Supplementary Information:

Uncertainty in determining Log_2Ratio (M) due to photoelectron shot noise and camera readout noise.

Two intensities are measured during image analysis: the foreground intensity, I_f, of an array element which includes the total light collected; and the local background I_b. The signal intensity I_s is given by $I_s = I_f - I_b$. This can be written as $I_s = I_{so} + \delta I_s = I_{so}(1 + \delta I_s/I_{so})$, where I_{so} is the mean value of hypothetical multiple measurements of I_s and δI_s is the deviation in the measurements. The imaging system makes contributions due to photoelectron counting statistics and readout noise that affects both the foreground and background measurements used for determination of I_s.

The fluorescence ratio, R, is given by $R = I_{st} / I_{sr}$, where t and r refer to the test and reference samples respectively. If the uncertainties in the intensities are small relative to the intensities themselves, then to first order :

$$R = I_{sot} / I_{sor} (1 + \delta I_{st}/I_{sot} - \delta I_{sr}/I_{sor}) = R_o(1 + \delta I_{st}/I_{sot} - \delta I_{sr}/I_{sor}).$$

Taking the logarithms one obtains:

$$Log_2R = Log_2R_o + Log_2(1 + \delta I_{st}/I_{sot} - \delta I_{sr}/I_{sor}) = Log_2R_o + (Log_2e)(\delta I_{st}/I_{sot} - \delta I_{sr}/I_{sor})$$

where the first order expansion Ln(1+x) = x for small x has been employed, and Log_2e adjusts for the switch from base 2 to base e for the logarithm. Thus the mean square deviation of the Log_2Ratio is given by,

$$\overline{(Log_2R - Log_2R_o)^2} = (Log_2e)^2\left[\overline{(\delta I_{st}/I_{sot})^2} + \overline{(\delta I_{sr}/I_{sor})^2}\right] \cong 2(Log_2e)^2\left[\overline{(\delta I_s/I_{so})^2}\right]$$

Where the bars indicate averaging over multiple measurements, and it is assumed for simplicity that the test and reference signal intensities, and thus their variances, are roughly the same.

The quantities that are actually measured are the I_f and I_b, and uncertainties in them due to random noise sources are uncorrelated. Thus:

$$\overline{(\delta I_s/I_{so})^2} = (1/I_{so})^2\left[\overline{(\delta I_f)^2} + \overline{(\delta I_b)^2}\right]$$

The contributions of the imaging system to the mean square deviation of I_f and I_b are due to the counting statistics of the photoelectrons and the camera readout noise, and can be estimated by representing them in terms of numbers of collected electrons. Since I_f is the average intensity for the array element in digital units, then the the total number of photoelectrons collected for the element is $N_f = g\alpha_f I_f$, where g is the conversion factor of the camera that relates the number of electrons in a pixel to the digital output of the camera, and α_f is the area of the array element in pixels. Thus

$$\overline{(\delta I_f)^2} = \left(1/(\alpha_f g)^2\right)\overline{(\delta N_f)^2} = \left(1/(\alpha_f g)^2\right)\left[g\alpha_f I_f + \alpha_f \nu_{ro}\right]$$

where the mean number of electrons has been used for the variance of the number of electrons as is appropriate for counting, and ν_{ro} is the mean square readout noise of the camera in $(\text{electrons})^2$. A similar relationship holds for the mean square deviation of the background intensity, but with the α_f replaced by α_b, the number of pixels used to determine the local background level. Since $I_f = I_{so} + I_b$, the mean square variation in Log2R due to the imaging system is:

$$\overline{(\delta \mathrm{Log}_2 R)^2} = (\mathrm{Log}_2 e)^2\left[2/(I_{so}\alpha_f g)\right]\left[1 + (1 + \alpha_f/\alpha_b)(I_b/I_{so} + \nu_{ro}/(gI_{so}))\right]$$

The total mean square variation of the Log2Ratio also includes a contribution due to the intrinsic variability in the array -- the standard deviation, SD_∞, that would be present if the signals were very bright. This adds to the equation above. Thus:

$$\mathrm{S.D.} = \sqrt{(\mathrm{Log}_2 e)^2\left[2/(I_{so}\alpha_f g)\right]\left[1 + (1 + \alpha_f/\alpha_b)(I_b/I_{so} + \nu_{ro}/(gI_{so}))\right] + (SD_\infty)^2}$$

The plot in the inset to Figure 8a shows this equation for $\alpha_f \sim 50$ pixels and $\alpha_b \sim 15$ pixels, and $SD_\infty = 0.06$. Measurements on our camera found $g = 2.7$ electrons / digital unit and $v_{ro} \sim 100$ electrons^2. I_{so} and A are related by $A = 0.5\mathrm{Log}_2 I_{st} I_{sr} \sim \mathrm{Log}_2 I_{so}$.