

Why in MetaFluor® software does the ratio not match the ratio of the two wavelengths?

Question:

I am using MetaFluor software to measure the calcium concentration using 340 and 380 wavelength images. When I compared the average intensities from the 340 image and from the 380 image the ratio for these two measurements did not equal the ratio measurement reported by the MetaFluor program. Why is that and which is more indicative of the true calcium concentration within the cells?

Answer:

The confusion you are having is because the MetaFluor program reports the average of the ratios and the ratio of the averages does not equal the average of the ratios.

For example if we had two pixels in the images

Pixel 1:	I340: 3	I380: 4
Pixel 2:	I340: 7	I380: 8

The ratio of the averages

The average of the two pixels in the 340 wavelength is 5 and the average in the 380 wavelength is 6. The ratio of ratio of the averages is $5/6$ or 0.8333.

The average of the ratios

The ratio of the first pixel is $3/4$ or 0.75. The ratio of the second pixel is $7/8$ or 0.875. The average of the two ratios is 0.8125.

If the ratio of the ratio value is indicative of the calcium concentration for each pixel and you want the average calcium concentration in the cell the average of the ratios (as reported by the MetaFluor program) should be used.

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PRODUCTS
MetaFluor®
MetaMorph®

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