

CHAPTER 20

Multilabel Counting Using Digital Overlay Technique

Heikki Kouru and Kenneth Rundt

ABSTRACT

The development of electronics has brought multichannel analyzers (MCA's) into liquid scintillation counting. However, the data analysis methods are not on the same level. The currently used window-based calculation methods cannot use all the information measured from the sample. In this presentation a new method called digital overlay technique (DOT) is introduced. This method efficiently uses the information available from the MCA.

In DOT, the shape of the sample spectrum is used for resolving multicomponent samples, by fitting the spectrum of each component to the measured composite spectrum. Furthermore, the fitting of a reference spectrum to the measured spectrum enables the monitoring of sample quality.

In this report the basic principles of DOT are illustrated. Measurements made by a liquid scintillation counter, the Wallac 1410, which uses this principle, are presented. Comparisons are made with a conventional window-based method. The results show that DOT gives the best precision irrespective of the activity ratio of the isotopes.

INTRODUCTION

Dual label liquid scintillation counting is currently a relatively common discipline for which almost all commercial instruments provide some kind of solution. Traditionally, liquid scintillation counting of dual-label samples is performed in two counting windows. The windows may either be fixed, moving,¹ or automatic. Fixed window counting is a fairly uncomplicated procedure accurate enough for samples of moderate quench and activity ratios. If the quench level of the samples within one batch varies immensely, fixed windows have to be exchanged for moving or automatic windows. In the method with moving windows the windows are first set by the user for an unquenched sample. The counter thereafter automatically moves the window limits for each sample prior to counting, according to the quench level of the sample. In the method with automatic windows, there are no counting windows during counting, but only a multichannel analyzer (MCA). The user need not specify

any window limits, only isotopes. After counting, the windows are selected from a table of window limits as a function of an external standard quench index for different isotope combinations. Moving windows and automatic windows have the advantage of providing quite robust methods for a wide quench range. However, window methods are not particularly suitable for counting multilabel samples containing more than two isotopes, as the windows have to be defined either by the user (moving windows) or by the manufacturer (automatic windows) through a tedious trial-and-error procedure. Furthermore, separate quench curves for different labeling situations are needed as the windows may be set differently. This means that ordinary single label quench curves just cannot be combined for multilabel counting when using moving or automatic windows.

During the last few years, three new methods for dual label counting have been introduced in commercial counters: Full Spectrum DPM by Packard,² Three-Over-Two introduced by Wallac Oy in the RackBeta LS counters in 1987, and Digital Overlay Technique introduced in 1988 by Wallac Oy in the new LS counter 1410. Three-Over-Two counts dual labeled samples with three fixed windows. The count rate of each isotope is determined by solving a set of three equations in two unknowns by using, e.g., the method of least squares. For dual label counting the number of windows with this method is three, or one more than the number of isotopes. This method can, in analogy, be extended to multilabel counting of n isotopes as well by always having the number of windows greater than the number of isotopes n . The number of windows may naturally be much higher than $n + 1$. The ultimate solution is when the number of windows approaches the resolution of the MCA; this is then equal to Digital Overlay Technique (DOT).

DOT is based on a methodology similar to spectral analysis in other branches of spectroscopy and analytical sciences. Oller and Plato³ were the first to mention spectrum analysis in connection with liquid scintillation counting. In their original paper they did not take quench into consideration though, nor did they describe how the composite spectrum was resolved mathematically. DOT makes use of a multichannel analyzer having 1024 channels.

Mathematically, DOT can be described as follows. Let S_1 and S_2 denote the normalized spectra of isotopes 1 and 2 respectively, and $s_{i,j}$ the normalized count rate of isotope i ($i = 1$ or 2) in channel j . A normalized spectrum is a spectrum for which the total count rate is equal to 1 cpm. For a dual-label test sample, let U denote the predicted spectrum and u_j the predicted count rate in channel j . Finally, let c_1 and c_2 denote the unknown, *true* count rates of the two isotopes in the sample. Then the following linear equation is valid for each channel:

$$u_j = c_1 s_{1,j} + c_2 s_{2,j} \quad j = 1 \dots m \quad (1)$$

or, for n isotopes

$$u_j = \sum_i^n c_i \cdot S_{i,j} \quad j = 1 \dots m \quad (2)$$

The number of equations that describe the whole spectrum of m channels is equal to m. For the general case of n isotopes it is most convenient to introduce matrix notation. Let S denote an n by m matrix comprising the n spectra S₁ to S_n and let C denote a vector comprising the n count rates c₁ to c_n. For the spectrum U the equation

$$U = S \cdot C \quad (3)$$

is then valid. Let Y denote the measured composite spectrum and, y_j the measured count rate in channel j. In the least squares solution the count rates c_i* are found by minimizing the squared difference between the measured and the predicted spectrum:

$$\chi^2 = \sum_{j=1}^m (y_j - \sum_{i=1}^n c_i \cdot s_{i,j})^2 \quad (4)$$

which is the least squares solution without weights. In matrix notation, the solution is

$$C = (S^T \cdot S)^{-1} \cdot S^T \cdot Y \quad (5)$$

The count rates c_i can thereafter be converted to activities a_i (or dpm_i) by using the familiar relation

$$a_i = c_i / e_i \quad (6)$$

where e_i is equal to the counting efficiency of isotope i, which must separately be determined from a quench curve. Although Equations 4 and 5 would be accurate enough in favorable situations, they should not be used when the count rates are low and the spectra overlap considerably. The next step is to introduce weighted least squares fit. Let W denote a weight vector comprising the, so far unknown, weight values. Equation 4 then becomes

$$\chi^2 = \sum_{j=1}^m w_j \cdot (y_j - \sum_{i=1}^n c_i \cdot s_{i,j})^2 \quad (7)$$

and the matrix solution becomes

$$C = (S^T \cdot W^{-1} \cdot S)^{-1} \cdot S^T \cdot W^{-1} \cdot Y \quad (8)$$

W now denotes a weight matrix in which all elements are equal to zero except the diagonal ones, which are equal to the weight values in vector W . The weight values can be chosen in many ways, but the optimal values can only be found through an iterative procedure. A better procedure in this case is to make full use of the maximum likelihood technique,^{4,5,6} of which least squares fit is a subclass. The maximum likelihood technique gives the minimum variance solution. The principle of MLT applied to LS counting is shortly as follows: radioactive decay is governed by Poisson statistics and the counts y_j in channel j are Poisson distributed (y_j is the mean value). The probability p_j for detecting k_j counts in channel j is:

$$p_j = (y_j)^{k_j} \cdot e^{-y_j} / k_j! \quad (9)$$

For a spectrum S the joint probability l is the product of p_j for all m channels:

$$l = \prod_{j=1}^m p_j$$

Combining Equations 2 and 10 leads to the conclusion that the probability l is a function of the component spectra S_i and the count rates c_i , or $l = l(S_i, c_i)$. In DOT, the shape of the spectra S_i are fixed, but the count rates are iteratively determined so that l achieves a maximum value. The spectra S_i are retrieved from the spectrum library as soon as the quench level of the sample has been determined with the external standard. In the LS counter 1410 there is a spectrum library comprising spectra that cover a large quench region of both chemical and color quench for six common isotopes. This library can be used as such or extended with the users own calibrations or "fine-tunings."⁷ The influence of color on counting efficiencies and spectrum shape, and the Wallac color quench monitor, has been dealt with elsewhere by the present authors.⁸

EXPERIMENTAL

This report is an account of a comparison between the traditional two fixed window method and DOT, under similar conditions and in the same instrument. Organic tritium and ¹⁴C were used in this study. Two calibration standards with the pure isotopes were prepared for fine tuning the Wallac library. Unknown samples were prepared with the activities shown in Table 1. The scintillation liquid was OptiScint HiSafe, a high flashpoint cocktail. The samples were quenched with carbon tetrachloride so that the counting efficiency of tritium was around 23% and the counting efficiency of ¹⁴C around 86%.

The samples were measured repeatedly 120 times and the counting time was one minute. All measurements were made on a typical 1410 LS counter. As this instrument is not equipped with any traditional window methods, the cpm

Table 1. Activity of the Two Isotopes of the Samples used in this Work. "dpm_{exc}" is the Activity of the Isotope in Excess and "dpm_{less}" is the Activity of the Other Isotope. "Ratio" is the Ratio Between These Two

dpm _{less}	dpm _{exc}	Ratio
95000	1500	64/1
95000	3000	32/1
95000	6000	16/1
95000	12000	8/1
95000	23800	4/1
95000	47500	2/1
95000	95000	1/1

values in five different windows for each isotope were printed out and converted to dpm off-line.

RESULTS AND DISCUSSION

The aim of this test was to find experimental evidence for our theoretical assumption that DOT is at least as good as or even better than any window method. For this reason, the standard deviation of the 120 dpm values was computed. The results are presented in Figures 1 to 4 as relative standard deviation (rDev) as a function of the activity ratio. rDev is defined according to the equation:

$$rDev = Dev_w / Dev_{DOT}$$

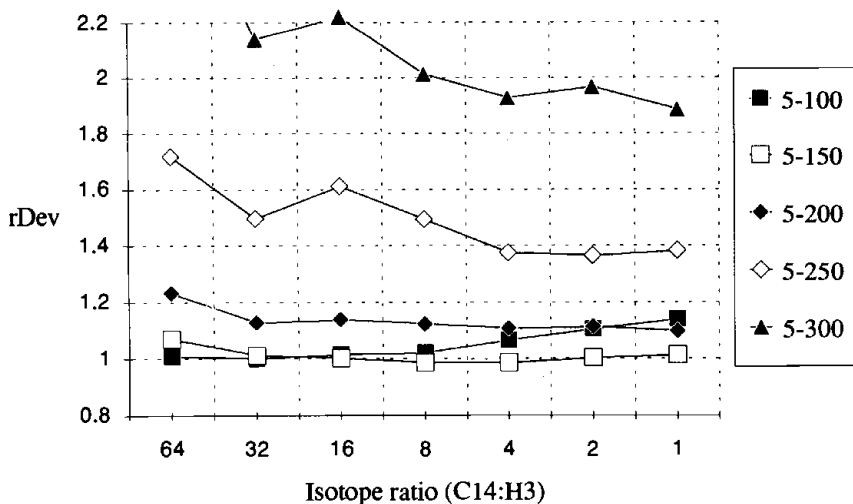


Figure 1. The relative standard deviation of dpm for ¹⁴C as a function of the activity ratio for a number of low window limits. rDev is defined in the text.

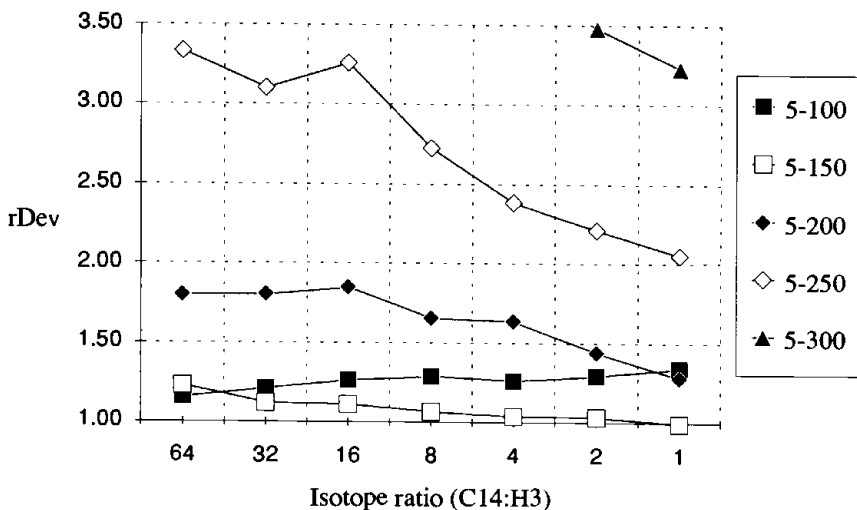


Figure 2. The relative standard deviation of dpm for tritium as a function of the activity ratio for a number of low window limits. rDev is defined in the text.

wherein Dev_w and Dev_{DOT} are equal to the standard deviations of the 120 dpm values for the two isotopes computed by using a windows method and DOT, respectively. In Figure 1, ^{14}C is in excess, and rDev is computed for ^{14}C while in Figure 2, ^{14}C is also in excess, but rDev is now computed for tritium. Accordingly, in Figures 3 and 4, tritium is in excess and rDev is computed for ^{14}C and tritium, respectively. These figures also mentioned the window limits of the

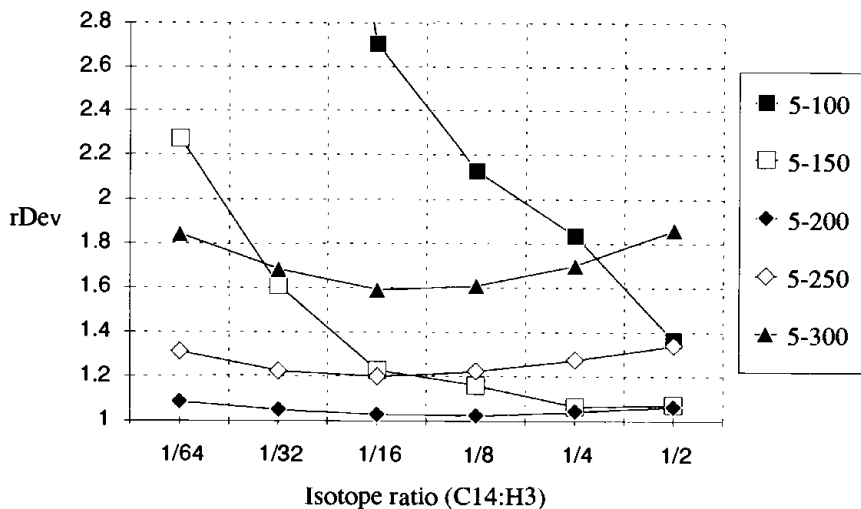


Figure 3. The relative standard deviation of dpm for ^{14}C as a function of the activity ratio for a number of low window limits. rDev is defined in the text.

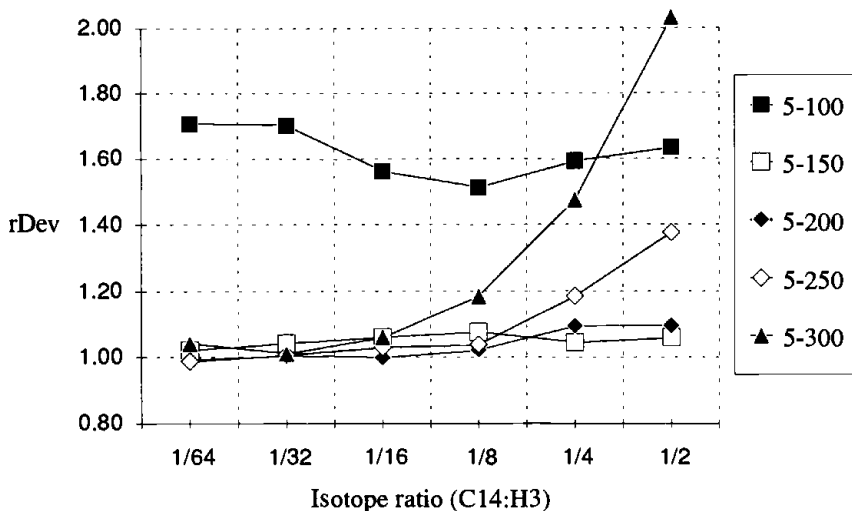


Figure 4. The relative standard deviation of dpm for tritium as a function of the activity ratio for a number of low window limits. rDev is defined in the text.

lower (A) of the two windows A and B. Window B extends from the upper limit of A up to channel 650.

The data in Figures 1, 2, and 4 states that, at the quench level assessed here, the narrow A window 5-150 gives a standard deviation almost as low standard deviation as DOT. However, Figure 3 shows that the window 5-150 is not the best when tritium is in excess. The data in these figures disclose one of the limitations of the traditional two-window method: there is no single window setting that gives as low standard deviations as DOT for a wide range of activity ratios.

One important conclusion that can be drawn from these figures is that selecting the correct limits for window A is very important when using two windows. If using an automatic method like moving windows, the unquenched windows must be set correctly and the user can do no more than hope that the program always chooses the best windows settings at different quench levels. With DOT, the user has no such worries, as the method always gives lower or equally low deviations as any window method.

SUMMARY

The advantages of DOT are the following:

1. Statistically, DOT is always better than or equally good as any combination of moving or automatic windows.
2. The calibrations are of general characters and may be used in any connection, single label or multilabel.

3. Counting is optimized for all conditions, such as varying quench levels or activity ratios.
4. The method is similar in the general multilabel case as in the special case of dual label.
5. The reference spectra are also used in a quality control manner to ensure that the unknown sample does indeed contain the assumed radioisotopes; they are also used to determine, in a qualitative manner, what radioisotopes are present in completely unknown samples.

REFERENCES

1. Nather, R.E., U.S. Pat. No. 4,029,401 (1977).
2. van Cauter, G.C., L.J. Everett, and S.J. DeFilippis, European Patent Appl. No. 0 202 185 (1986).
3. Oller, W.L. and P. Plato, "Beta Spectrum Analysis: A new Method to Analyze Mixtures of Beta-Emitting Radionuclides by Liquid Scintillation Techniques," *Int. J. Appl. Rad. Isot.*, 23:481-485 (1972).
4. Orth, P.H.R., W.R. Falk, and G. Jones. "Use of the Maximum Likelihood Technique, for Fitting Counting Distributions," *Nucl. Inst. and Meth.*, 65:301-306 (1968).
5. Ciampi, M., L. Daddi, and V. D'Angelo. "Fitting of Gaussians to Peaks by a Maximum Probability Method," *Nucl. Inst. and Meth.*, 66:102-104 (1968).
6. Awaya, T. "A New Method for Curve Fitting to the Data with Low statistics Not Using the χ^2 -Method," *Nucl. Inst. and Meth.*, 165:317-323 (1979).
7. Kouru, H. "A New Quench Curve Fitting Procedure: Fine-Tuning of Spectrum Library," in *New Trends in Liquid Scintillation Counting and Organic Scintillators*, (1989).
8. Rundt, K., T. Oikari, and H. Kouru. "Quench Correction of Colored Samples in LSC," in *New Trends in Liquid Scintillation Counting and Organic Scintillators*, (1989).