

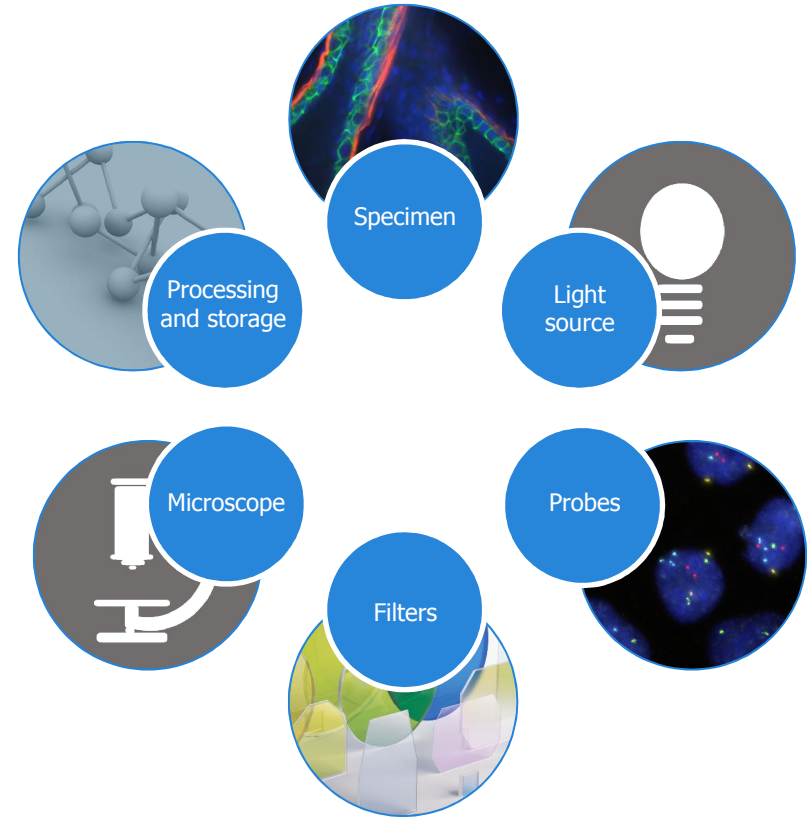
FLUORESCENCE FILTERS FOR FISH

FLUORESCENCE IN SITU HYBRIDIZATION

What You Need To Know For
Optimal Performance



Many **factors** influence the quality of FISH preparations and the images obtained from them.



Fluorescence Filters

How do **Fluorescence Filters** affect the most important image characteristics when viewing FISH samples so that you can:



Score slides more easily?



Reduce uncertainty?



Reduce eye strain?



Save time?



Increase throughput?

Fluorescence Filters

Filters do two things:



1 TRANSMIT

(allow to “pass” through) the desired wavelengths of light (color), and the desired amount (brightness) of light

AND



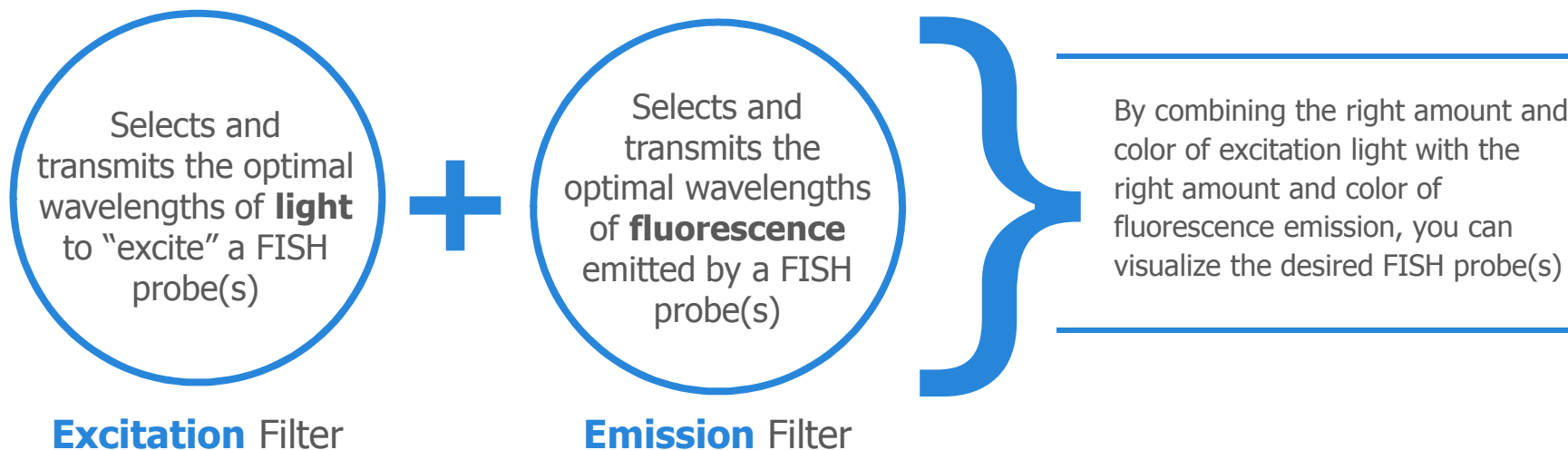
2 REJECT

(block) undesired light to a very high degree (high OD, or Optical Density)

The combined effect is a filter with a very high signal/noise ratio

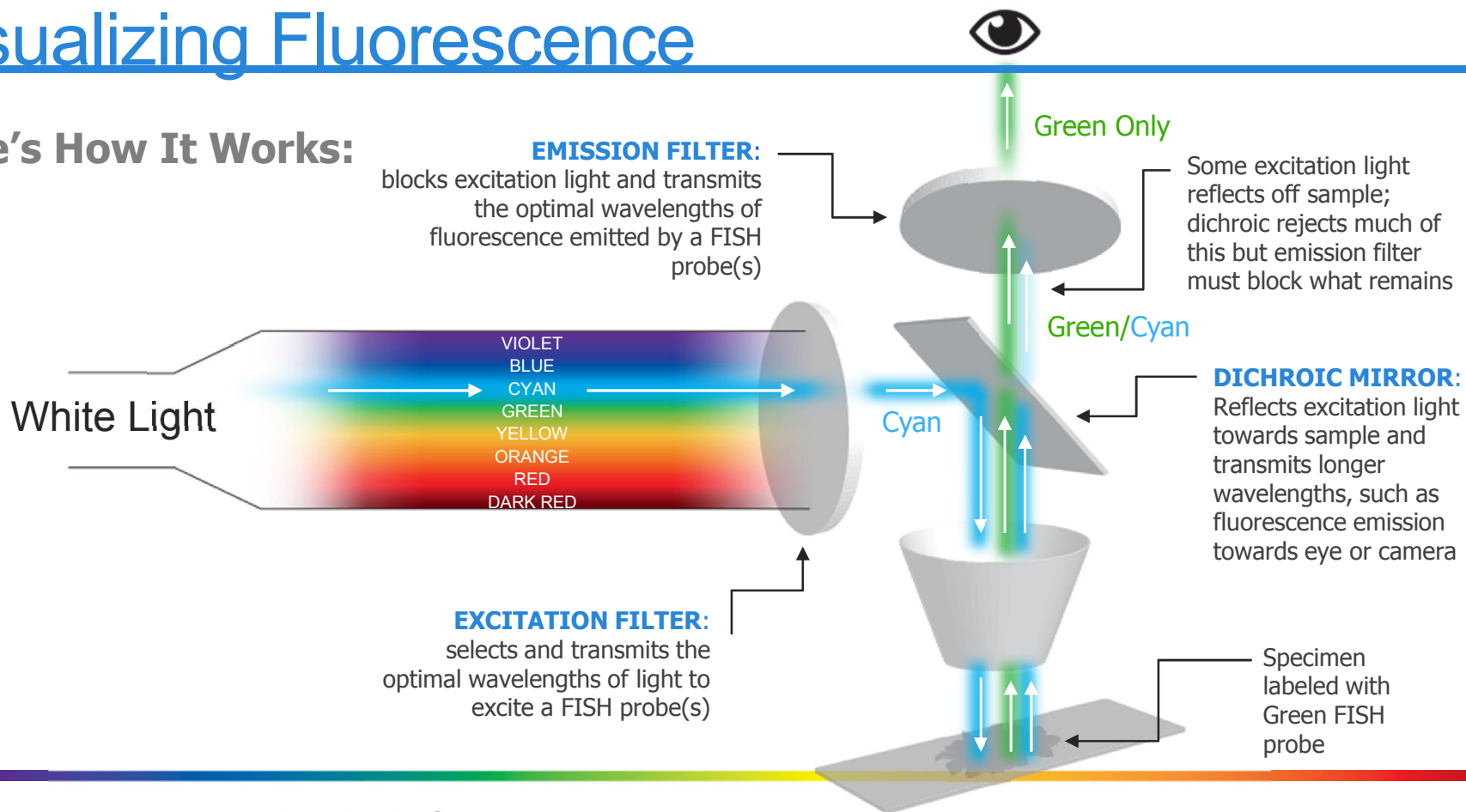
Fluorescence Filters

Two of these filters in a set work together to visualize fluorescence:



Visualizing Fluorescence

Here's How It Works:



Fluorescence Filters

By transmitting the most effective wavelengths of light, fluorescence filters influence the **4 most important aspects of FISH images**:



Contrast



Brightness



Color
Separation



Registration
(or alignment)

Contrast

Fluorescence Signal vs. Background Noise

This is the Signal/Noise ratio of the image.



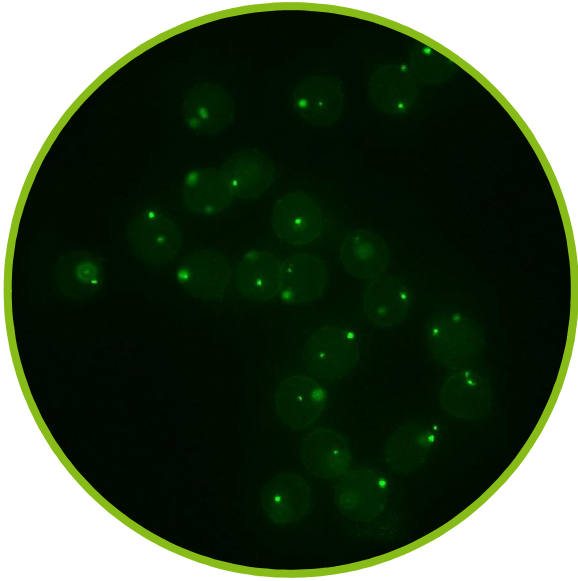
Low Signal/Noise



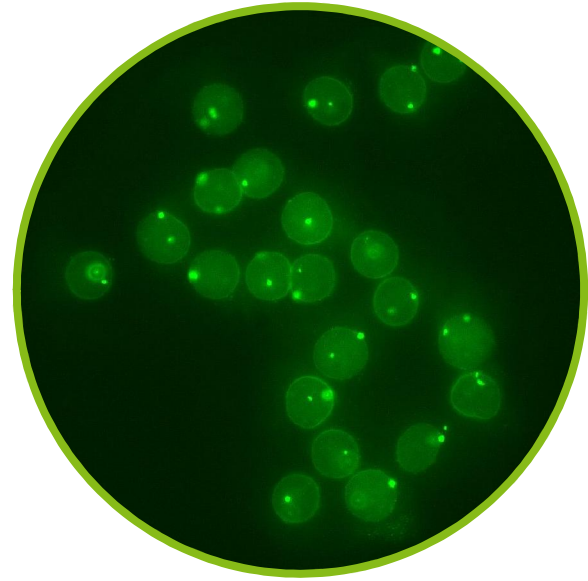
High Signal/Noise



Contrast



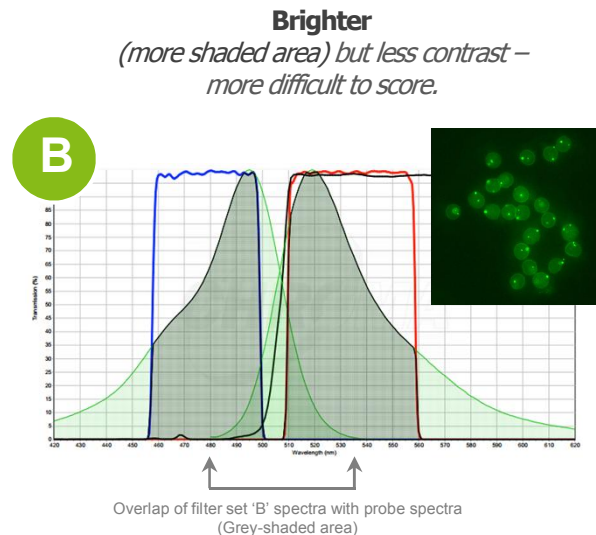
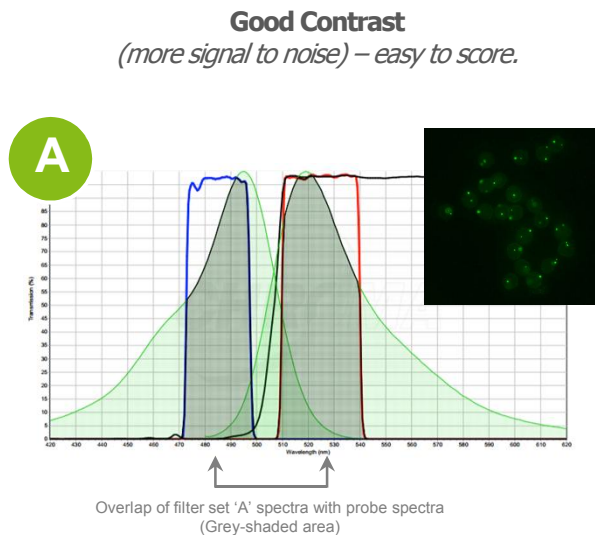
Good Contrast



Inadequate Contrast

Contrast

A Tale of Two Filter Sets



- Excitation filter transmission spectra
- Emission filter transmission spectra

Green FISH probe excitation and emission spectra

Contrast

If Image 'B' is brighter, why is the contrast lower?



Because the **noise**
is **higher...**

There is always background
fluorescence from various sources:

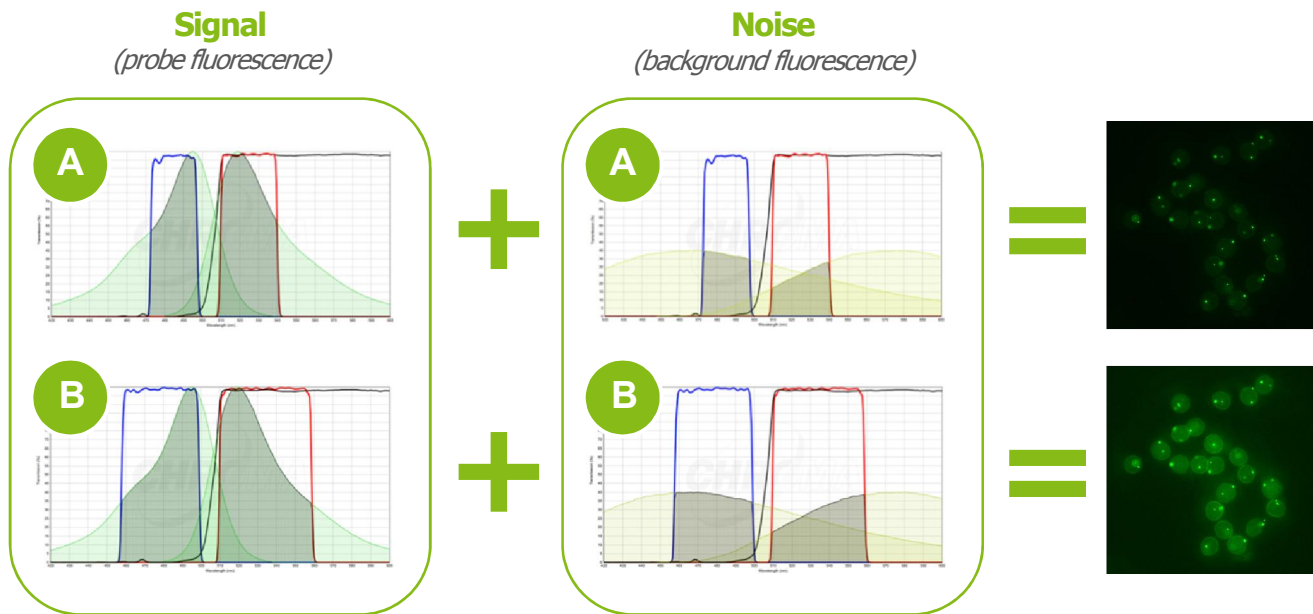
Cells and tissues themselves are **autofluorescent**

Fixatives and other reagents are **autofluorescent**

Fluorescent probes "stick" non-specifically

Contrast

'A' has better Signal/Noise ratio = **MORE CONTRAST**



'B' will detect
91% more
signal than 'A'

but...

'B' will detect
226% more
noise than 'A'

Brightness

Total intensity of fluorescence signal

Overall result of degree of excitation of sample (*dichroic and excitation filter*) **plus** level of transmission of fluorescence (*dichroic and emission filter*)



Low Transmission



High Transmission



Color Separation

Detect desired color(s) while rejecting undesired colors

Optimal filter combinations allow for best performance



! **Problem:**

Poor Color Separation
reduces certainty
and wastes time

Poor Filter choice



💡 **Solution:**

Maximize
Color Purity

Appropriate Filter choice



✓

Benefit:
Distinguish between colors
more easily and exclude
unwanted colors

Color Separation

Optimal color separation is not always simple

Considerations:

Requires appropriate combinations of probe colors in specimens in order to reliably separate colors

Also dependent on quality of specimen processing and balancing the degree of labeling of the different probe colors

Often involves trade-offs between brightness and the degree to which undesired colors are rejected

Registration

Images are in register (aligned) with each other

Each filter set produces images that are aligned with images from other filter sets



! **Problem:**

Images offset from each other...which cell is signal coming from?

Poor filter materials



💡 **Solution:**

Align images by using better filters

High Quality substrates



✓

Benefit:

Reduce uncertainty and save time by allowing you to score cells more easily

Registration

Filters are only one factor in image registration

Considerations:

Factors such as the microscope filter turret are also responsible for proper image registration

Image registered filters do not introduce any mismatch but they cannot fix lack of registration caused by other sources

Typically only a factor when using a camera

Multiband sets for simultaneous viewing of multiple colors are by definition “aligned with themselves”

THANK YOU

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Chroma optical filters and related products may be purchased online or via phone. For more information, please e-mail sales@chroma.com, visit www.chroma.com or call **800.824.7662**.

