Technical note DOC 513-012

HIDEX 300 SL™ TDCR LSC *Version 1.0*

DIGITAL LEAD SHIELD - improving assay sensitivity by spectral fitting

The method is deterministic double label counting method where one component (CPM of background) is known. Calibration of the assay is performed by measuring spectral distribution of active sample (A), spectral distribution of background (B) and total background CPM. Poisson-weighted least squares fitting is employed for unknown samples to find the most probable isotope CPM (A). If there is difference between spectral distribution of A and spectral distribution of B, the uncertainty (fluctuation) of A CPM improves. The improvement is similar to “normal” background subtraction in instrument with lower B CPM. As consequence, the assay sensitivity is improved.

Method

1. Calibration

   Method set-up:

   - Set several windows over whole isotope ROI, covering both triple and double spectrum.
   - Preset MikroWin parameter template includes 4 windows: 2 for triple spectrum and 2 for double spectrum.

   Calibration samples:

   - Prepare A sample (>1000 CPM) and B sample, compositionally similar to unknowns.
   - Count in the set windows, the software stores:

     Proportion (percentage) of A in each window = A spectral distribution

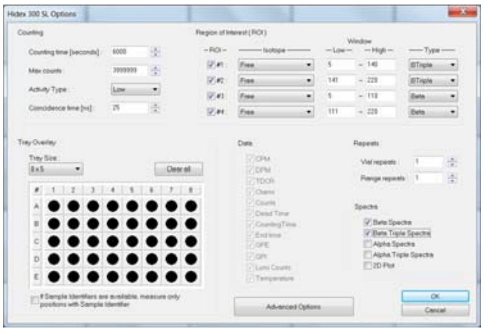
     Proportion (percentage) of B in each window = B spectral distribution

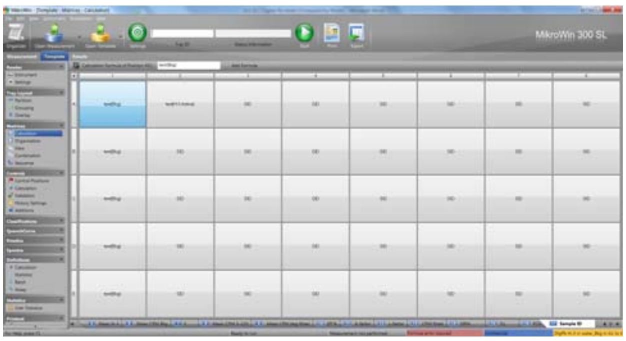
     Total B (CPM) in the whole ROI.

2. Unknowns

   - Count with the set windows.
   - Software calculates the most probable value of A CPM with Poisson-weighted least squares fitting, using stored A spectral distribution, B spectral distribution and B CPM.

Result calculation algorithms with suggested instrument settings for H-3 in water are delivered as pre-defined MikroWin parameter file templates.





3. Algorithms

Normalized relative proportions of A and B in each window:

$a_1 + a_2 + \cdots + a_n = 1$ and $b_1 + b_2 + \cdots + b_n = 1$

where a_i and b_i refer to proportions of A and B in window i, respectively.

Unknown samples (known a_i, b_i, B, unknown A)

For counts in each window (C_i) hold

$C_1 \approx a_1 A + b_1 B;\ C_2 \approx a_2 A + b_2 B \quad etc.$

Least squares fitting is adopted to find A. Weighted least squares sum is

$$Q = w_1[C_1 - (a_1 A + b_1 B)]^2 + w_2[C_2 - (a_2 A + b_2 B)]^2 + \cdots + w_n[C_n - (a_n A + b_n B)]^2$$

Established weights for Poisson distributed counts are

$w_i = 1/variance = 1/C_i$

To minimize least squares sum, differentiate Q with respect to A and equate to zero.

$$\frac{\partial Q}{\partial A} = -2w_1 a_1[C_1 - (a_1 A + b_1 B)] - 2w_2 a_2[C_2 - (a_2 A + b_2 B)] - \cdots - 2w_n a_n[C_n - (a_n A + b_n B)] = 0$$

Solving for A yields

$$\boxed{A = \frac{1 - LB}{K}}$$

where

$$K = \frac{1}{C_1}a_1^2 + \frac{1}{C_2}a_2^2 + \cdots + \frac{1}{C_n}a_n^2 = \sum \frac{1}{C_i}{a_i}^2$$

$$L = \frac{1}{C_1}a_1 b_1 + \frac{1}{C_2}a_2 b_2 + \cdots + \frac{1}{C_n}a_n b_n = \sum \frac{1}{C_i}a_i b_i$$

Uncertainty of activity, ΔA

As known from statistics, uncertainty (standard deviation) of A (ΔA) is:

$$\Delta A = \sqrt{\sum \left(\frac{\partial A}{\partial C_i}\right)^2 \Delta C_i^{\ 2}}$$

From differential calculus:

$$\frac{\partial A}{\partial C_i} = \frac{\partial A}{\partial K}\frac{dK}{dC_i} + \frac{\partial A}{\partial L}\frac{dL}{dC_i} = \frac{(1-LB)}{K^2}\frac{a_i^2}{C_i^2} + \frac{B}{K}\frac{a_i b_i}{C_i^2} = \frac{a_i}{K C_i^{\ 2}}\left[a_i\frac{1-BL}{K} + b_i B\right]$$

$$= \frac{a_i}{K C_i^{\ 2}}(a_i A + b_i B)$$

Inserting in above and noticing that with Poisson statistics, $\Delta C_i^{\ 2} = C_i$

$$\Delta A = \frac{1}{K}\sqrt{\sum \frac{a_i^{\ 2}(a_i A + b_i B)^2}{C_i^{\ 3}}}$$

Sensitivity analysis

For background level samples $A = 0$ and $C_i = b_i B$

$$\Delta A_{A=0} = \frac{B}{K}\sqrt{\sum \frac{(a_i b_i)^2}{C_i^{\ 3}}}$$

$$K_{A=0} = \frac{1}{B}\left(\frac{a_1^2}{b_1} + \frac{a_2^2}{b_2} + \cdots + \frac{a_n^2}{b_n}\right)$$

$$\Delta A_{A=0} = \sqrt{\frac{B}{\frac{a_1^2}{b_1} + \frac{a_2^2}{b_2} + \cdots + \frac{a_n^2}{b_n}}}$$

Direct background subtraction has uncertainty $\sqrt{B}$. From above, the uncertainty of fitted result behaves as if direct background subtraction with lower effective background B_{eff}:

$$\boxed{B_{eff} = FB} \qquad \boxed{F = \frac{1}{\frac{a_1^2}{b_1} + \frac{a_2^2}{b_2} + \cdots + \frac{a_n^2}{b_n}}}$$

$F < 1$, or $B_{eff} < B$. Only if every $a_i = b_i$ (isotope and background spectra identical), $F = 1$.

That is: ASSAY SENSITIVITY IS IMPROVED.

4. Example

For HIDEX 300 SL, normal model, utilizing both triple coincidences (triples) and pure double coincidences (pure doubles = doubles not participating in triples).
Isotope H-3. Sample: 8 mL water + 12 mL AquaLight cocktail, plastic vial.
Whole ROI 5 - 200, 4 windows. Window contents non-overlapping.

W1: triples in channels 5-140.
W2: triples in channels 141-200.
W3: pure doubles in channels 5-110.
W4: pure doubles in channels 111-200.

H-3 calibration sample result (CPM):
W1: 15082, W2: 10181, W3: 34812, W4: 13094, Total 73169 CPM (38 % efficiency).
a1 = 15082/73169 = 0.2061
a2 = 10181/73169 = 0.1391
a3 = 34812/73169 = 0.4758
a4 = 13094/73169 = 0.1790

Background sample result (CPM), 2 hours counting:
W1: 1.37, W2: 3.35, W3: 6.19, W4: 8.97, Total 19.88 CPM
b1 = 1.37/19.88 = 0.0692
b2 = 3.35/19.88 = 0.1685
b3 = 6.19/19.88 = 0.3114
b4 = 8.97/19.88 = 0.4510

Background reduction factor (F)

$$F = \frac{1}{\frac{0.2061^2}{0.0692} + \frac{0.1391^2}{0.1685} + \frac{0.4758^2}{0.3114} + \frac{0.1790^2}{0.4510}} = 0.655$$

and

B_{eff} = FB = 0.655 x 19.88 CPM = 13.0 CPM

Uncertainty of the unknown sample would behave as if background dropped from 19.9 CPM to 13.0 CPM on direct background subtraction.

5. Summary

- Improvement of assay sensitivity by spectral fitting
- Reduction about 30 % on full H-3 ROI
- With Hidex 300 SL super low level instrument:
    - o Bkg from 6 - 7 CPM down to 4 - 5 CPM in 35 % window
    - o FOM from 200 to above 300
- Delivery as MikroWin parameter-file template
  (templates are designed for constant quench conditions)

Table arranged data

Sample Id	CPM tot 5 - 220	Eff. %	F	CPM Bkg Fitted	CPM fitted	DPM	TU
Bkg (A1)	5,9		0,7097	4,18			
H-3 Active (A2)	208843	36,9			208874	565442	1,0E7
A03	6,0				0,1	0,3	6,1
A04	5,9				0,2	0,5	8,7
A05	6,6				0,8	2,1	37,5
A06	7,2				1,4	3,8	67,2
A07	8,5				2,8	7,5	132,0
A08	10,1				4,3	11,8	207,1
B01	5,8				-2,8E-3	-7,6E-3	-0,1
B02	316,0				310,0	839,2	14773,8
B03	7,1				1,4	3,7	65,7
B04	7,1				1,2	3,3	58,4
B05	6,0				0,2	0,6	9,7
B06	6,7				0,9	2,4	42,3
B07	6,3				0,5	1,4	25,3
B08	6,0				0,2	0,5	9,4
C01	5,9				6,3E-2	0,2	3,0
C02	314,5				308,3	834,6	14694,3
C03	6,3				0,6	1,5	27,0