News & Tips on Microscopy NUMBER 4

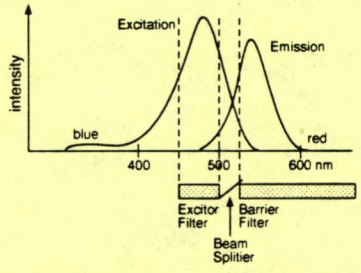
THE ZEISS CORNER

FLUORESCENCE NO. 1: THE FILTER SET

Fluorescence microscopy is based on the high-energy excitation of a chemical component within your sample, followed by a lower-energy emission. The chemical component (fluorochrome) can be:

1. a natural part of the system which will autofluoresce or
2. a dye or probe attached to highlight a specific feature (membrane vs. cytoplasm) or chemical activity (calcium metabolism).

Each fluorochrome can be described by two characteristic curves: excitation and emission.



CHOOSE THE CORRECT FILTER SET.

To convert a microscope to a fluorescence system, you need:

1. an exciter filter which passes only the high energy excitation light
2. a dichroic beam splitter which directs the light downward toward the specimen
3. a barrier filter to remove any extraneous light
4. a high-intensity gas discharge light source.

The filter set must be appropriate for the fluorochrome being used.

RECOMMENDATION FOR DOUBLE LABELING.

If you are using more than one fluorochrome, make sure that the emission curve of one does not overlay the excitation curve of the next, causing spontaneous internal fluorescence. Typical culprits: FITC + Rhodamine. A better alternative: use probes with greater spectral separation such as FITC + Texas Red.

Contact us and we'll send you a helpful chart of recommended filter sets for common fluorochromes. And for all your needs in fluorescence microscopy, choose the leader—Zeiss.

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ZEISS

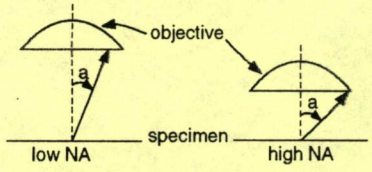
SEE WHAT YOU'RE MISSING

News & Tips on Microscopy NUMBER 5

THE ZEISS CORNER

FLUORESCENCE NO. 2: THE OPTICS

1. **Use the highest numerical aperture (NA) and lowest magnification (M).** Intensity (I) of the image is related to NA and M, $I = NA^4/M^2$. Therefore, use an objective with the lowest M and highest NA, such as the Zeiss Plan-Neofluar 40X/1.3 oil objective. NOTE: higher NA will mean a shorter working distance.



2. **Use oil immersion objectives.** Oil objectives make use of physics to capture many of the rays which would usually escape collection. **Caution:** make sure to use a non-fluorescing immersion oil.

3. **Use objectives with as few internal elements as possible.** The greater the correction in an objective, the more internal lenses it must have. Each new lens gobbles up light. The best choice: Zeiss high-quality "neofluar" objectives.

4. **Remember, optics have spectral responses, too.** Interested in working just outside the typical 380-700 nm spectral range? Check with your Zeiss rep for the response of your optics. Some transmit down into the near ultra-violet, as low as 340 nm. For deeper UV, exchange necessary components for quartz.

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