Linearity of FISH

Carol's notes

Method one: addition and subtraction

Reading the Gamma 5000-14 manual and looking at the equations in the material added to the end (page 69), as well as the signal values in the workbook example, I infer:

The method 2.2 is used; the apertures are in the ratios

1:1:2:3:5:8:13:21:34:55:89:144:233:377:610: etc. The first two (smallest) apertures are identical, the third is the sum of the previous two, the fourth is the sum of the previous two, and so forth. As the series progresses the ratio of successive apertures approaches the factor 1.62. Apertures A and I in the Gamma instrument are in the 1:1 ratio. The aperture designations are in order of increasing flux in the worksheet. So what is 1:1:2:3:5:8:13:21:34:55:89:144 is the same as A:I:B:J:C:K:D:L:E:M:F:N etc.

A key to linearity method is that every measurement, with one aperture or a different aperture or both apertures, is subject to nonlinearity. But the method here assumes for the smallest aperture (1 or A), the system is linear. After that, an allowance is made for nonlinearity. The equations on page 69 make sense to me the way the notation is used. The notation on the worksheet is more confusing to me. Hence we get for the differences:

$$k_1 \equiv 0 \quad \left[= k_{\mathrm{A}}\right]$$
$$k_2 = N_{1'} + N_1 + 2k_1 - N_{1,1'} = N_{1'} + N_1 - N_{1+1'} \quad \left["1"+"1"-"2" = k_{IA}\right]$$

since $k_1 = 0$. Note the term is $2k_1$ since we have two independent measurements of the "1" aperture. Bootstrapping one aperture to the next this way, and realizing a difference term must be included to represent the nonlinearity for each measurement, we get

$$k_3 = N_1 + k_1 + N_2 + k_2 - N_{1+2} = \left(N_1 + N_2 - N_{1+2}\right) + k_2 \quad \left[\text{or}"1"+"2"-"3"+k_2 = k_{\mathrm{IB}}\right]$$
$$k_5 = N_2 + k_2 + N_3 + k_3 - N_{2+3} = \left(N_2 + N_3 - N_{2+3}\right) + k_2 + k_3 \quad \left[\text{or}"2"+"3"-"5"+k_2 + k_3 = k_{\mathrm{JB}}\right]$$
$$k_8 = N_3 + k_3 + N_5 + k_5 - N_{3+5} = \left(N_3 + N_5 - N_{3+5}\right) + k_3 + k_5 \quad \left[\text{or}"3"+"5"-"8"+k_3 + k_5 = k_{\mathrm{JC}}\right]$$

And so forth. The worksheet error is they use the uncorrected differences, the terms $N_{\mathrm{X}} + N_{\mathrm{Y}} - N_{\mathrm{X,Y}}$ and neglect the correction factor – they summed the two previous terms from the leftmost column and should have used the rightmost column labeled. The percent linearity at each summed signal is $100 * k_{\mathrm{X+Y}} / N_{\mathrm{X+Y}}$ and the percent linearity should be plotted as a function of summed signals, $N_{\mathrm{X+Y}}$ to demonstrate the results. Note this method limits the possible combinations, e.g. there are no measurements pairs outside the sequential order. Another concern is it appears the apertures are located off axis, which means the system must have excellent, beam uniformity, conservation of throughput (no vignetting) , along with excellent source stability.

After reading the other documents:

White, Clarkson, Saunders and Yoon (2008) and Saunders and Shumaker (1984) were the most helpful to me. Sanders (1972) reviews everything, but I do not understand where his correction factors come from (the multiplicative technique), or why it is equivalent to the method in the Gamma manual. I think we should not attempt that formulation of correction factors.

My guess: The FISH results at low signal appear nonlinear because the background is not known well enough.

It is seen that the Gamma method is a doubling method and thus is limited in coverage and spacing. However, we do appear to have enough equations to do a linear least squares – anybody up for this? (Howard says it is the way to go.) Look at Saunders and Shumaker's Eqn. (6) and the Gamma worksheet, we get:

$$\phi_{\mathrm{A}} = r_0 + s_{\mathrm{A}} + r_2 s_{\mathrm{A}}^2$$
$$\phi_{\mathrm{A}} + \phi_{\mathrm{I}} = r_0 + s_{\mathrm{IA}} + r_2 s_{\mathrm{IA}}^2$$
$$\phi_{\mathrm{I}} = r_0 + s_{\mathrm{I}} + r_2 s_{\mathrm{I}}^2$$
$$\phi_{\mathrm{I}} + \phi_{\mathrm{B}} = r_0 + s_{\mathrm{IB}} + r_2 s_{\mathrm{IB}}^2$$
$$\phi_{\mathrm{B}} = r_0 + s_B + r_2 s_{\mathrm{B}}^2$$
$$\vdots$$

Howard says since our unknowns are the fluxes and the polynomial coefficients, to write it this way:

$$s_{\mathrm{A}} = \phi_{\mathrm{A}} - r_0 - r_2 s_{\mathrm{A}}^2$$
$$s_{\mathrm{IA}} = \phi_{\mathrm{A}} + \phi_{\mathrm{I}} - r_0 - r_2 s_{\mathrm{IA}}^2$$
$$s_{\mathrm{I}} = \phi_{\mathrm{I}} - r_0 - r_2 s_{\mathrm{I}}^2$$
$$s_{\mathrm{IB}} = \phi_{\mathrm{I}} + \phi_{\mathrm{B}} - r_0 - r_2 s_{\mathrm{IB}}^2$$
$$s_B = \phi_{\mathrm{B}} - r_0 - r_2 s_{\mathrm{B}}^2$$
$$\vdots$$

And then we can make a nice 17x17 (15 unknown fluxes plus two polynomial coefficients) matrix equation $\overline{S} = \overline{U}\,\overline{\Phi}$ where $\overline{\Phi}$ is a 17x1 column vector:

$$\phi_{\mathrm{A}}$$
$$\phi_{\mathrm{I}}$$
$$\phi_{\mathrm{B}}$$
$$\vdots$$
$$\phi_{\mathrm{O}}$$
$$r_o$$
$$r_2$$

$\overline{S}$ is a 29x1 column vector:

$$s_{\mathrm{A}}$$
$$s_{\mathrm{IA}}$$
$$s_{\mathrm{I}}$$
$$\vdots$$
$$s_{\mathrm{O}}$$
$$s_{\mathrm{OH}}$$
$$s_{\mathrm{H}}$$

And $\overline{U}$ is a 29x17 matrix:

$$\begin{matrix} 1 & 0 & 0 & \cdots & 0 & -1 & -s_{\mathrm{A}}^2 \\ 1 & 1 & 0 & \cdots & 0 & -1 & -s_{\mathrm{IA}}^2 \\ 0 & 1 & 0 & \cdots & 0 & -1 & -s_{\mathrm{I}}^2 \\ & & & \vdots & & & \\ 0 & \ldots & 0 & 1 & 1 & -1 & -s_{\mathrm{OH}}^2 \\ 0 & 0 & \cdots & 0 & 1 & -1 & -s_{\mathrm{H}}^2 \end{matrix}$$

Solution for the fluxes and coefficients in $\overline{\Phi}$ is by non linear least squares; for the matrix solution according to the PDF file Howard sent: $\overline{\Phi} = \left[\left(\overline{U}^T \overline{U} \right)^{-1} \overline{U}^T \right] \overline{S}$

We should apply this method to the Gamma data. To test the algorithm, make some simulated data (choose polynomial coefficients, standard deviations, generate some noisy data, apply the method, compare to input).

Thoughts?