



Ratio Calculation with MetaFluor

Abstract

A description of how MetaFluor calculates ratios

Discussion

MetaFluor computes the ratio by taking the average of (pixel in wavelength 1 / pixel in wavelength 2) for all the pixels in the region. If either pixel of wavelength 1 or wavelength 2 is zero or is outside of the threshold range, that pixel is discarded.

An Example

Consider a region of interest that is 6 pixels wide by 1 pixel high.

The pixels of the region in the Wavelength 1 image are as follows:
[50, 75, 100, 125, 150, 175].

For the purpose of the example, assume the Low threshold = 60 and the High threshold = 160.

MetaFluor will threshold Wavelength 1 to obtain:
[--, 75, 100, 125, 150, ---].

MetaFluor will average all of the valid (not thresholded away) pixels to produce the Average intensity of Wavelength 1. In this case, the valid pixels are 75+100+125+150, divided by a count of 4 valid pixels, results in the Average for this region in Wavelength 1 of 112.5.

The pixels of the region in the Wavelength 2 image are as follows:
[25, 25, 25, 25, 100, 100].

For the purpose of the example, assume the Low threshold = 0 and the High threshold = 50.

MetaFluor will threshold Wavelength 2 to obtain:
[25, 25, 25, 25, ---, ---].

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MetaFluor will average all of the valid (not thresholded away) pixels to produce the Average intensity of Wavelength 2. In this case, the valid pixels are 25+25+25+25, divided by a count of 4 valid pixels, results in the Average for this region in Wavelength 2 of 25.

MetaFluor will compute the Ratio by only ratioing those pixels in the region that were valid in both wavelengths. To recap, the pixels for each wavelength were:

[--, 75, 100, 125, 150, ---] (Wavelength 1)

[25, 25, 25, 25, ---, ---] (Wavelength 2)

The pixels that will be ratioed are:

[--, 75/25, 100/25, 125/25, ---, ---].

Therefore the Ratio will be the average of 3+4+5, which is 4.

Implications

If you are logging average intensities as well as the ratio, in the above example you will have logged:

- Wavelength 1 Average = 112.5
- Wavelength 2 Average = 25
- Ratio = 4

One could assume that the Ratio should be the result of a divide of 112.5 by 25, which would be 4.5. By the method described above, however, MetaFluor produces a ratio of 4 because it is not using the raw intensity but is excluding pixels which were not valid (outside the threshold range) in either wavelength.