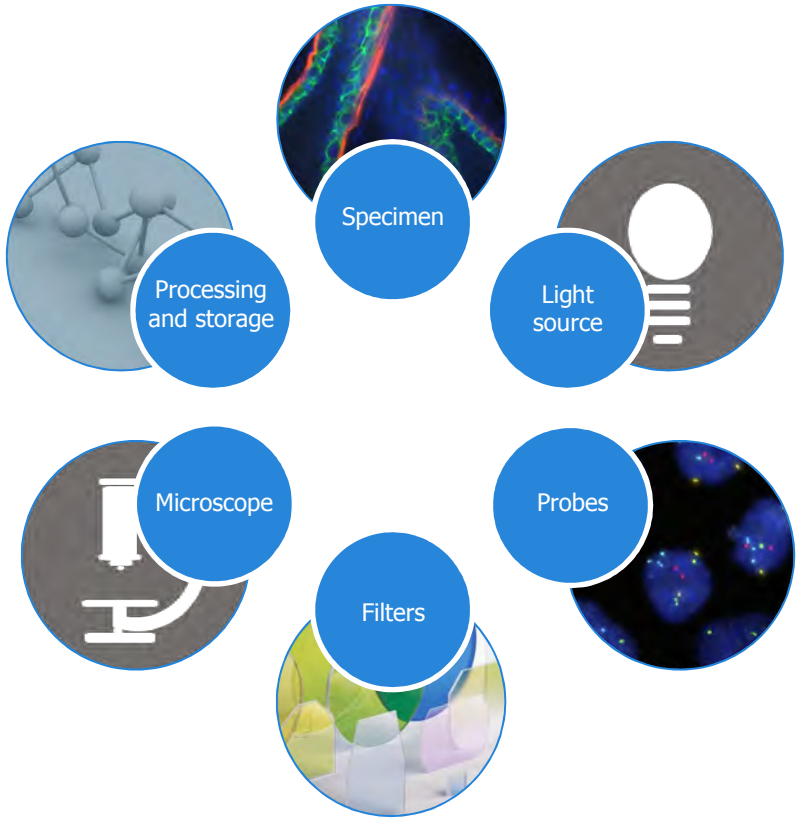
FLUORESCENCE FILTERS FOR FISH

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

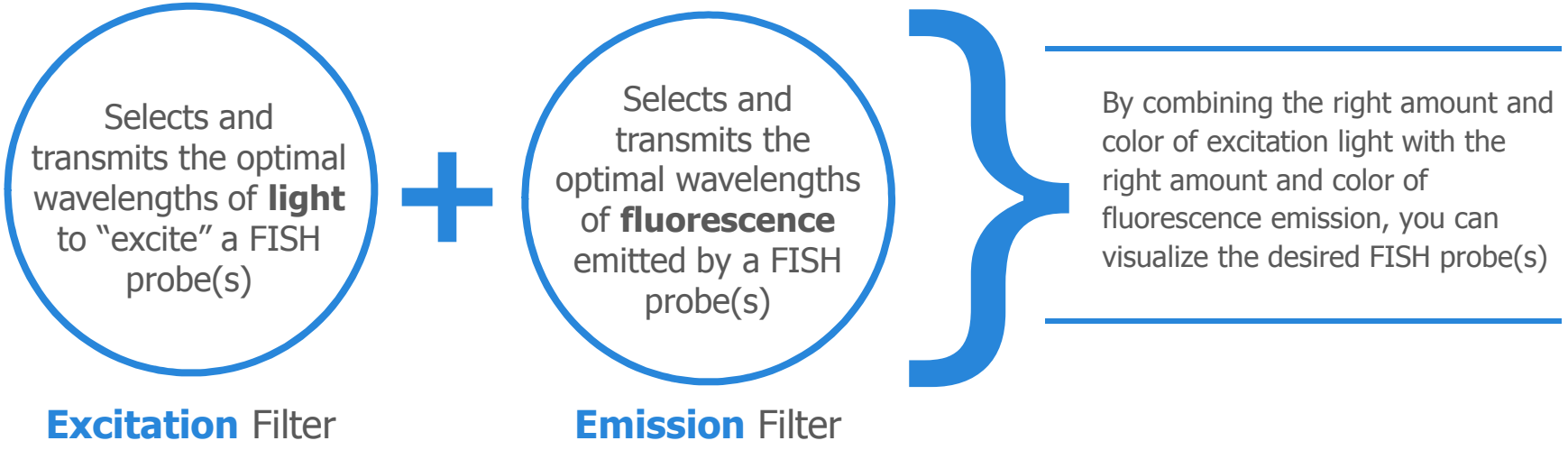
2 REJECT

(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

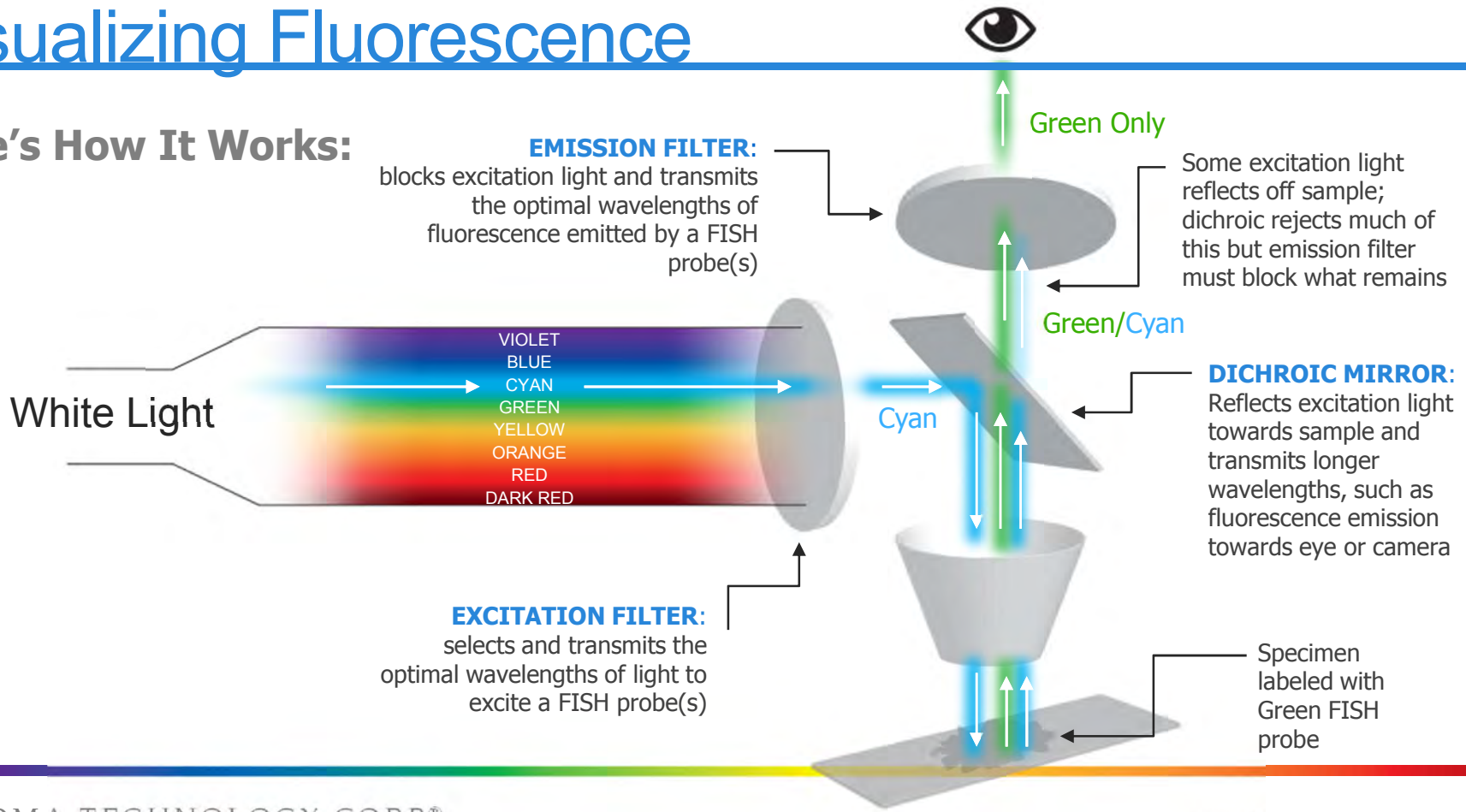
Fluorescence Filters

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

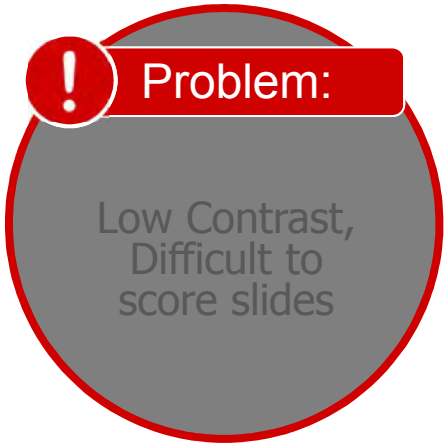
Color Separation

Registration *(or alignment)*

Contrast

Fluorescence Signal vs. Background Noise

This is the Signal/Noise ratio of the image.



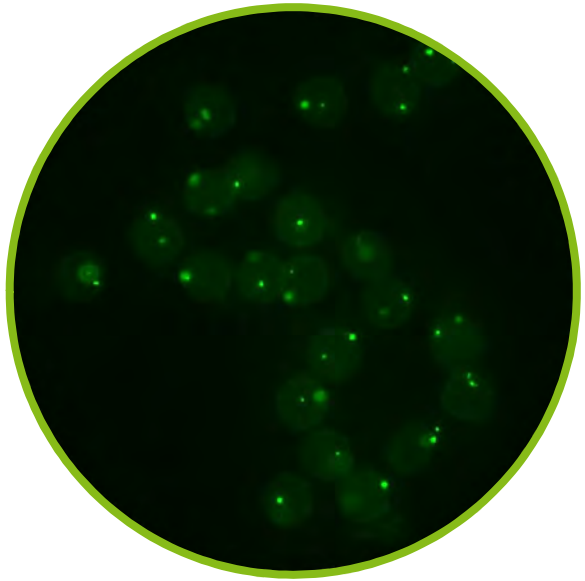
Low Signal/Noise

High Signal/Noise

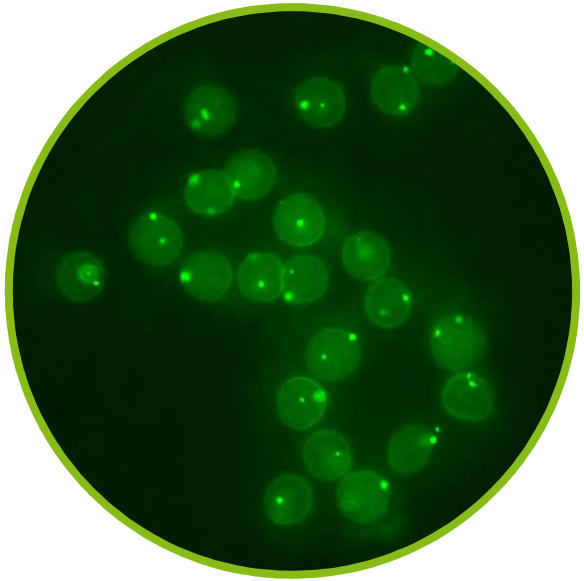
Benefit:

Allow you to more easily see the difference between real signal and background

Contrast



Good Contrast

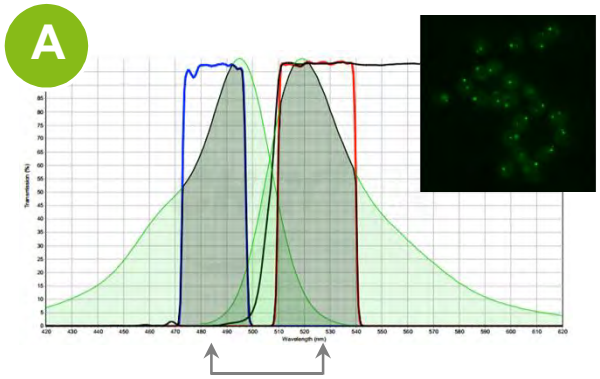


Inadequate Contrast

Contrast

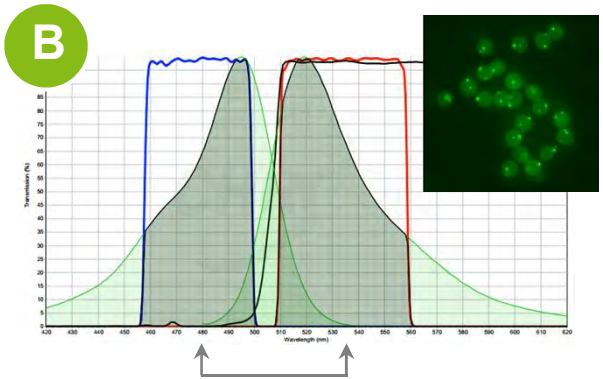
A Tale of Two Filter Sets

Good Contrast
(more signal to noise) – easy to score.



Overlap of filter set 'A' spectra with probe spectra
(Grey-shaded area)

Brighter
(more shaded area) but less contrast – more difficult to score.



Overlap of filter set 'B' spectra with probe spectra
(Grey-shaded area)

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**

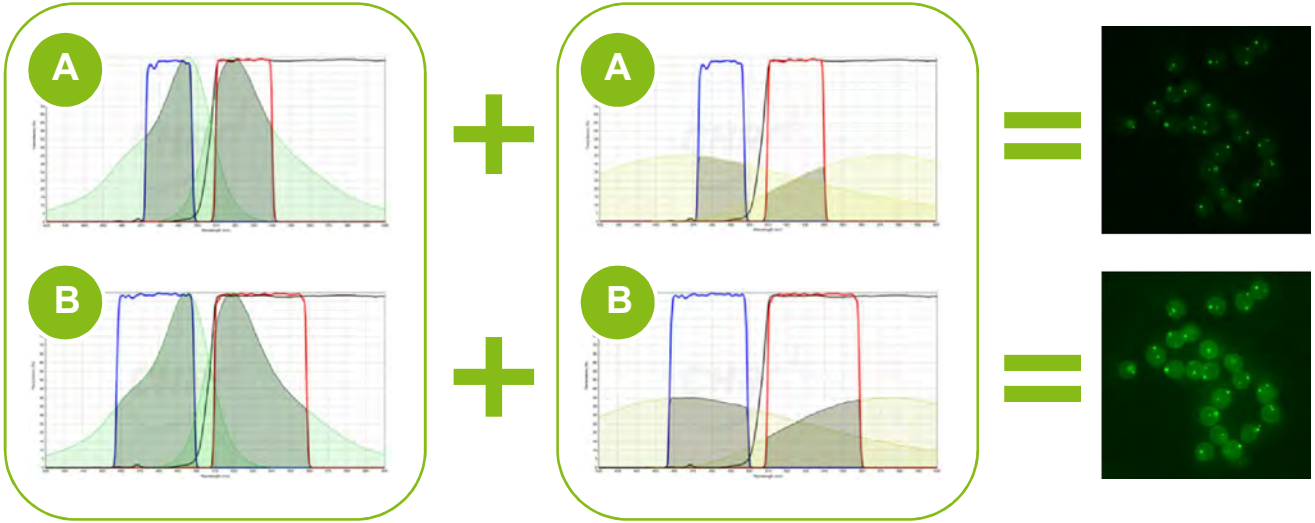
Fluorescent probes "stick" non-specifically

Contrast

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

Signal
(probe fluorescence)

Noise
(background fluorescence)



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

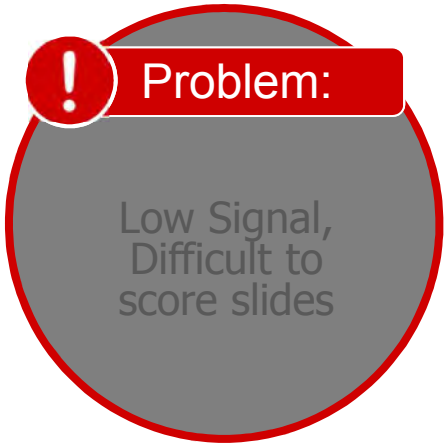
but...

'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

Benefit:

Save time, increase throughput and reduce eye strain with brighter images

Color Separation

Detect desired color(s) while rejecting undesired colors

Optimal filter combinations allow for best performance

Poor Filter choice

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

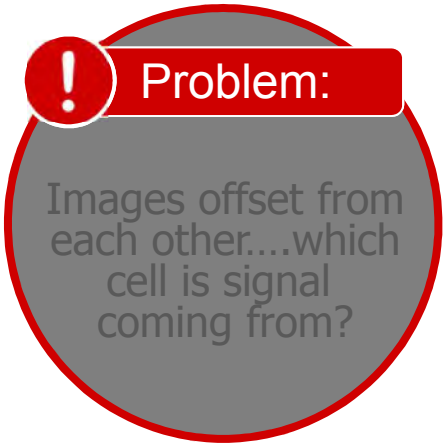
Also dependent on quality of specimen processing and balancing the degree of labeling of the different probe colors

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



Benefit:

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

Poor filter materials

High Quality substrates

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**.