Algorithms for cross-talk suppression in fluorescence microscopy

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CROSS-TALK, or bleed-through, is the incomplete separation of fluorescence emission from different flourochromes at image capture. Fluorescence emission, intended to be associated with a particular wavelength, may therefore give rise to detected intensities also at other wavelengths. This can be caused either by fluorescence emission spectra having components outside the main intended wavelength range and/or by incomplete spectral separation of the different detected wavelengths. The effect is proportional to the intensity of the signal. Stable methods for suppression of cross-talk are dependent on image capturing techniques and hardware settings. Hardware solutions for avoiding cross-talk are typically expensive and have to be adapted to the specific image capturing apparatuses.

In the image analysis based method proposed by us, an input image is transformed into a one-dimensional histogram, referred to as the angle histogram. The axes representing "pure" components can be selected arbitrarily and may, e.g., correspond to the green and red directions in a Hue-Saturation-Intensity diagram, i.e., 0° for red and 120° for green. We assume that red and green channels are examined, but this could be any pair of channels. By expressing the pixel vectors as linear combinations [1] of the smallest, α_R, and the largest, α_G, dominating angles of the angle histogram, an image compensated for cross-talk can be produced.

To illustrate the method cross-talk is simulated by adding the red channel multiplied by a factor representing the degree of cross-talk to the green channel of an image and adding Gaussian white noise. If we examine the angle histogram (Fig.1A) of series of red, colocalized and green objects as shown in Fig.1B, one can see that the dominating angle of the "pure" colours is not situated in immediate vicinity of 0° and 120°; the red peak has been shifted from 0° to 43° due to cross-talk. Cross-talk to the red channel is significantly smaller since the green peak is shifted from 120° to 112°. This means that a pixel showing only "red" emitted light also contributes to the "green" intensity and vice versa.

Images free of cross-talk are produced by making the correct linear combination of the original channels. As can be seen in Fig.1C, the proposed method has resulted in suppression of cross-talk while preserving true colocalization.

Together with the proposed method, a novel method for quantification and detection of colocalization, based on segmentation of the angle histogram, has been developed [2]. Colocalization is particularly important for revealing information on how and where biomolecules such as proteins and protein complexes interact within a cell, as well as in which sub-cellular compartments they are present.

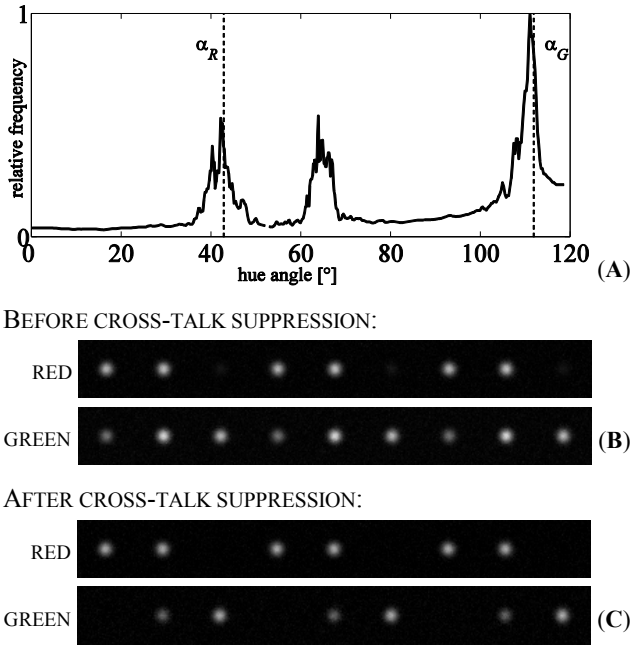

Fig.1. **(A)** Weighted angle histogram compensated for uncertainty of digital intensity values [2]. The histogram shows relative frequency of angles, representing colour spectrum varying from red (0°) to orange to yellow (60°) to yellowish green to green (120°). **(B)** Analysed artificial fluorescence microscopy image showing 9 objects (three series of red, colocalized and green). **(C)** The result of cross-talk suppression – "pure" red objects are not visible in the green channel, and vice versa, and colocalized objects are preserved.

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REFERENCES

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