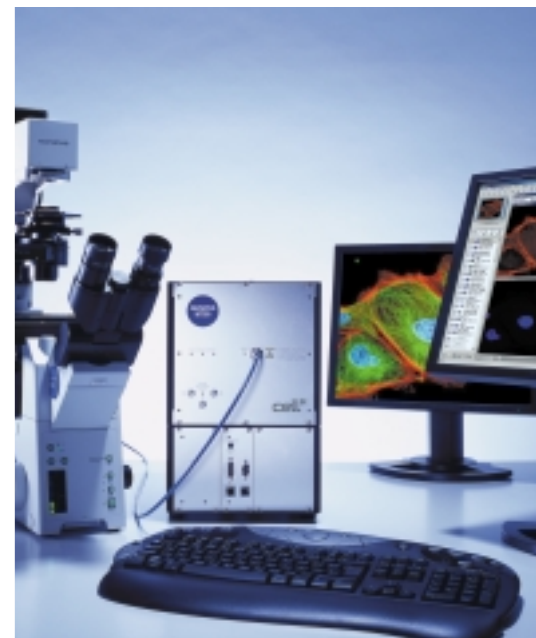
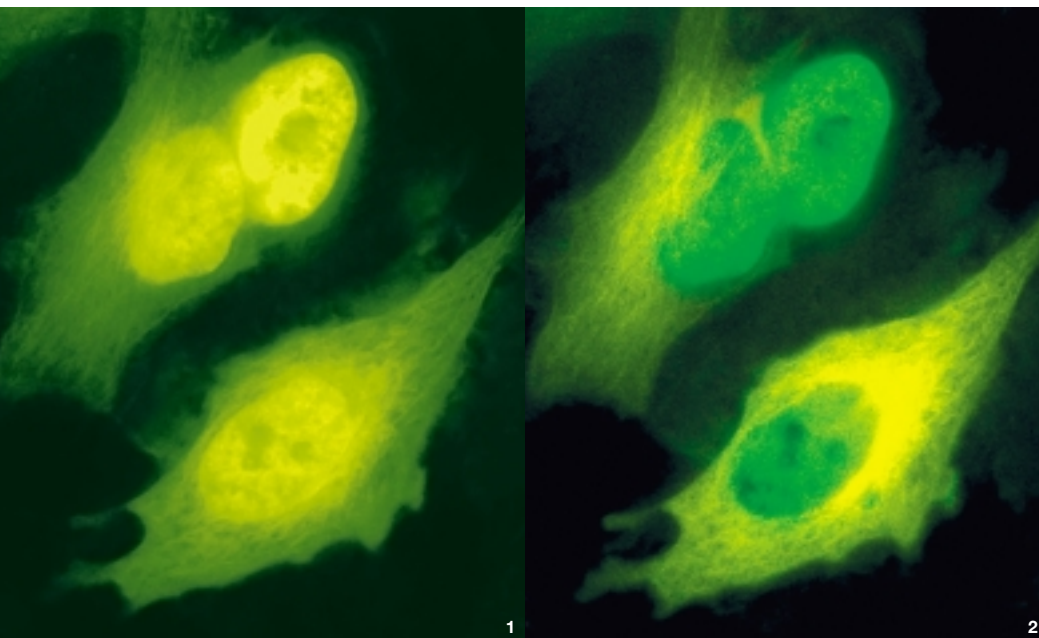
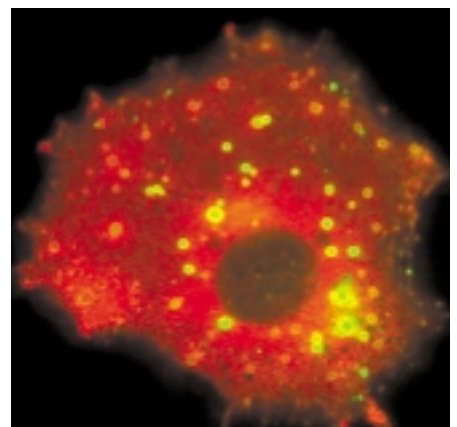
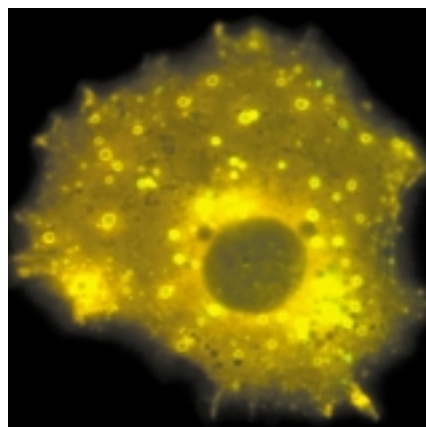
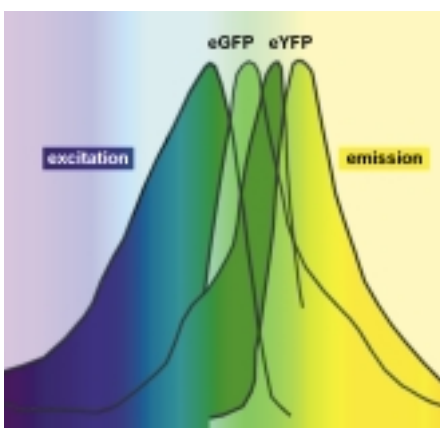


Spectral Unmixing



Resolution Enhancement in Multi-Color Fluorescence Imaging

- new combinations of fluorochromes and GFP variants in multi-labeled samples become possible
- spectrally very similar fluorochromes can be distinguished
- FRET becomes more easily visible if acceptor channel reveals structures upon donor excitation
- facilitation of colocalization studies by removal of bleed-through artifacts
- no cosmetics: revealing information inherent to images
- linear method: quantitative intensity analysis still possible with increased precision
- no additional hardware necessary
- easy-to-use two-step software module



• **Background and Problem:**

A large variety of fluorescent dyes and an increasing number of fluorescent protein variants are available in cellular biology to selectively label sub-cellular structures and biomolecules. Pronounced spectral overlap of the excitation and emission characteristics often limits the possibilities to combine these fluorescent markers within one sample. For this reason, for example, it was nearly impossible so far to optically distinguish GFP- and YFP-labeled structures in the same specimen. Even in the already »classical« combination CFP/ YFP, as for example used in FRET investigations, spectral bleed-through is a severe problem. CFP excitation also directly excites YFP to a considerable degree and thus YFP structures always become visible in the CFP channel, even if no energy transfer occurs. This has to be corrected in FRET studies, which strongly complicates the entire application.

• **Solution:**

Linear Spectral Unmixing is a technique applied, for example, in airborne or satellite borne environmental imaging and remote sensing to facilitate or enable sophisticated analysis. cell[®] is the first imaging system to implement such a method into wide-field fluorescence microscopy. With this powerful technique it becomes possible to ascertain the contribution of different fluorochromes to the total signal and, by chromatic redistribution of the intensity, to restore a clear signal for each color channel undisturbed by emission from the other fluorochromes.

• **Prerequisites:**

No additional hardware is necessary. Linear Unmixing is an easy-to-use two-step software module. The only prerequisite is a straightforward calibration by use of single-labeled samples.

• **Results:**

Spectral Unmixing does not create artificially embellished images. The chromatic information inherent to the data is used to redistribute the fluorescence intensity pixel by pixel while the total pixel intensity is maintained. Thus, quantitative analysis is not only still possible but delivers more significant data.

• **Applications:**

The use of new fluorescent protein combinations now results in sharply contrasted images, for example GFP+ YFP. In FRET studies, for example using the CFP/YFP combination, the occurrence of FRET becomes more immediately obvious in the images (even before quantitative analysis is performed). Bleed-through artifacts are avoided in colocalization studies.