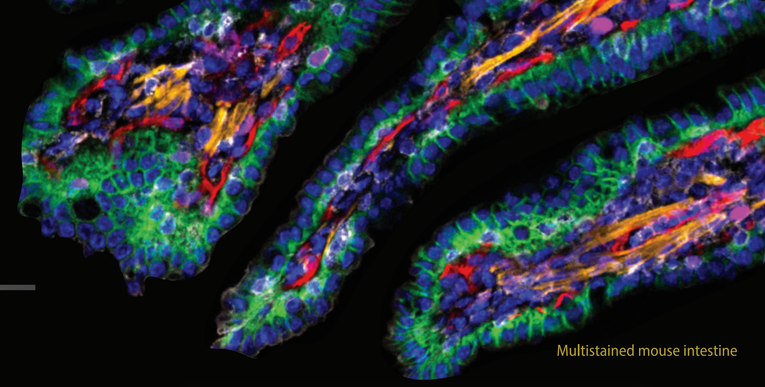
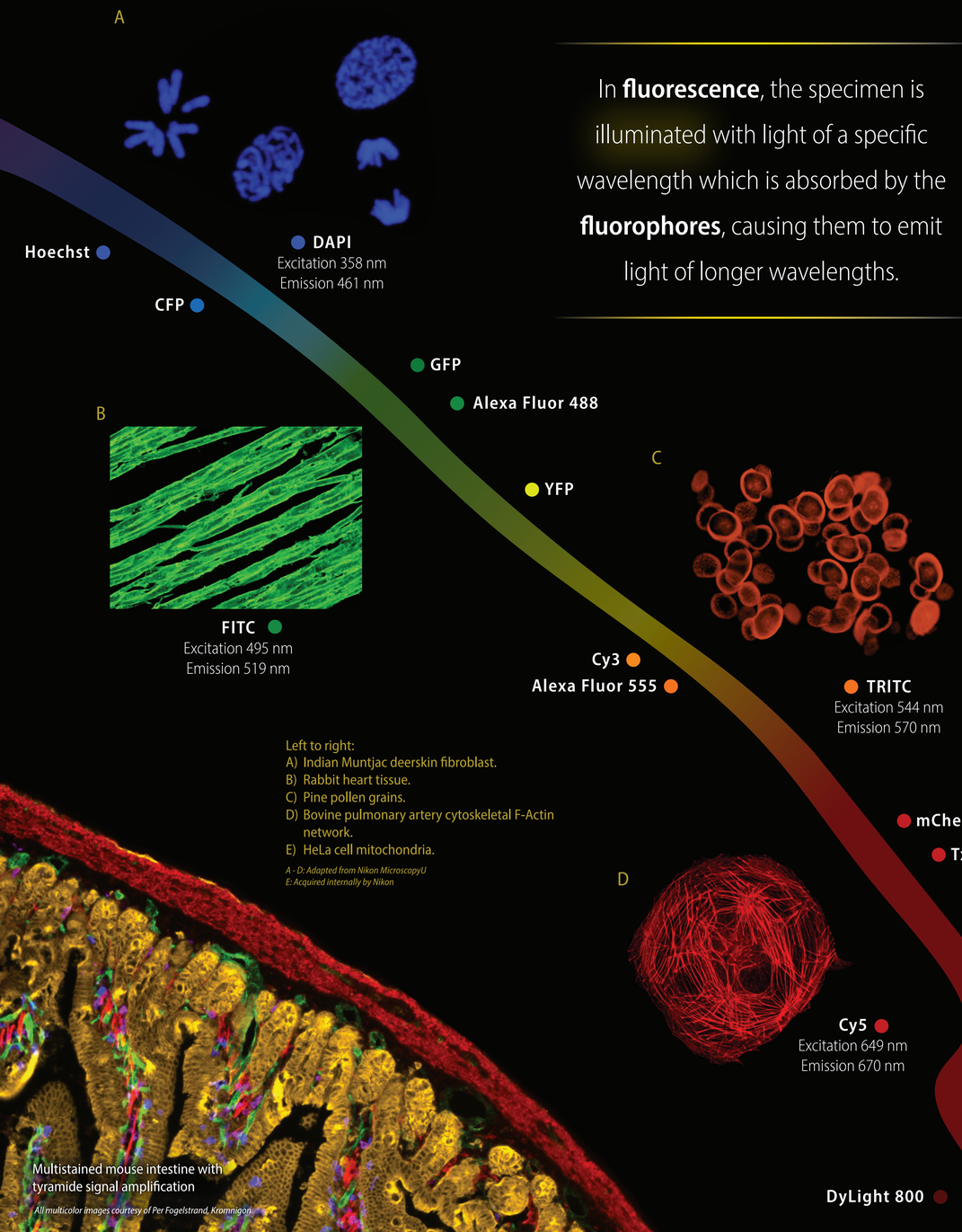
Illuminating Biology in Colour

A PRACTICAL GUIDE TO SMARTER FLUORESCENCE IMAGING



Multistained mouse intestine

COMMON FLUOROPHORES FOR MICROSCOPY



In **fluorescence**, the specimen is illuminated with light of a specific wavelength which is absorbed by the **fluorophores**, causing them to emit light of longer wavelengths.

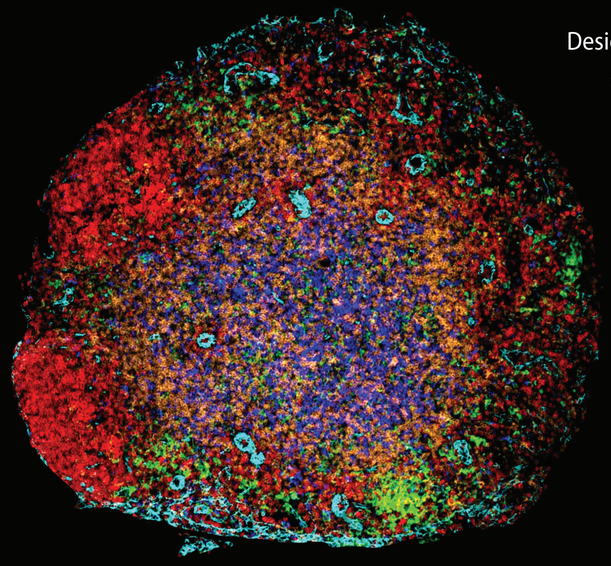
Left to right:
A) Indian Muntjac deerskin fibroblast.
B) Rabbit heart tissue.
C) Pine pollen grains.
D) Bovine pulmonary artery cytoskeletal F-Actin network.
E) HeLa cell mitochondria.

A - D: Adapted from Nikon MicroscopyU
E: Acquired internally by Nikon

Multistained mouse intestine with tyramide signal amplification

All multicolor images courtesy of Per Fogelstrand, Kromnigon

MULTI-COLOR IMAGING AND FILTER MANAGEMENT



Immune cells and endothelium in mouse lymph node

Designing Multicolor Experiments

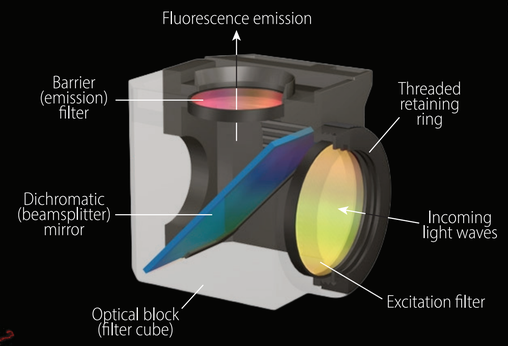
When combining multiple fluorophores, spectral overlap can lead to bleed-through and false signals. Proper filter management ensures accurate separation of channels.

Key considerations:

- Choose fluorophores with well-separated emission peaks (≥30–40 nm apart).
- Match filters to fluorophore spectra: Use excitation and emission filters that align with peak wavelengths.
- Use dichroic mirrors strategically: They split light efficiently between channels.
- Plan for laser lines: Ensure compatibility with available excitation sources.

Tip: Always check the full spectral profile, not just peak values, when selecting fluorophores and filters.

FLUORESCENCE FILTERS



PRODUCT INFORMATION
Learn more about Nikon's Fluorescent Filter Cubes

HOW TO MINIMIZE BLEED-THROUGH?

Bleed-through (also known as crossover) can occur when fluorophores have overlapping emission spectra, causing unwanted signal between channels.

Good fluorophore and filter choices, plus careful acquisition, help you avoid it.

- **Select fluorophores with minimal spectral overlap.**
- **Use narrow-band filters** for emission detection.
- **Adjust acquisition settings**: Lower laser power and optimize detector gain.
- **Sequential imaging**: Capture each channel separately instead of simultaneously.
- **Apply spectral unmixing** if available in your imaging software.

Quick Rule:
Greater separation between emission peaks = less bleed-through.

Shedding New Light On **MICROSCOPY**

Nikon MicroscopyU
Learn more about fluorophore-filter compatibility