TopCount *Topics*

TCA-013

Crosstalk

Abstract

Sample-to-sample crosstalk is an optical and physical phenomenon observed in single and multiple detector radioisotope counting systems. Crosstalk can lead to counting artifacts which reduce the accuracy of the sample measurement. The TopCount Microplate Scintillation and Luminescence Counter has been designed to eliminate or minimize these artifacts. Experimental results are presented which demonstrate the success of the TopCount design in avoiding the experimental artifacts caused by crosstalk.

Introduction

Analysis of samples labeled with beta emitters is traditionally done using liquid scintillation counters (LSC's). The decay energy of the nuclide is converted into photons in the scintillation medium, which are then counted by highly sensitive photomultiplier tubes (PMT's). Traditional LSC's are constructed so that the sample being counted is optically isolated from other samples contained in the instrument; thus, light produced in a neighboring sample cannot interfere with the measurement. This basic design is also utilized in the construction of luminometers used for assay of luminescent labels. Isotopes such as ^{125}I and ^{32}P emit penetrating radiation (gamma rays or high energy beta particles), which can travel between samples and excite scintillations, thereby causing counting interferences. The traditional approach taken to reduce this excitation involves shielding individual samples from one another with highly absorbent materials. Elaborate algorithms are sometimes used to correct for excitation by neighboring samples, but are error-prone. The interferences caused by insufficient isolation (optical) or shielding (physical) are collectively known as crosstalk.

The increased use of the microplate format has resulted in the placement of up to 96 radiolabeled or luminescent samples in very close proximity to each other. The advent of microplate scintillation counters such as the TopCount has made it possible to measure simultaneously up to 12 labeled samples directly in the microplate. TopCount was designed with strict specifications to prevent or minimize artifacts caused by crosstalk. Reflective optics ensures that each of the 96 samples in a microplate is optically isolated from all neighboring wells. Furthermore, the use of dense, radiation absorbing material in the instrument and in the microplates themselves significantly reduces the penetration of radiation from a labeled well to its neighbors.

This paper describes the design of TopCount in detail. Experimental results are presented which demonstrate the excellent performance of this design in preventing crosstalk in both isotopic and non-isotopic microplate assays.

Design

Traditional LSC's use transmission optics, whereby the sample is placed in a transparent or translucent counting vial. Photons produced in the sample travel through the vial wall and are reflected by a highly efficient reflector into a pair of PMT's. The TopCount employs single-PMT time-resolved scintillation counting.[1] This design makes it possible to view each sample in a microplate with only one PMT placed directly above the microplate well. The microplate itself is constructed of rigid plastic containing white pigments which make the wells highly opaque and reflective. In this design, illustrated in Figure 1, the microplate well acts as both the sample vial and the reflector so that all photons pro-

Packard Instrument Company 800 Research Parkway, Meriden, CT 06450 Tel. 1-800-323-1891 203-238-2351 TX: 643251 FAX: 203-235-1347

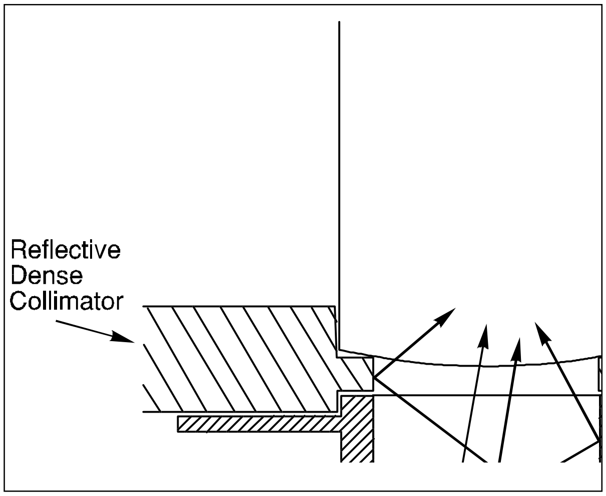

Figure 1.
Illustration of reflective optics used in the TopCount Microplate Scintillation Counter. Not to scale.

duced in the well are reflected into the single PMT. To provide accurate positioning and total optical isolation, a reflective collimator is placed between the well and the PMT. In addition to reflecting photons, this collimator protects the PMT from contamination and/or damage. These design parameters allow a very efficient and accurate collection of photons produced in the microplate well, and eliminate optical crosstalk.

Another consequence of this design is the radiation absorption properties of the system. While traditional counters make use of heavy lead shielding, this is not practical for microplate-based systems. The many microplates recommended for use in TopCount are thick-walled rigid plates containing colorants which produce an opaque white or black finish. These pigments, in conjunction with wall thickness, help attenuate the transmission of radioactive decay energy between neighboring wells. This, together with the solid metal collimator, permits a significant reduction in the crosstalk due to penetrating radiation.

Experimental

Several types of microplates (Table 1) are commercially available in white or black for use in TopCount. Crosstalk was evaluated for a selection of these plates under realistic assay conditions using several radionuclides (^{3}H, ^{125}I, ^{14}C, ^{32}P) and a luminescent signal. Stock solutions of the four radionuclides were used to make two dilutions at the following approximate specific activities:

Dilution A: 100000 DPM/10 µL

Dilution B: 1000 DPM/10 µL

10 µL aliquots of the dilutions were spotted into microplate wells in the pattern shown in Figure 2 and scintillation cocktail was added to all wells. For the luminescent experiment, dilutions of xanthine oxidase, simulating low and high signal levels, were pipetted into microplate wells with the same pattern, and a luminol and iron-EDTA based signal reagent was added to

Microplate	Supplier	Color	Applications
PicoPlate	Packard	White	Liquid Scintillation
OptiPlate	Packard	White	Liquid Scintillation, CL
CulturPlate	Packard	White	Cell Culture-LS, CL
ViewPlate	Packard	White or Black	Cell Culture-LS, CL
LumaPlate	Packard	White	Solid Scintillation
UniFilter	Packard	White	Filter Counting
MultiScreen	Millipore	White	Filter Counting
Microlite	Dynatech	White	Cell Culture-LS, CL
LitePlate	Packard	Black	CL Assays
MicroFLUOR	Dynatech	Black	CL Assays

Table 1.
Partial list of TopCount microplates; CL is an abbreviation for chemiluminescent.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B							B		B		B	
C			A					A	A	A		
D							B	A	B	A	B	
E								A	A	A		
F			B				B		B		B	
G												
H												

Figure 2.
Microplate sample pattern for crosstalk experiments.

all wells.[2]

Each plate was counted under typical conditions on the TopCount Microplate Scintillation Counter after sealing with TopSeal film. Preset counting windows for each nuclide, stored in the instrument prior to factory shipment, were used in all experiments. Parallel aliquots of each radioactive solution were prepared in vials and assayed for DPM on a conventional LSC.

The pattern of high and low activity samples illustrated in Figure 2 represents assay conditions where the maximum signal is approximately 100 times the minimum, a dynamic range typical of real assays. The pattern on the left side of the plate allows us to evaluate the count rates of the samples without any potential for crosstalk from surrounding wells, and simulates a situation of a single high activity sample surrounded by low activity samples. The crosstalk from the high activity sample in well C3 into surrounding eight wells with background correction, is referred to as individual well crosstalk (Table 2).

The pattern on the right side of the plate in Figure 2 simulates a low activity sample surrounded by high activity samples. The crosstalk from the eight neighboring wells into the central low activity well, is referred to as cumulative crosstalk (Table 2).

Besides providing objective measures of crosstalk (Table 2), these patterns simulate situations in screening assays in which relatively

	Individual well (a)	Cumulative (b)
H-3 in PicoPlate	not detectable	not detectable
C-14 in MicroLite plate	not detectable	not detectable
I-125 in UniFilter plate	0.11%	1.28%
P-32 in LumaPlate	0.22%	1.88%
Chemiluminescence	0.007%	not detectable

a. $$\left(\frac{\text{Avg CPM of 8 wells around well C3 - Avg CPM of the surrounding 16 wells}}{\text{CPM of the central active well C3}}\right) \text{X100}$$

b. $$\left(\frac{\text{CPM of well D9 - CPM of well F3}}{\text{Average CPM of the 8 wells neighboring well D9}}\right) \text{X100}$$

Table 2.
Summary of crosstalk with TopCount.

	1	2	3	4	5	6	7	8	9	10	11	12
A	18	14	11	9	10	13	11	13	23	21	13	15
B	16	15	9	10	21	13	350	9	430	20	390	11
C	14	15	41058	11	13	16	20	40303	41125	41289	15	14
D	18	16	14	10	17	15	415	40063	388	42328	395	21
E	13	11	10	10	16	18	13	40805	41803	40427	11	15
F	15	12	457	10	14	8	414	11	407	15	375	12
G	12	16	10	10	19	14	17	12	21	18	17	17
H	10	19	12	9	17	11	9	12	18	20	7	15

Figure 3.
^{3}H crosstalk on PicoPlate, CPM.

rare positive samples are surrounded by negative samples.

Results and Discussion

A check for ^{3}H crosstalk in the PicoPlate is shown in Figure 3. The count rates (CPM's) of the individual samples without any crosstalk contribution from neighboring wells can be seen in wells C3 and F3. The same count rates are observed on the right side of the microplate, where high and low activity samples are interspersed. No crosstalk between wells of any kind is observed. Counting efficiencies as determined by DPM determination on conventional LSC averaged 33%.

^{14}C crosstalk was evaluated using the Microlite plate. The results for this experiment are shown in Figure 4. Again, there are no crosstalk errors in measurement of the low activity samples, even with neighboring samples containing 100x more activity. Counting efficiency was 94%.

Because many filter assays involve the use of ^{125}I labels, the UniFilter plate[3] was used to evaluate crosstalk for this nuclide (Figure 5). The UniFilter plate is an integrated filtration plate designed to prevent migration of the label from well-to-well. Here, a small amount of crosstalk is observed, due to the penetrating nature of gamma emissions. The individual well crosstalk to surrounding wells was about one part in 1000 (0.11%), and the cumulative crosstalk of eight surrounding wells into the central well was

	1	2	3	4	5	6	7	8	9	10	11	12
A	19	16	16	12	19	16	15	12	24	17	16	11
B	14	17	11	11	24	16	981	17	1030	40	1194	12
C	22	14	95188	6	12	18	14	105311	105931	106707	26	16
D	14	13	11	7	19	15	911	97102	1001	100640	1654	13
E	15	11	8	8	14	15	146	100331	105310	100979	18	14
F	16	16	1007	9	15	16	1302	11	1004	19	1050	14
G	19	17	8	13	24	16	13	11	22	22	20	12
H	16	12	11	11	40	19	18	12	15	17	11	13

Figure 4.
^{14}C crosstalk on Microlite microplate, CPM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	13	26	21	17	29	22	28	43	55	50	33	25
B	20	39	129	15	39	40	425	164	610	184	447	39
C	13	72	40487	112	29	42	139	37856	40313	41161	134	38
D	29	31	132	18	39	45	546	38746	890	39659	556	48
E	15	19	11	9	35	39	137	39206	40217	40694	114	42
F	9	19	382	8	22	39	381	152	585	130	401	36
G	17	19	25	21	19	24	35	44	55	44	29	35
H	15	18	15	22	18	16	17	22	23	28	21	24

Figure 5.
^{125}I crosstalk on UniFilter plate, CPM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3	6	10	7	9	7	7	51	30	27	11	6
B	7	92	359	110	9	18	845	462	1505	498	887	17
C	5	347	96062	351	10	12	471	96407	98898	100234	430	12
D	5	79	318	100	12	29	1399	98988	2551	93023	1417	17
E	5	6	13	12	8	19	478	98016	98789	98813	447	10
F	3	9	703	9	10	18	827	433	1218	503	818	17
G	4	6	9	6	5	11	18	29	33	42	18	11
H	2	6	4	5	4	7	7	8	7	7	5	7

Figure 6.
^{32}P crosstalk on LumaPlate, CPM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	213	213	245	225	206	148	113	153	173	120	207	60
B	173	270	832	213	161	173	31855	1847	54398	1747	46753	233
C	173	599	2824662	387	206	219	1440	3536639	3338967	3620460	633	93
D	161	232	483	283	199	199	59346	3412323	55867	3632547	43788	100
E	296	238	219	245	199	213	1500	3445719	3708672	3505933	567	93
F	303	206	59883	206	277	264	59553	713	48435	533	42460	120
G	528	187	219	251	1373	283	173	200	193	207	160	113
H	180	283	219	238	232	238	153	153	173	1013	507	147

Figure 7.
Chemiluminescence crosstalk on black MicroFLUOR microplate, CPS.

1.3%. This is significantly less than other sources of experimental error such as pipetting and counting statistics. Counting efficiencies in this experiment averaged 54%.

Next, a LumaPlate solid scintillator microplate[4] was used to evaluate ^{32}P crosstalk. Here again, (Figure 8), a small amount of crosstalk is observed due to the extremely high energy (1.7 meV) of the beta particle. Individual well crosstalk was 0.22% and cumulative crosstalk was 1.9%. This error is small when one considers the many other sources of experimental error involved in microplate assays. Counting efficiencies averaged 80%.

Finally, a black MicroFLUOR plate was used in the evaluation of crosstalk for chemiluminescence applications (Figure 6). Because of extremely high photon flux, a few photons are seen in neighboring wells. However, the individual crosstalk was only 0.007%, and the cumulative crosstalk was not detectable due to pipetting error. Thus the assay dynamic range can be 10^4 or more. These results demonstrate that TopCount is capable of measuring luminescent samples with minimal crosstalk interference.

Conclusions

The TopCount Microplate Scintillation and Luminescence Counter can be used to accurately assay radio-labeled and luminescent samples in various microplates with virtually no sample-to-sample crosstalk. The results of this investigation are summarized in Table 1. Crosstalk for liquid and solid scintillation counting in TopCount has been shown previously [4,5] to be insignificant for low energy radionuclides and less than 1% for high energy radionuclides. The use of single-PMT, time-resolved scintillation counting and reflective optics prevents optical crosstalk, whereas the design of the instrument and microplates minimizes physical crosstalk of penetrating radiation. This approach is preferred over complex and error-prone crosstalk reduction algorithms, which are limited by poor sample counting statistics, mathematical errors, variability due to varying experimental conditions, and labor-intensive calibration procedures.

References

1. TopCount Topics #3, Theory of TopCount Operation, Packard Instrument Company.

2. Baret, A., Fert, V., and Aumaille, J., (1990), Application of long-term enhanced xanthine oxidase-induced luminescence in solid-phase immunoassays. Anal. Biochem. 187: 20-26.

3. TopCount Topics #10, Receptor Binding Assays, Packard Instrument Company.

4. TopCount Topics # 2, Solid Scintillation Counting, Packard Instrument Company.

5. TopCount Topics #5, Counting Aqueous samples, Packard Instrument Company.