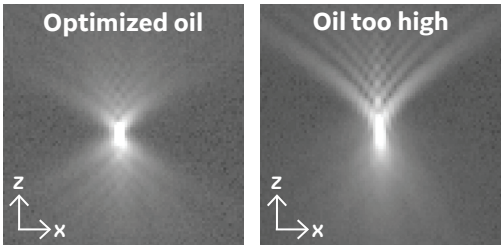
DeltaVision™ OMX V4/SR

Immersion Oil Optimization

Spherical aberration (SA) is one of the most common aberrations in fluorescence light microscopy and it leads to degradation of image quality and significantly decreased image contrast. SA can be especially challenging because, even on the most optimized microscope, SA can easily be induced by a refractive index (RI) difference between the objective immersion medium and the sample.

With oil immersion objectives, this type of aberration can be minimized by selecting immersion oil with an RI that closely matches the sample characteristics. Continue reading to learn how to recognize SA in your sample and how to optimize oil selection to minimize SA and maximize image quality.



Point Spread Function (PSF) imaged with optimized oil (left) and mismatched oil (right). The PSF, measured by imaging a 100 nm bead, represents signal emitted from a point source and is a good way to visualize aberration.

Factors that affect optimal oil refractive index

- **Temperature:** Due to variations in viscosity with changing temperature, the immersion oil RI for a sample at 25°C will differ from what should be used at 37°C. For each 3°C increase in temperature, oil RI should be increased by ~0.002.
- **Coverslip Thickness:** High resolution objectives are corrected for #1.5 coverslips (170 µm thick). Using any other thickness coverslip will require a large change in oil RI to compensate (i.e. #1.0 coverslip requires increasing RI by ~0.010, which is a lot!).
- **Sample Thickness:** With oil objectives, there is only a thin region where the PSF will not exhibit SA and, ideally, this region should be centered at the middle of the sample. For each 5µm increment into the sample, oil RI must increase by 0.002 (which corresponds to one step in the GE immersion oil kit).
- **RI of the Mounting Medium:** From aqueous medium (~1.33) to glycerol based mountants (~1.47), mounting medium RI varies widely. This variation will also change the oil RI required for optimal imaging.
- **Excitation Wavelength:** The amount of SA present will vary slightly in each channel. Best practice for Structured Illumination (SIM) imaging is to optimize oil for the longest wavelength in an experiment.

Selecting the correct refractive index immersion oil

With so many variables to consider, selecting the correct RI oil may seem complex. To simplify oil selection, GE has a free Oil Calculator App. Input information about your unique experiment and the calculator generates a recommended RI oil to use.

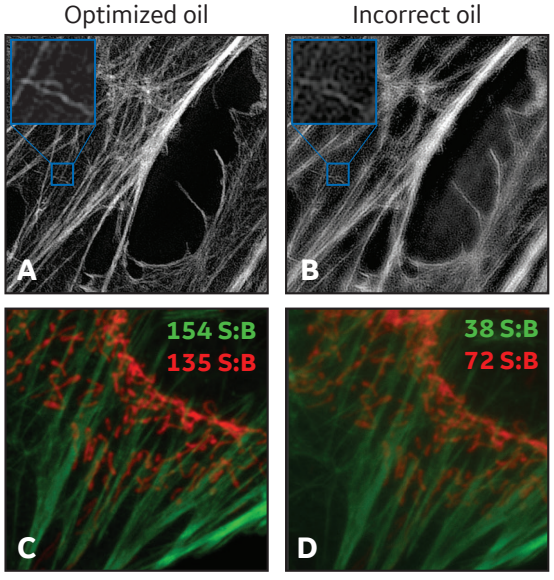
The Oil Calculator App[1] is compatible with Apple and Android devices and is available as a Web App[2]. For more information, visit www.gelifesciences.com and search for "Oil Calculator."

How much does oil selection really affect image quality?

The short answer is, A LOT! Spherical aberration will dramatically affect the quality of SIM reconstruction as well as the quality of the raw data collected.

Reconstruction artifacts and/or poor reconstruction: Honeycombing artifacts are the most common challenge in SIM imaging. If honeycomb artifacts are present in reconstructed data, it is an indication that there is an asymmetry of the signal within the raw data, most commonly caused by oil mismatch or photobleaching. The reconstructed images at right (A and B), were imaged identically except A was acquired with optimized oil and B with incorrect oil. Notice that cellular structure is masked in image B. If honeycombing is present but oil matching is correct, check for photobleaching, improper z stack centering or other signal variations through the z stack.

Decreased image contrast in raw data: The widefield images (C and D) at right were acquired with identical parameters except C was acquired with optimized oil and D with incorrect oil. As you can see from the S:B displayed for each channel, imaging with an incorrect oil RI leads to significantly decreased S:B (~4 fold in green and ~2 fold in red). In a real experiment, one would have to increase laser power or exposure time to achieve the same S:B, increasing the likelihood of photobleaching and phototoxicity.



[1] http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-us/service-and-support/immersion-oil-calculator/

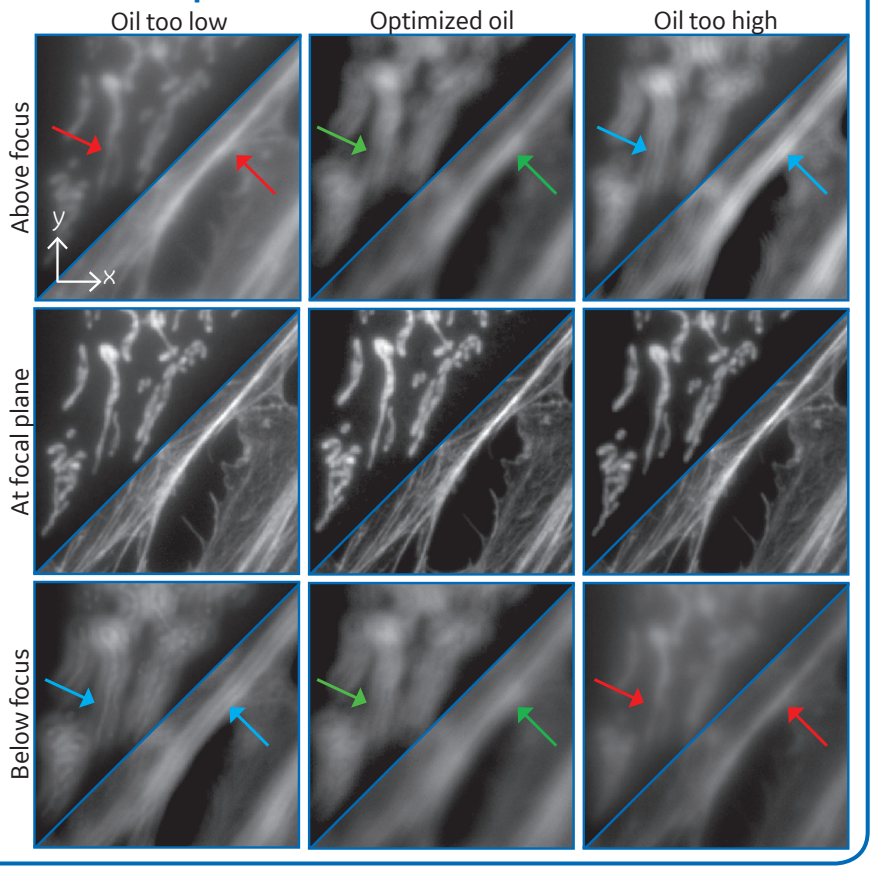
[2] http://www.gelifesciences.com/webapp/wcs/stores/servlet/catalog/en/GELifeSciences-us/service-and-support/immersion-oil-calculator-web-app/

How will I recognize spherical aberration in MY sample?

The images at right show how SA presents in an image with puncta (top left in each panel) or fibrous structures (bottom right). This two channel data was aquired of the same cell with three different RI oils; too low for the sample, optimized and, too high. Snapshots of each image stack are shown above, below and at the focal plane.

When you're optimizing oil for your sample, keep the following in mind:

- SA isn't always obvious at the focal plane (center row). Examine z sections above and below focus to see it.
- SA looks different depending on the structure:
  - *Puncta:* look for airy disks (concentric rings eminating from a point source) above and below focus. If they are visible on one side (blue arrows) and not the other (red arrows) or are heavier on one side, oil is not matched well.
  - *Fibers:* look for ringing (doubling of fiber in out-of-focus planes) around the fiber. If ringing is uneven above and below focus, oil is not matched well.
- When oil is matched well (center column) notice that you see airy disks/ringing around objects on BOTH sides of the focal plane (green arrows). This is what we strive for when matching oil!



Evaluating oil selection

The oil calculator provides a great starting point but you may have to optimize oil RI with a new sample or when experimental conditions change. Follow the steps below to visualize SA in raw data and determine how oil should be adjusted to minimize SA.

1. **Adjust image display scaling:** To better visualize low intensity patterns that are a sign of SA, click on the ***Image Histogram*** icon in the image window and adjust the third number on the ***Display Min/Max/Exp*** line from 1.0 to 0.3 - 0.5.
2. **Visually evaluate spherical aberration:** Use either method below, depending on what you're most comfortable with. If evaluating SA with SIM raw data is challenging, consider generating a widefield image first (***Process | Generate Widefield from SI Image***).
   - Evaluate SA in the **Image Window**
     1. Scroll up and down through z and look for airy disks around puncta and ringing around fibers.
     2. Zoom and turn channels on/off as needed. Does the spread of light look even above and below the focal plane?
   - Evaluate SA in the ***Orthogonal Viewer***
     1. In the image window, select ***Tools | Orthogonal Viewer***.
     2. In the ***Orthogonal Viewer*** window, select ***Options*** and verify that "Coverslip at bottom right" is selected.
     3. Position the crosshair over a structure of interest and examine the y/z and x/z views. Does the spread of light look even above and below the focal plane?
3. **Adjust oil refractive index:** If you see SA in your image, remember that *High is too High and Low is too Low*.
   - If the spread of light is heavier *above* the focal plane, then *High is too High* and you should step *down* in oil.
   - If the spread of light is heavier *below* the focal plane, then *Low is too Low* and you should step *up* in oil.
   - The magnitude of the difference between the spread of light on either side of the focal plane indicates how far you should adjust oil RI. If there is only a slight difference, move only one or two oils. If it is significant, take larger steps.

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