Common SIM Artifacts – Tips for Successful SIM Imaging

Patrina Pellett, PhD
OMX Applications Scientist
GE Healthcare, Cell Technologies
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imagination at work

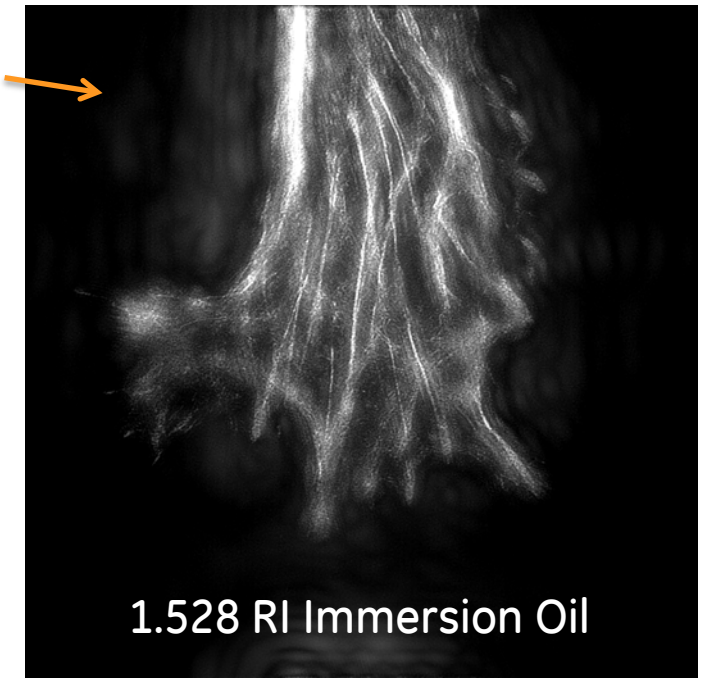
Common Structured Illumination Artifacts
2 main artifacts & their causes

1. Haloing/doubling
   - Caused by asymmetrical point spread function:
   - Spherical aberration
   - Photobleaching
   - Cutting structures off
2. Honeycomb
   - Caused by diffuse labeling or bad signal to noise
   - Increase signal to noise
   - Adjust Wiener filter constant

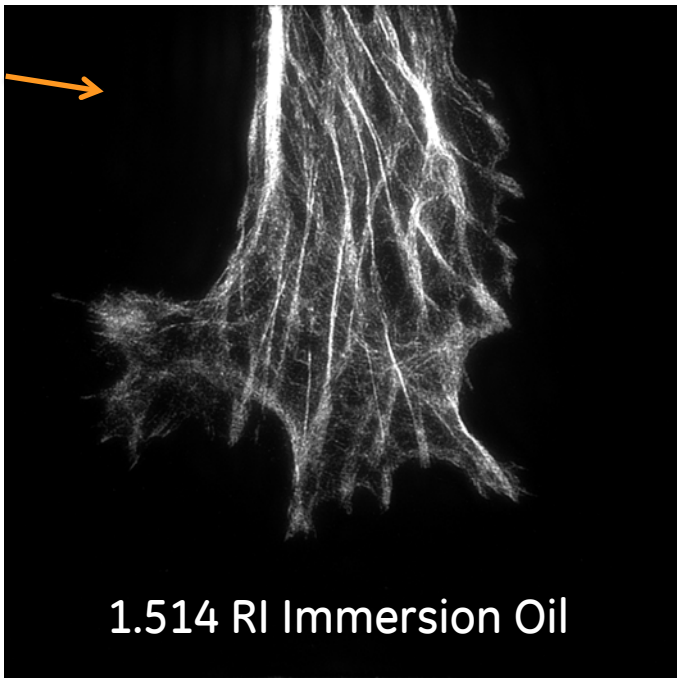
Haloing/Doubling Cause 1: Spherical aberration due to refractive index mismatch

Haloing/Doubling Artifact
Caused by asymmetry in point spread function

Haloing/doubling artifact

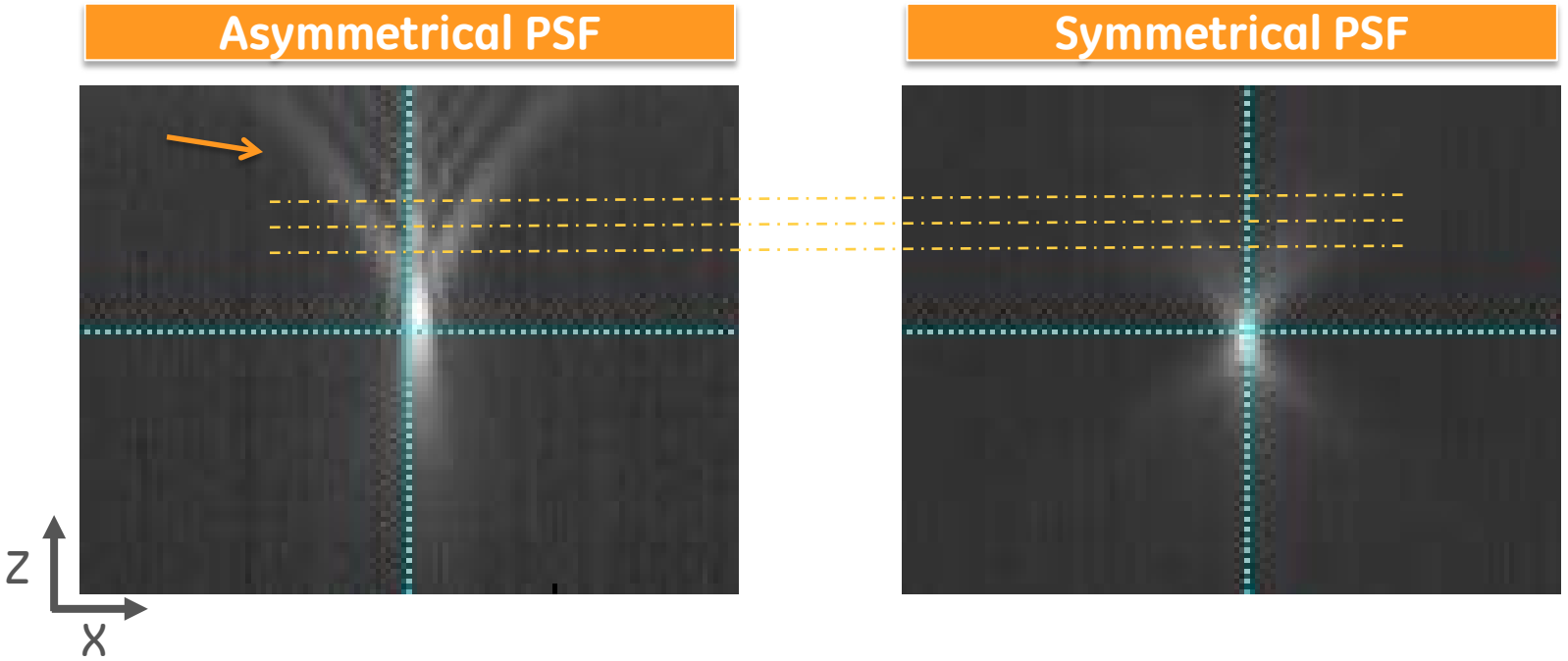


Artifact free



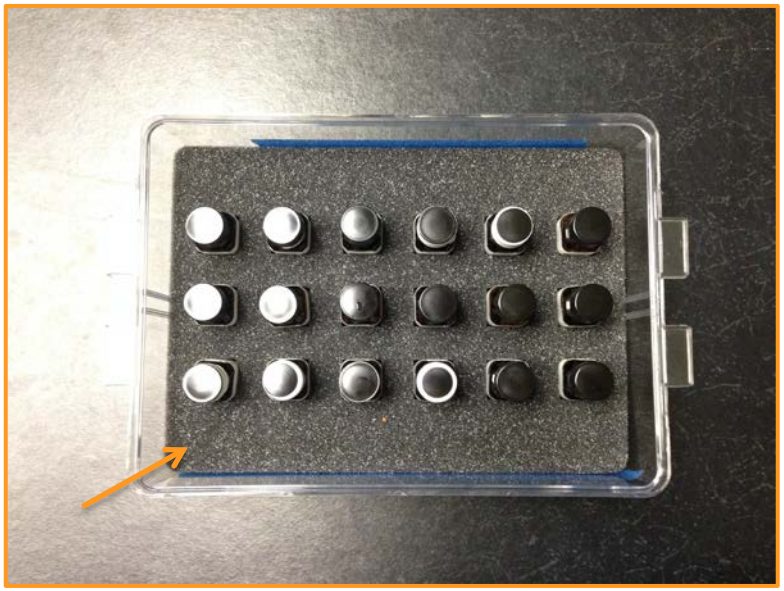
Spherical aberration caused by refractive index mismatch results in asymmetrical point spread function

Haloing/Doubling Artifact
What causes this?



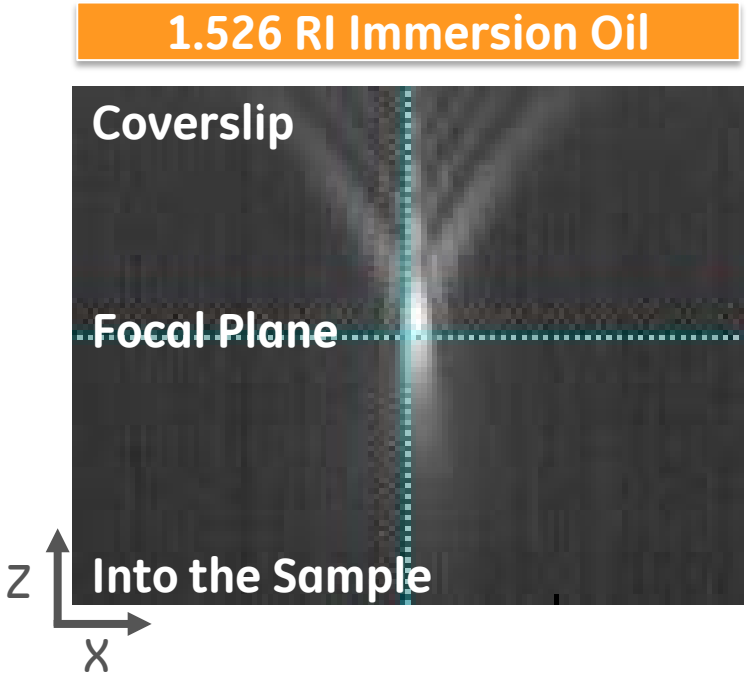
SIM algorithm assumes a perfectly matched PSF! When it detects out of focus light from mismatched PSF, it assumes this is real signal & reconstructs it

Haloing/Doubling caused by Spherical Aberration
Minimize by matching the RI of immersion oil to your sample



The OMX comes with an oil kit with 18 oils with different refractive indices ranging from 1.500 to 1.534 in increments of 0.002

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts



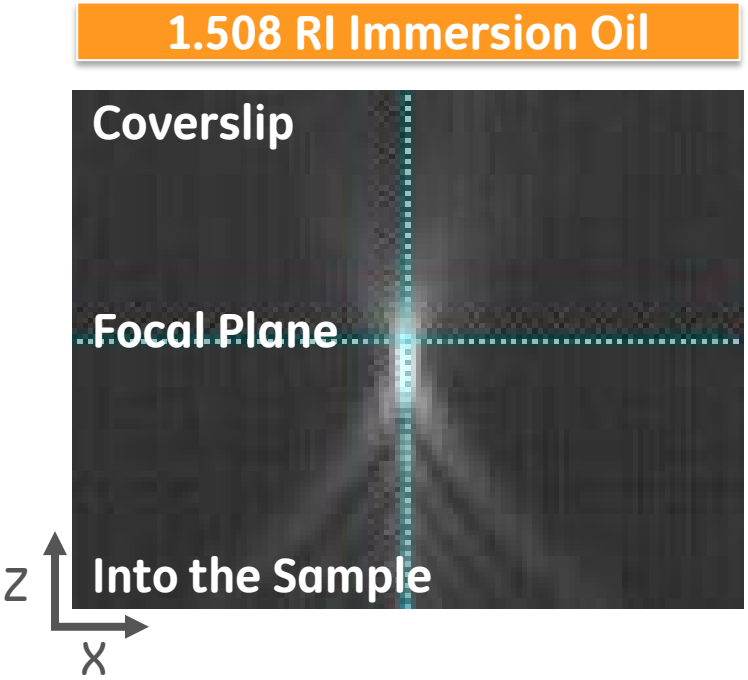
If the flaring/ asymmetry of the PSF is towards the coverslip or 'up', the RI of the oil is too high! Go down in oil!

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts

1.526 RI Immersion Oil

If the flaring/ asymmetry of the PSF is towards the coverslip or 'up', the RI of the oil is too high! Go down in oil!

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts



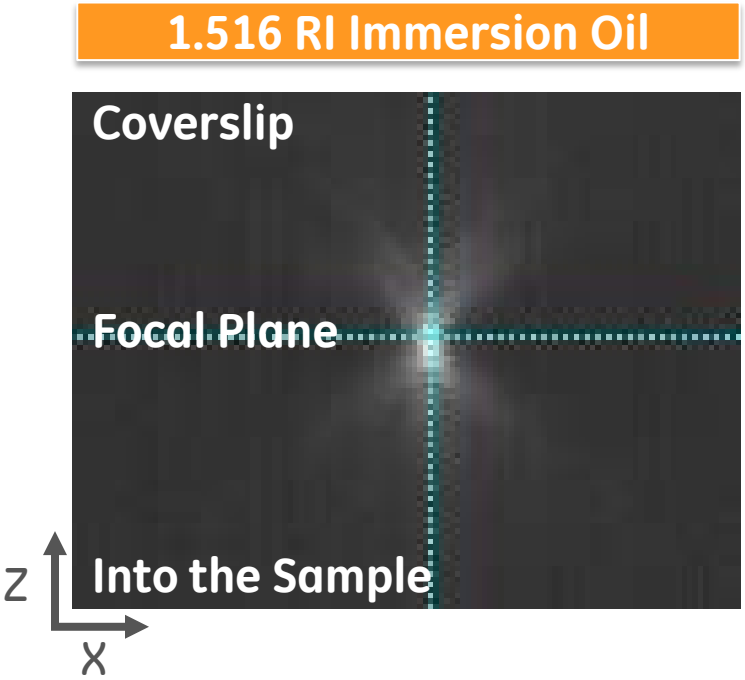
If the flaring/ asymmetry of the PSF is away from the coverslip or 'down', the RI of the oil is too low! Go up in oil!

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts

1.508 RI Immersion Oil

If the flaring/ asymmetry of the PSF is away from the coverslip or 'down', the RI of the oil is too low! Go up in oil!

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts



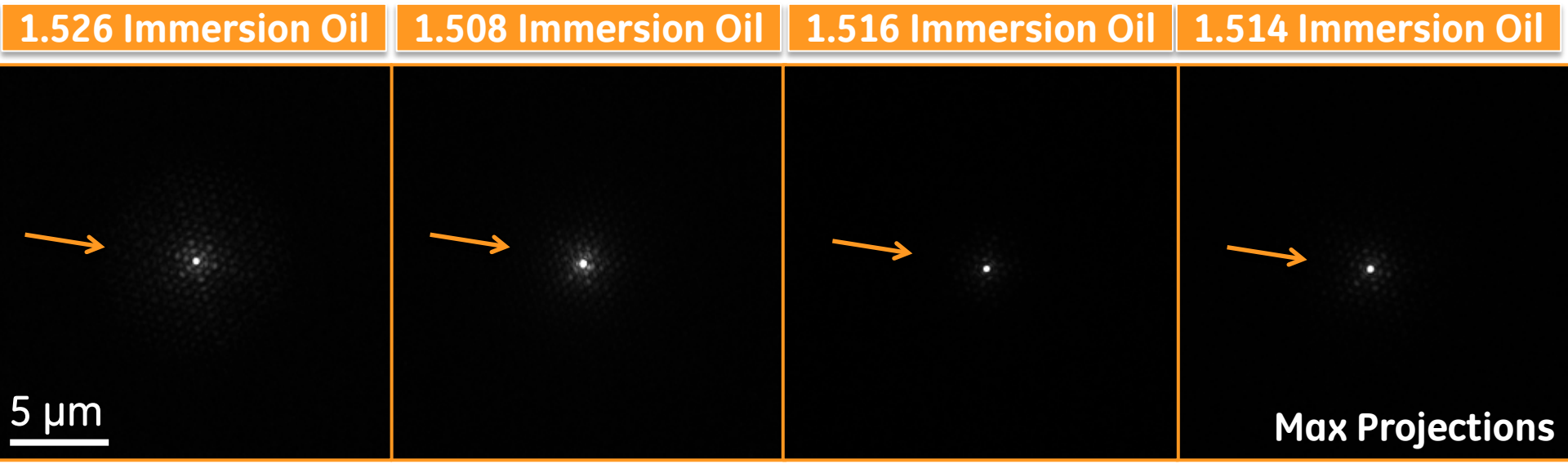
If the flaring of the PSF is symmetrical on both sides of the focal plane, the oil is matched!

Haloing/Doubling Caused by RI Mismatch
How to find the best oil to minimize artifacts

1.516 RI Immersion Oil

If the flaring of the PSF is symmetrical on both sides of the focal plane, the oil is matched!

Haloing/Doubling Caused by RI Mismatch
Affects of RI mismatch on SIM reconstructions



Haloing or doubling artifacts are minimized in SIM reconstructions when the oil used results in a symmetric PSF

Haloing/Doubling Caused by RI Mismatch
Matching oil for multi-color samples

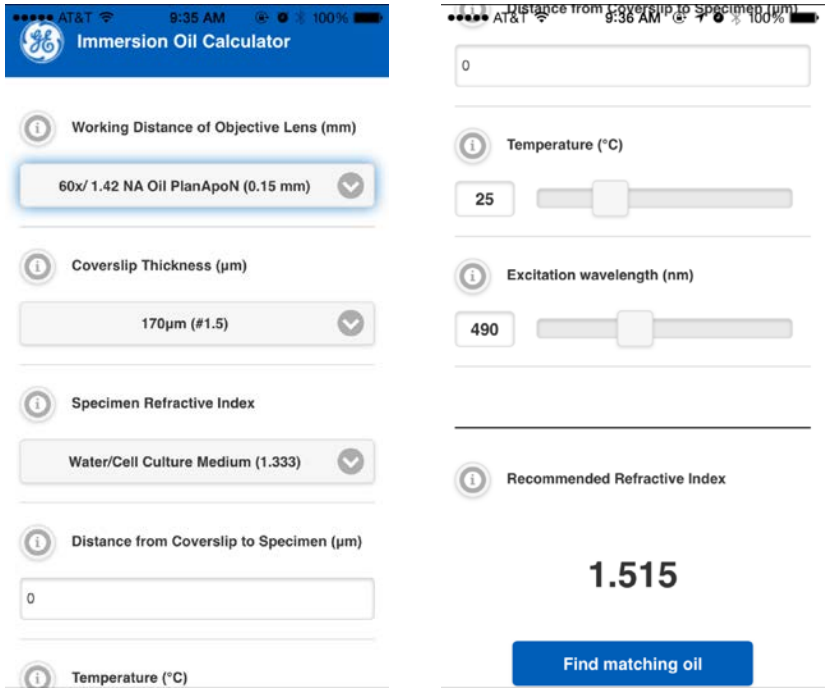
- Can only match the oil for 1 wavelength
- Meaning the wavelengths with suboptimal oil matching will have some haloing/doubling artifacts
- What can be done about this?
  1. Match for the most red shifted wavelength because the blue shifted wavelengths are more forgiving
  2. Match for the color you care the most about

Haloing/Doubling Caused by RI Mismatch
Which oil should you start with?

Excitation Wavelength (nm)	Imaging at the coverslip	Imaging + 5µm from coverslip	Imaging + 10µm from coverslip
405	1.510	1.512	1.514
488	1.512	1.514	1.516
568	1.514	1.518	1.520
642	1.518	1.520	1.522

For imaging at 37°C increase oil by +0.008 or 4 steps in oil kit
For every + 5µm imaging into the sample go up 0.002 or 1 step in the oil kit

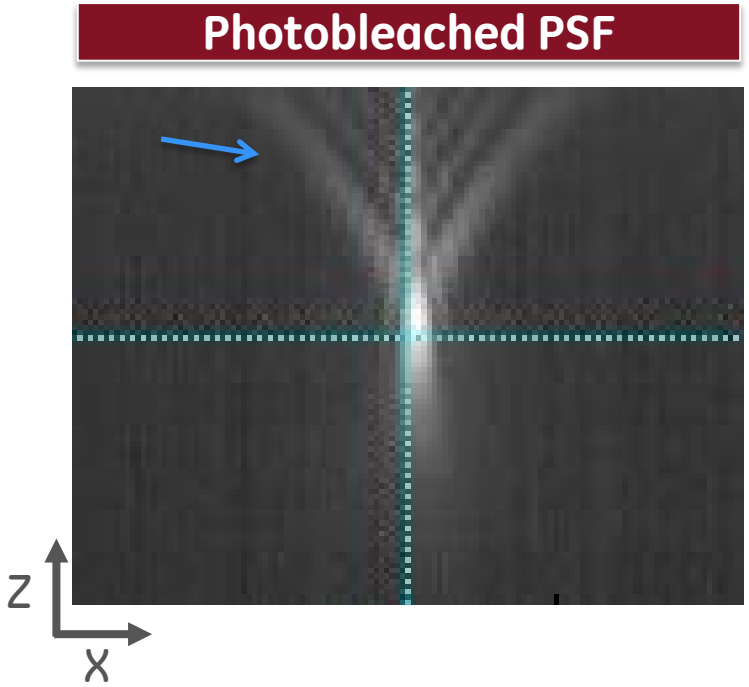
Tools to Estimate the RI Oil for your Sample
GE Oil Immersion Calculator (it's free!)



Search for 'GE oil immersion calculator' in the app store
Or visit www.gelifesciences.com/oilcalculator

Haloing/Doubling Cause 2: Photobleaching

Common Structured Illumination Artifacts
What causes the haloing or doubling artifact?



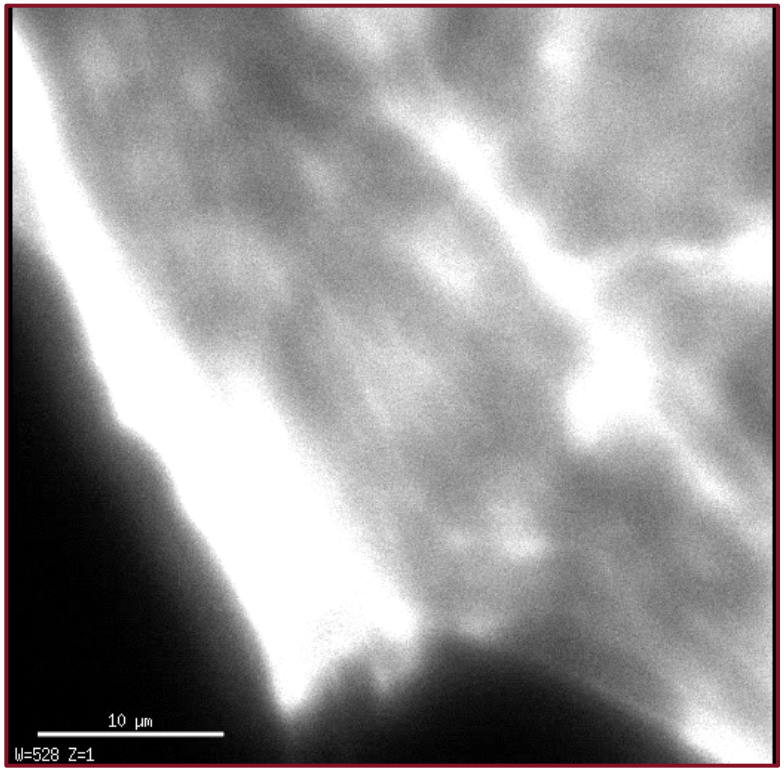
When photobleaching occurs, lose part of PSF

Haloing/Doubling Caused by Photobleaching Gives asymmetrical PSF

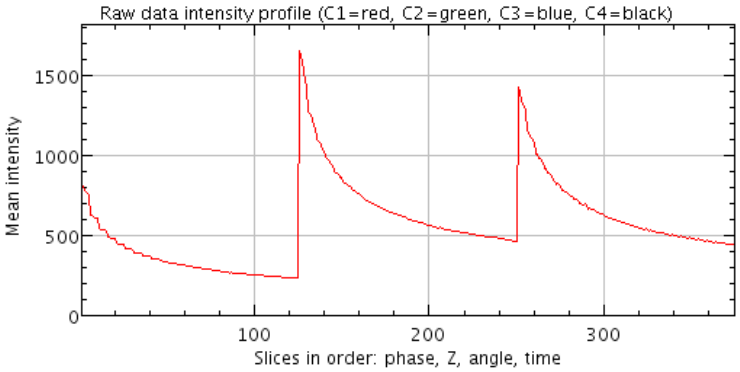
- Do not bleach more than ~30% across the stack
- Imaging parameters to change to minimize photobleaching:
  - Lower %T, increase exposure
  - Increase %T, decrease exposure
  - Smaller z stack (fewer # of optical sections)
  - Use all channel then z imaging setting
  - Use antifade reagents

Haloing/Doubling Caused by Photobleaching
Need less than 30% from top to bottom

Raw SIM Data with Bleaching



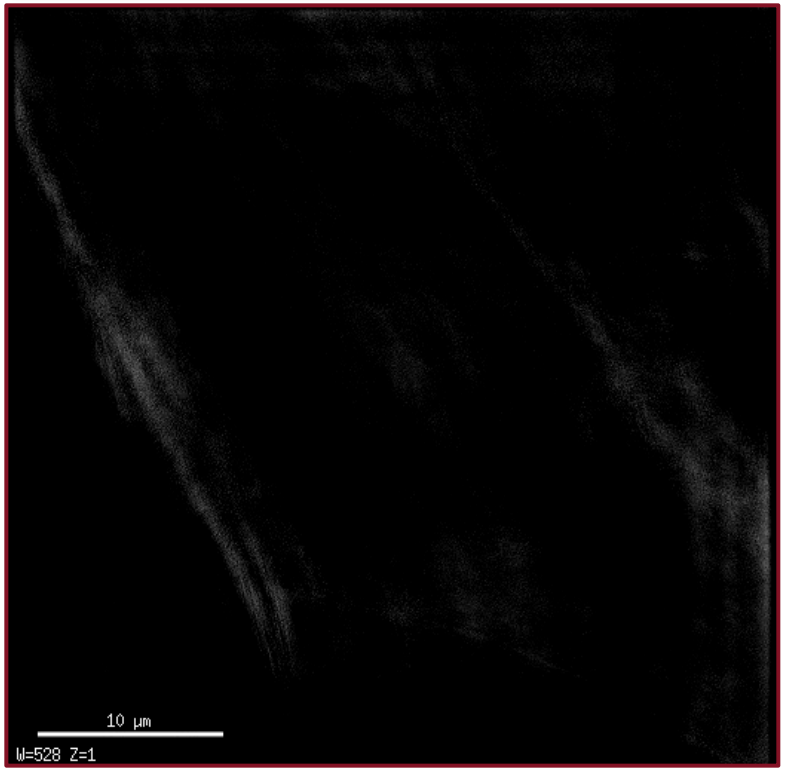
Intensity profile



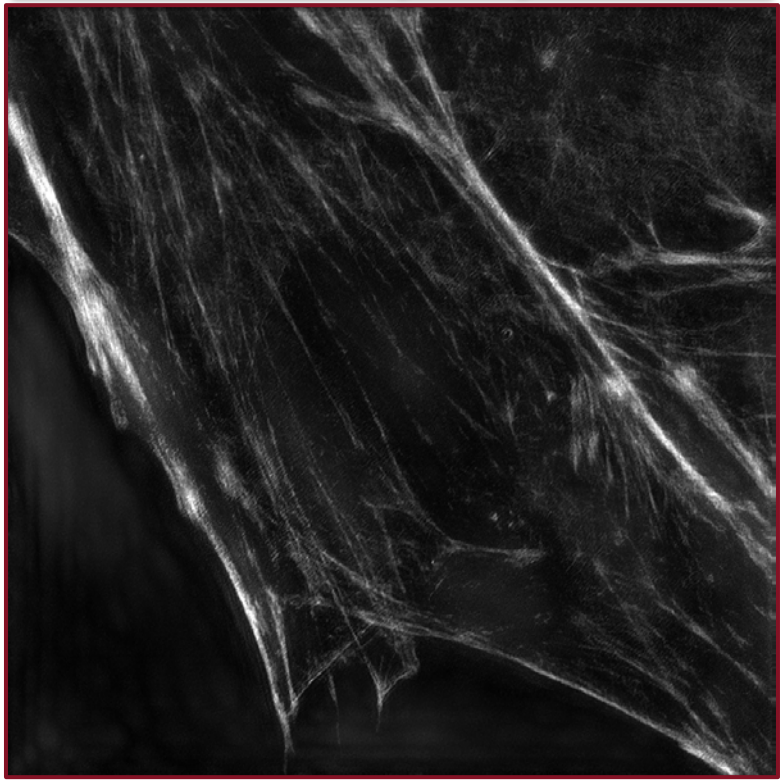
Stack starts around 1500 counts & ends at ~500 (25ms exposure, 15%T)

Haloing/Doubling Caused by Photobleaching
Need less than 30% from top to bottom

Z-stack Movie 3D-SIM



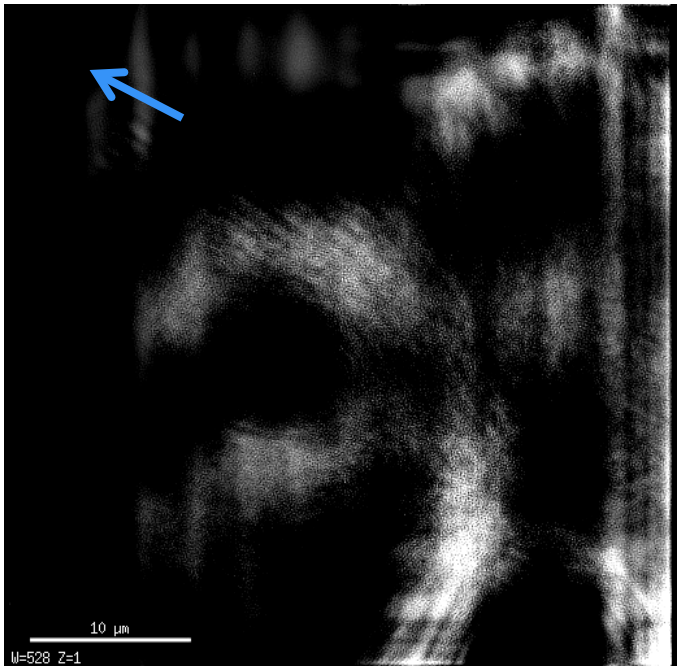
Max Intensity Projection



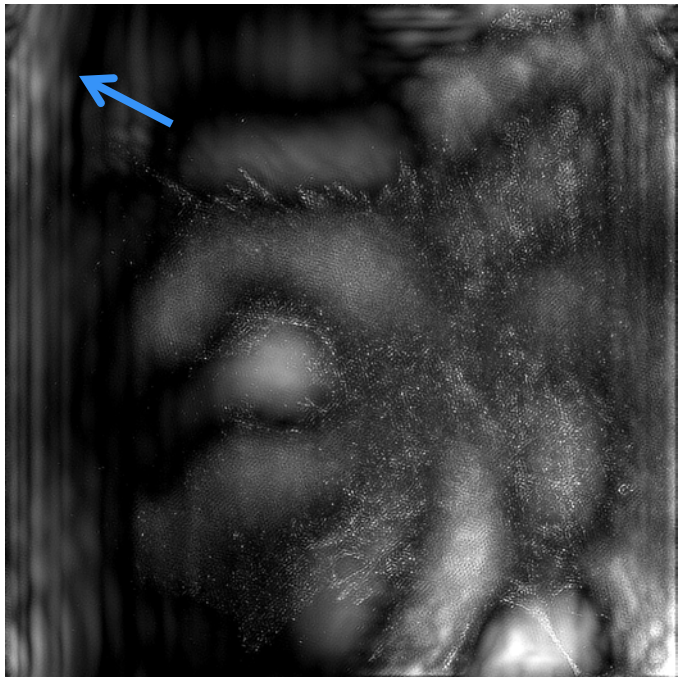
Ensure that bleaching is minimized

Haloing/Doubling Caused by Photobleaching
In extreme cases will get lines/square edges

Z stack movie



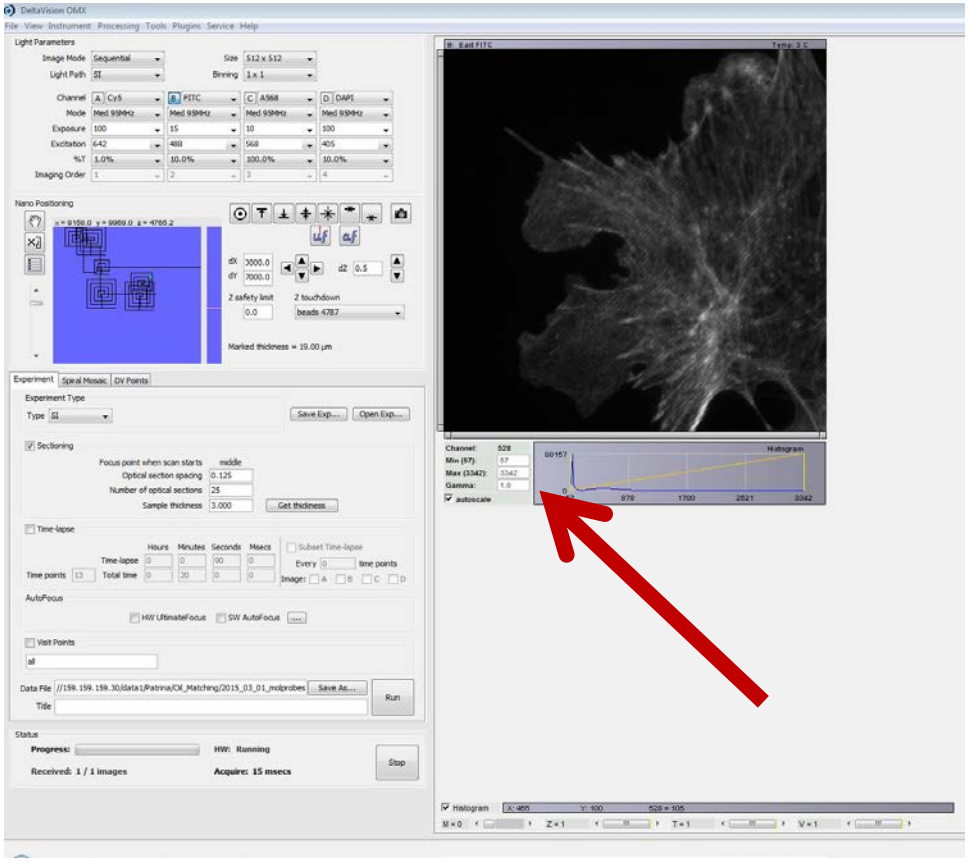
Max Projection



Sample bleached by ~90% while acquiring a 3 µm z stack

Haloing/Doubling Caused by Photobleaching
Pay attention to max counts during acquisition

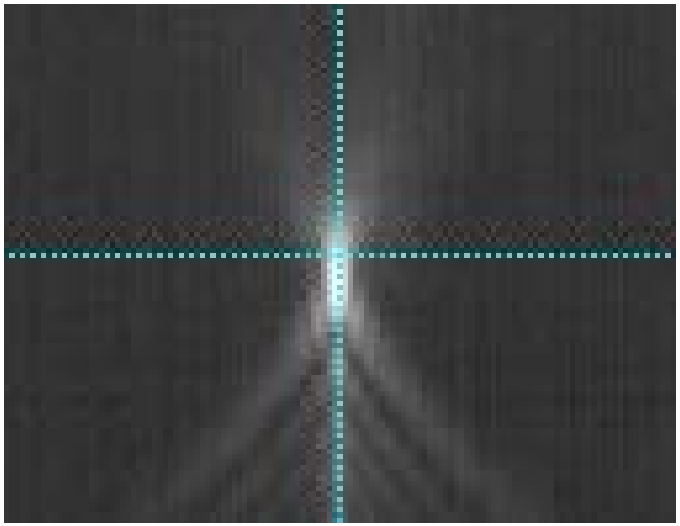
- Take note of max intensity count before starting experiment
- Watch how values change during acquisition
- Adjust imaging parameters to keep bleaching under 30%



Haloing/Doubling Cause 3: Cutting Structures off

Haloing/Doubling Caused by Cutting Off
Cutting off structures give asymmetrical PSF

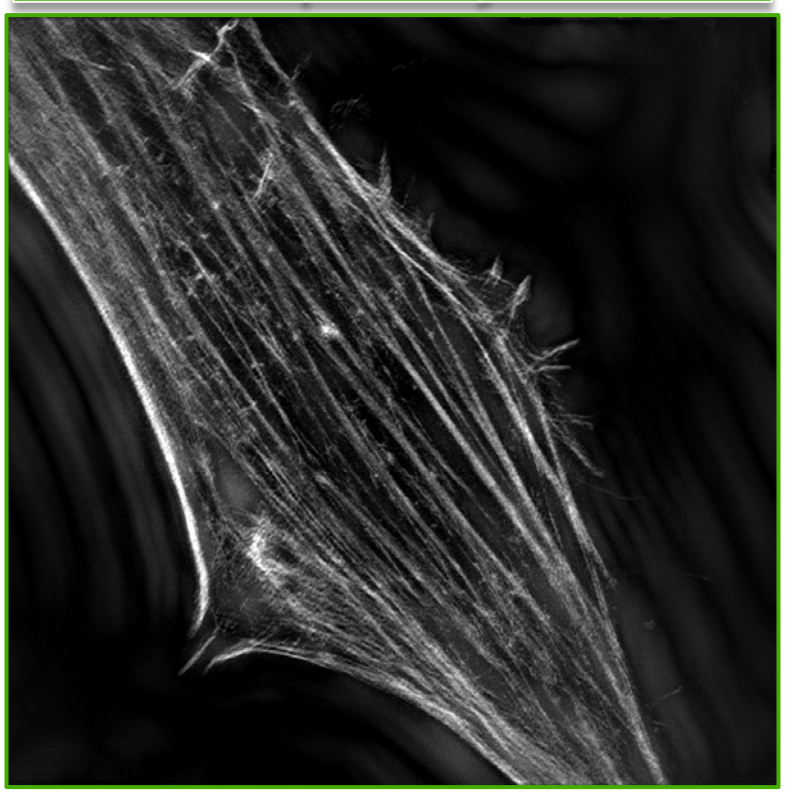
Cut off PSF



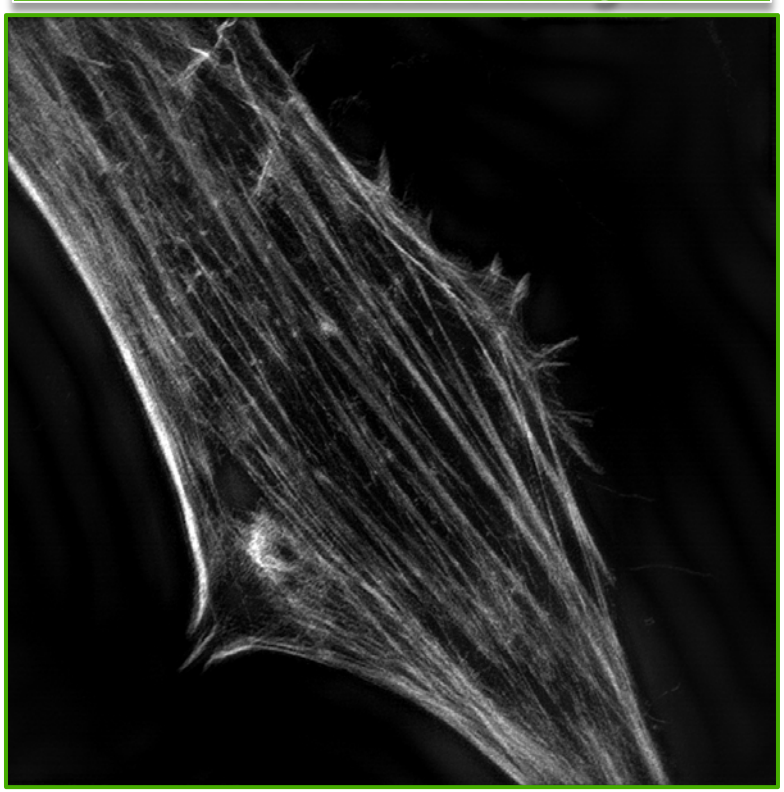
Part of the PSF is lost when cut off a bright structure

Haloing/Doubling Caused by Cutting Off
Cutting off structures give asymmetrical PSF

Z stack partially cut off



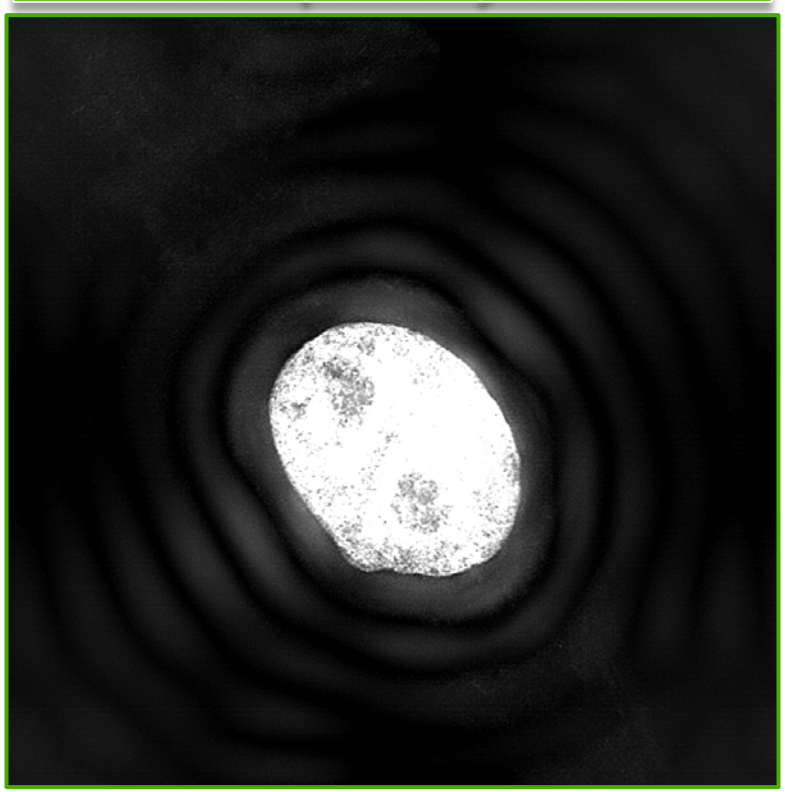
Entire structure imaged



Pro tip: Set up z stack until you can no longer see the stripe pattern

Haloing/Doubling Caused by Cutting Off
Cutting off structures give asymmetrical PSF

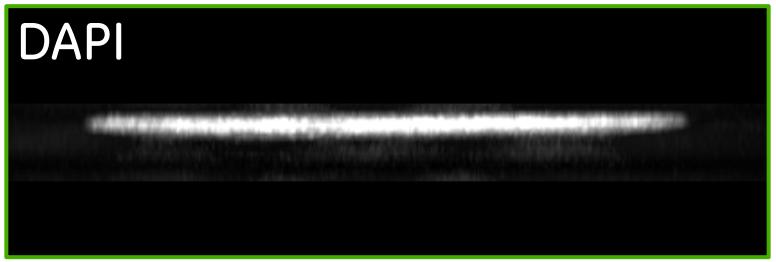
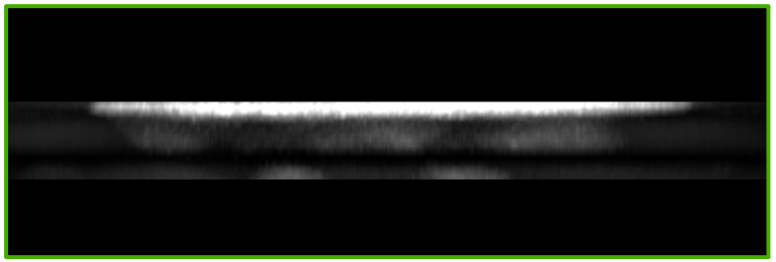
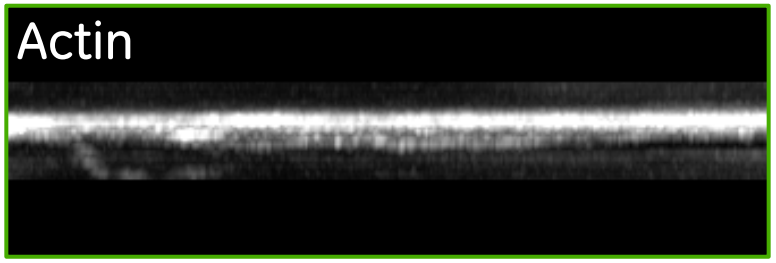
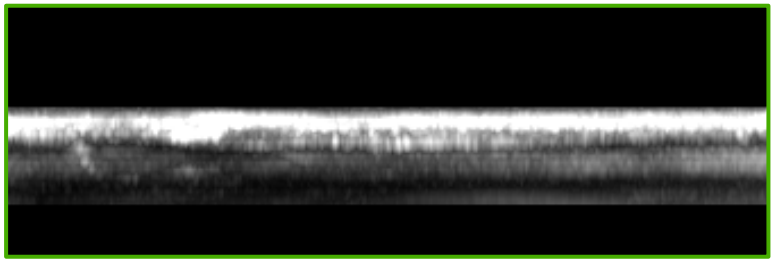
Z stack partially cut off



Entire structure imaged

Pro tip: Set up z stack until you can no longer see the stripe pattern

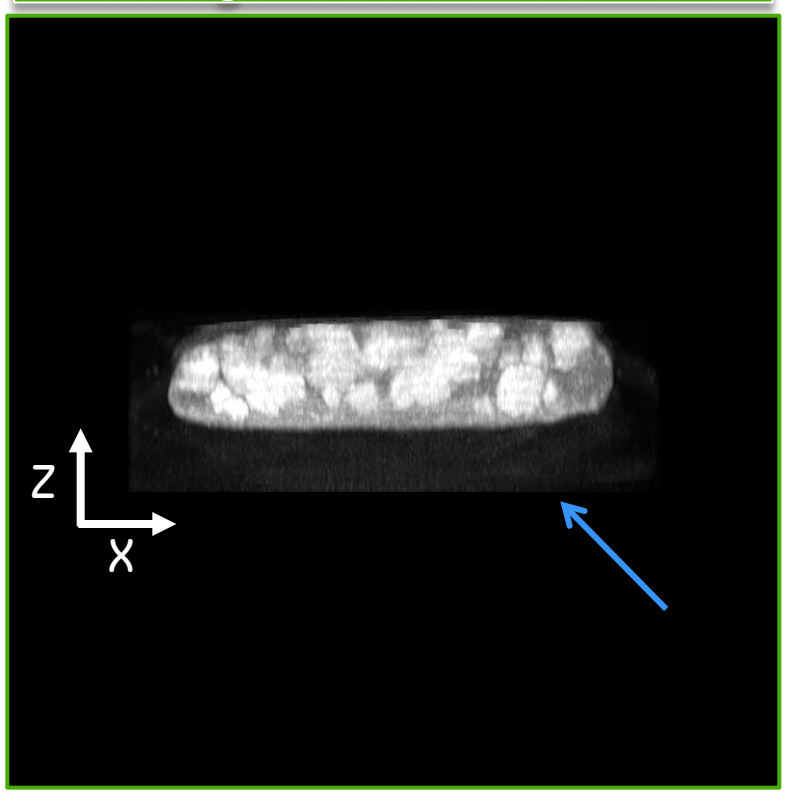
Haloing/Doubling Caused by Cutting Off
Cutting off structures give asymmetrical PSF

Z stack partially cut off	Entire structure imaged
DAPI 	
Actin 	

Ensure that entire structure is captured in Z stack

Haloing/Doubling Caused by Cutting Off
Image entire structure

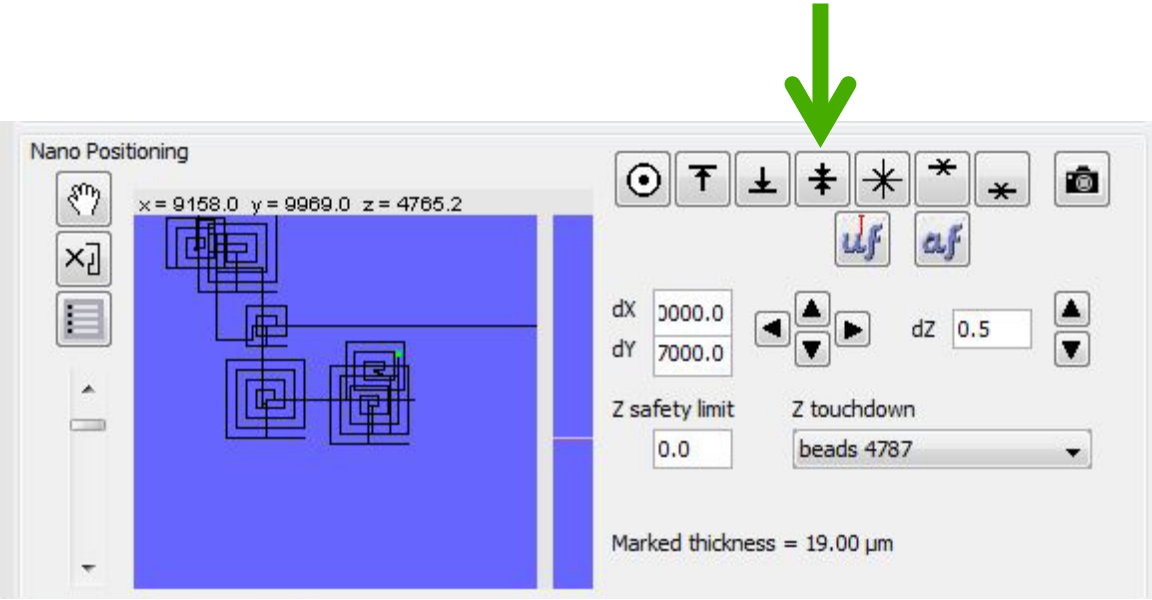
Orthogonal View - Nucleus



Part of nucleus is cut off, resulting in artifacts

Haloing/Doubling Caused by Cutting Off
Image entire structure

- Common with DAPI/nuclear labels
- Set Z stack so striped pattern is no longer visible
- Make sure click ‘visit middle’ button

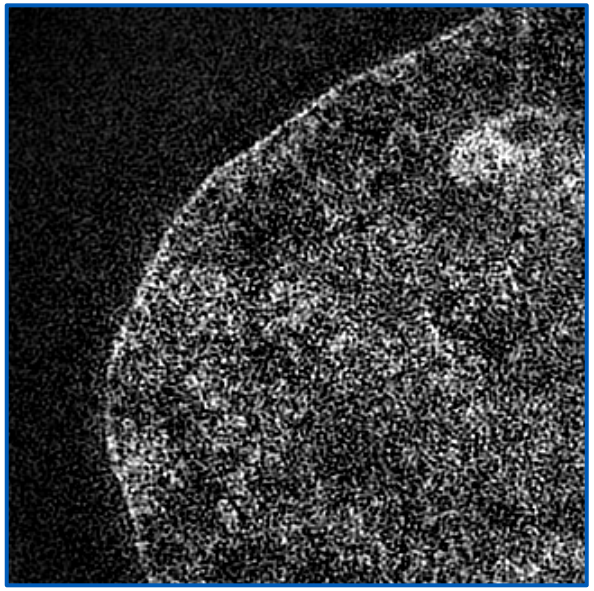


Common SIM Artifacts: Honeycomb

Common SIM Artifacts: Honeycomb
Diffuse labeling & bad S/N gives honeycomb

- Regular/hexagonal pattern
- Common with diffuse labeling or samples with bad S/N
- Caused by:
  - Little to no mid range frequencies to detect (imaging the SIM pattern)
  - Masking of the SIM pattern by high background
  - Going from Fourier space to real space

Typical honeycomb

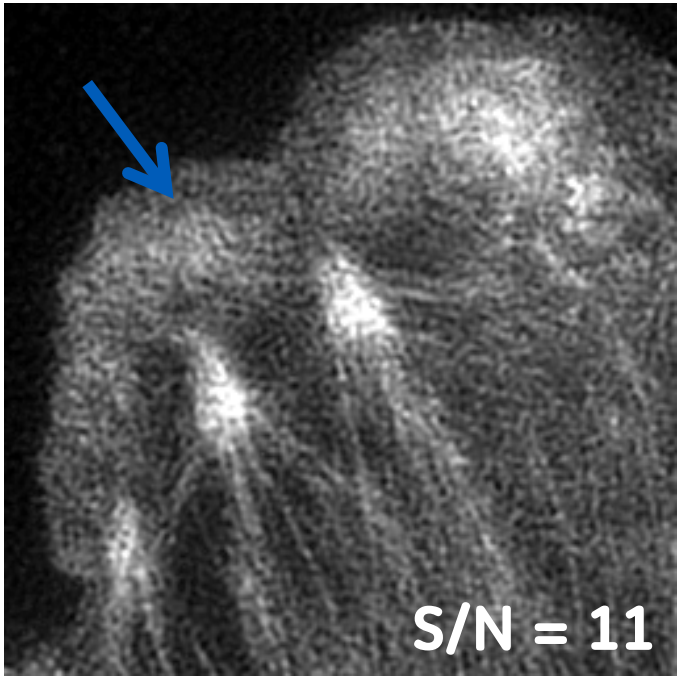


Common SIM Artifacts: Honeycomb
How to minimize the honeycomb artifact

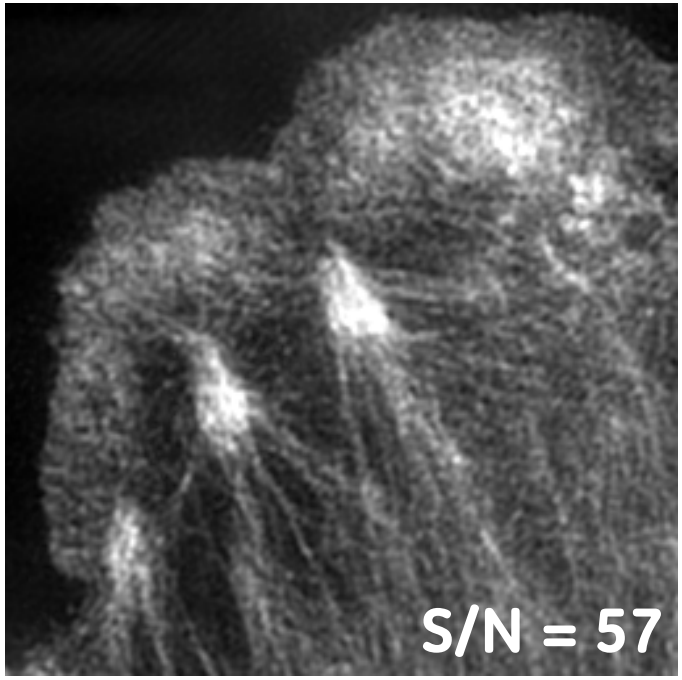
- Sometimes you can't:
  - Not all samples are appropriate for SIM
  - Diffuse labeling & high background will give honeycomb
  - SIM works best with samples that have discrete structures
- Increase S/N without photobleaching
- Increase Wiener filter constant
  - This removes high frequency data
  - Decreases resolution!
- Have good controls

Common SIM Artifacts: Honeycomb
Get best S/N to help minimize honeycomb

Low Signal to Noise



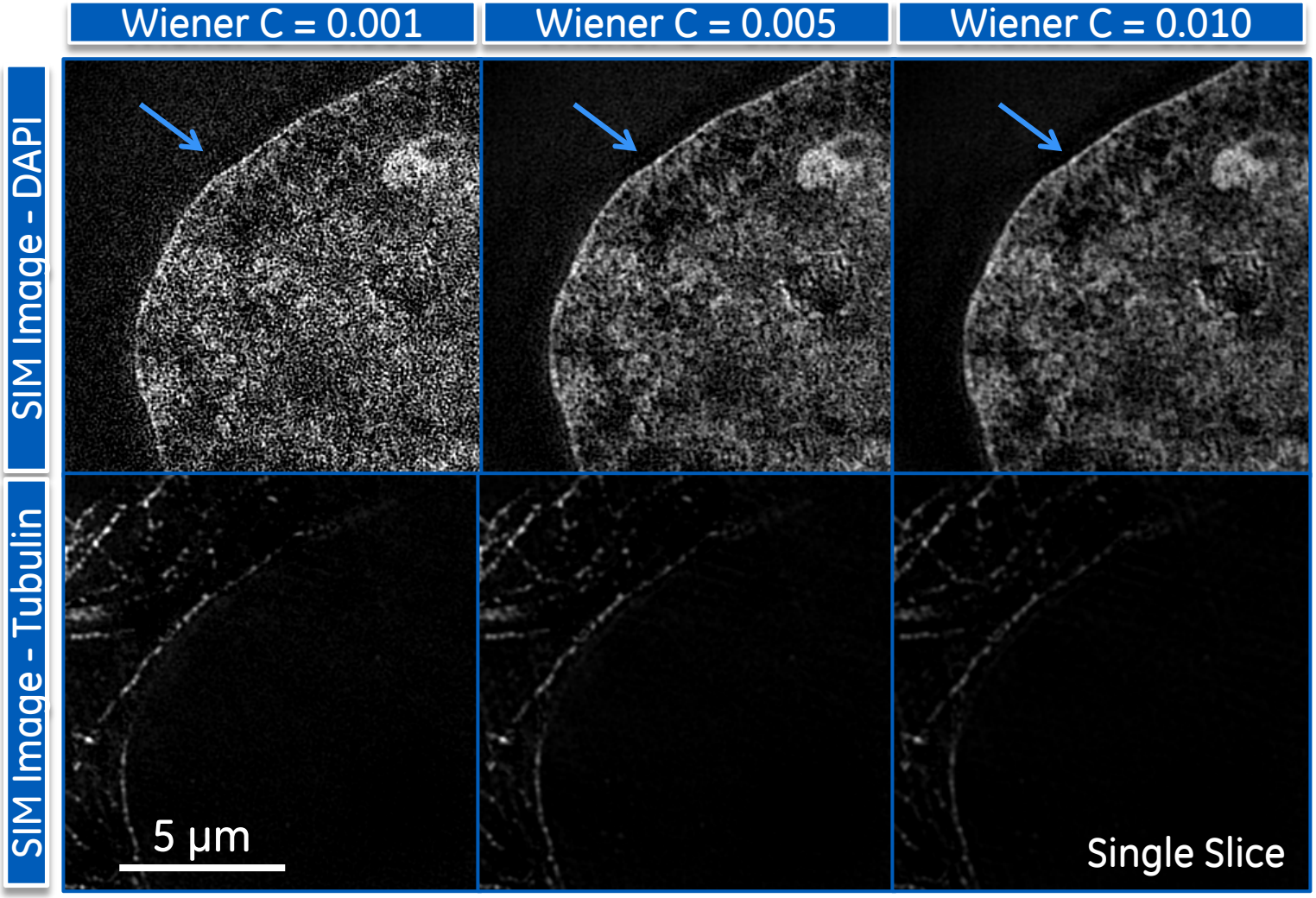
High Signal to Noise



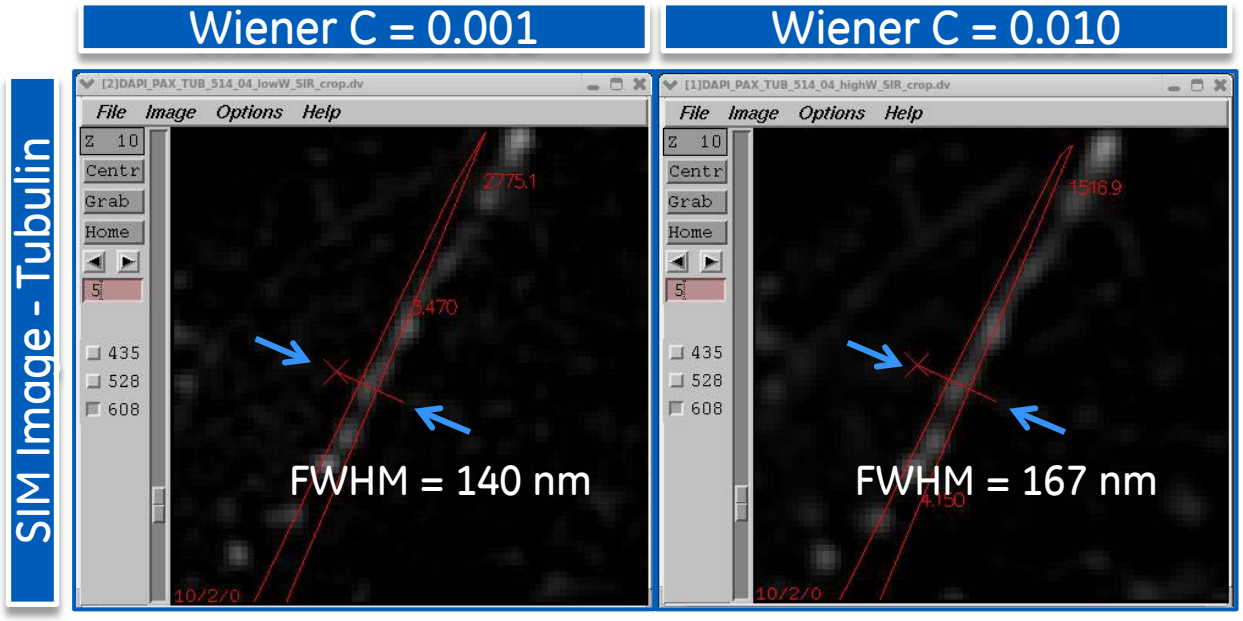
Increase %T or exposure until the background signal starts increasing

Common SIM Artifacts: Honeycomb

Increase Wiener C to reduce honeycomb



Adjusting the Wiener Filter Constant
Increasing this constant decreases resolution



Reconstructing with the higher Wiener filter constant resulted in a loss of 27nm in resolution!

Common SIM Artifacts: Honeycomb
Affect of using different Wiener Filter Values

- As this constant is increased:
  - The honeycomb pattern starts to decrease
  - The DAPI signal starts to look smoother and less noisy
- **By increasing this constant you remove high frequency info! You lose resolution by doing this!**
- Typical values are 0.001-0.003
- For diffuse labeling or bad S/N use 0.005 or higher

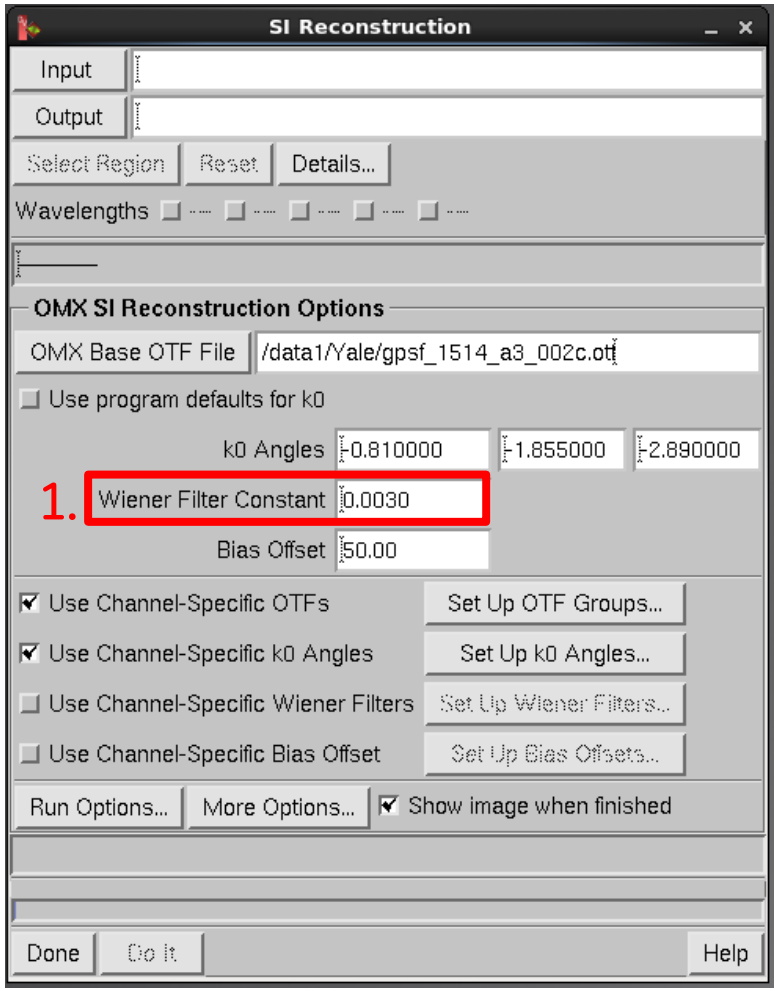
Adjust to Wiener Filter to Minimize Honeycomb
How to adjust the Wiener filter constant

1. In SI reconstruction window adjust values in the 'Wiener Filter Constant' box

2. For data with good signal to noise ratio typical values are 0.001-0.003

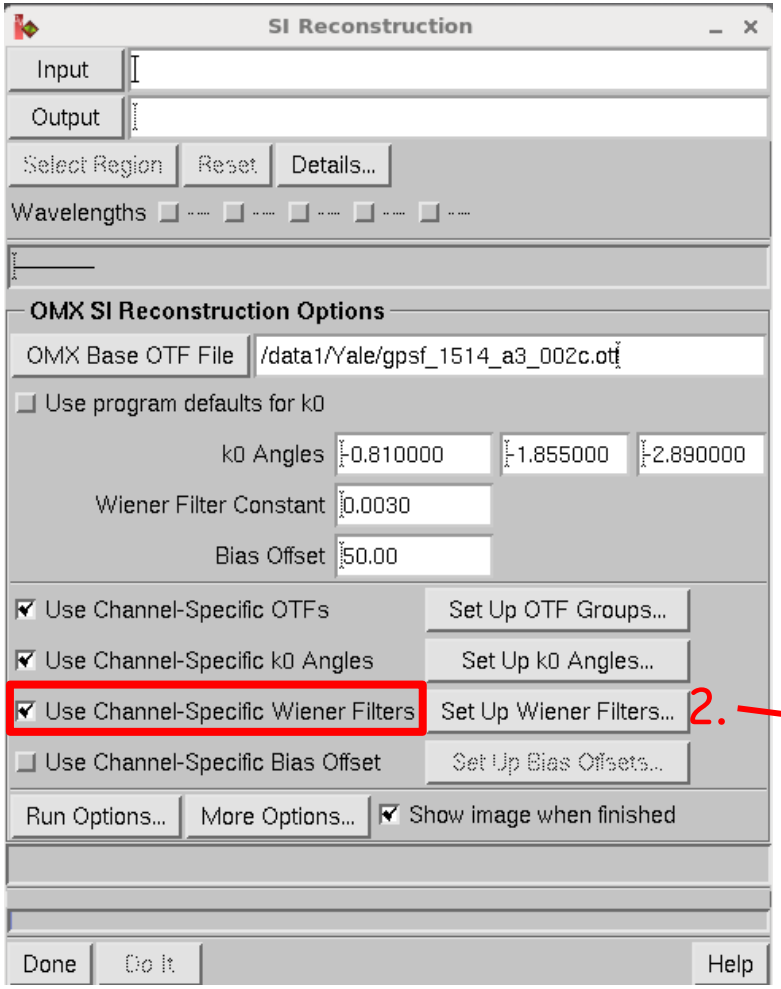
3. Adjusting values in this box applies the same Wiener Filter Constant to all channels

4. For structures with diffuse labeling or bad signal to noise, try increasing this value to 0.005 or higher

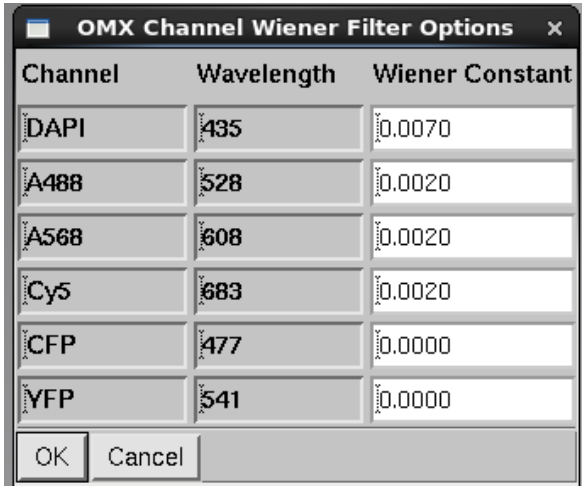


Adjust to Wiener Filter to Minimize Honeycomb

How to use channel specific Wiener Filters



1. In the SI Reconstruction tool, check the 'Use Channel-Specific Wiener Filters'
2. Click on 'Set Up Wiener Filters' to open the OMX Channel Wiener Filter Options
3. Input Wiener Filter constants for each channel, i.e. higher values for diffuse signals (DAPI) & lower values for other channels



Common SIM Artifacts: Honeycomb
How to minimize the honeycomb artifact

- Not possible with all samples!
- Increase signal to noise without photobleaching
- For diffuse labeling increase Wiener filter constant
  - Use 0.001-0.003 for normal samples
  - Use 0.005+ for diffuse labeling (results in a loss of resolution!)
  - **Report this value in the methods section of your paper!**

SIM Summary

Tips for Successful SIM Imaging
Summary

- 3 things to master:
  1) Determining the best immersion oil for your sample
  2) Adjusting imaging parameters to minimize bleaching
  3) Imaging the entire structure
- Get highest signal to noise ratio possible without photobleaching

Have questions? Need help? Need support?
Call or email me!

Patrina.pellett@ge.com

713-826-0697

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GE Healthcare Bio-Sciences Corp.
100 Results Way
Marlborough, MA 01752 USA
www.gelifesciences.com