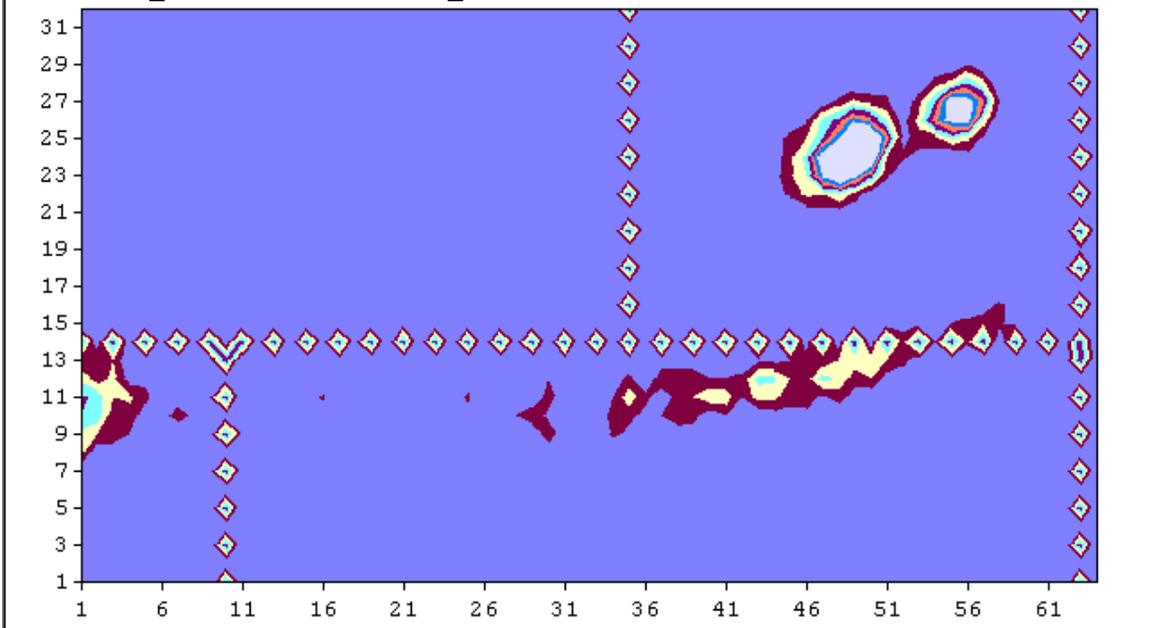
TRIATHLER

- INSTANT DPM.
- DUAL MCA.
- ADVANCED SPECTRUM ANALYSIS.
- CPM/DPM COUNTING.
- DUAL- LABEL COUNTING.
- ALPHA- BETA SEPARATION.
- DIRECT MEASUREMENT OF P-32.





Alpha-Beta separation



- measdata
 - scpetrum
 - spectrum_1003
 - spectrum_3003
 - spectrum_3003
 - spectrum_3003
 - spectrum_3003
 - alpha
 - alpha_130320C
 - alpha_130320C



TRIATHLER APPLICATIONS

Wipe tests

Tritium wipe test

	Wad	Glass Fiber	Paper
^3H eff. %	7 %	20 %	5 %
BKG (CPM)	50	50	50

The swab material can dramatically affect counting efficiency. Glass Fiber gives clearly the best efficiency.

Wipe tests

Alpha wipe test

	Paper	Mixed ester	Glass fiber
Alpha eff. %	75	85	85
Bgn 47 mm (CPM)	0.2	< 0.1*	0.9
Bgn 25 mm (CPM)	0.2	< 0.1*	0.25

*) no Rn-daughters present

TRIATHLER LSC Counter

- Isotopes up to 2000 keV.
- Efficiency 45% H-3 BG 100 CPM.
- 50%Reduction in BG with internal lead-shield.
- Different types and sizes of vials.
- Excel spreadsheet interface software.

TRIATHLER

Gamma Counter

- Isotopes up to 1000 keV.
- MultiLabel counting.
- Spill-over correction.
- Excel spreadsheet software.
- > 70% Efficiency I-125.
- External detector for high-energy isotopes.

TRIATHLER APPLICATIONS

- Radon-222/ Radium-226 in water/air.
- U-234/U-238 in water.
- P-32 as tracer in ground-water.
- Alpha-Beta separation.

SUMMARY TRIATHLER

- Portable.
- 4-Technologies in 1 instrument.
- Alpha-Beta separation.
- Easy-to-use.
- No risk for mechanical failures.