

Point Spread Function on Zeiss Confocal Microscopes

Acquire Image:

Use slide from De. PSF Beads Green 0.17 micrometers. 170nm.

Set correction collar if objective has one

Make sure to use correct immersion solution [Water not "regular" oil if it is a water objective]

Remove DIC Slider

Pinhole needs to be Open

1. Since beads are so small and hard to see first use Standard slide to get focus.
2. Change to bead slide and focus on beads
3. Need zoom of at least 4x
4. Use Profile to check for saturation. Adjust detector gain until levels are around 100
5. Use Info button to check scaling. Want scaling to be 0.05
6. Move stage so the bead you are measuring is always in the center of the view field. Do not move around crop box and center beads within that.
7. At 4x image, click crop [gives you 8x zoom].
8. Click Edit ROI. Draw box around just one bead
9. Scan fast x/y
10. Set Z stack. Use mark first/last to set up. Slice interval of 0.1
11. Image. This will scan bead much faster
12. You will need to do this with at least 5 beads.

Analysis of Bead Images

Lateral Resolution:

Use Fiji

File → Open

Select File

Image → Color Channels Tool → Switch to Gray Scale → Grays

Image → Zoom to zoom in image

Find Z slice with most in focus position

Draw line through a bead

Analyze → Plot profile

Click "List" in graph

Check for Max value

Take half of max value

Find values for that number in Plot Values

Subtract those numbers

This is your Lateral Resolution

Information when taking image: 1.1 NA, 488nm, BP 500-550 filter. 525 emission.

Lateral Resolution is emission :

Axial Resolution:

Use Fiji

File → Open

Select File

Image → Color Channels Tool → Switch to Gray Scale → Grays

Image → Zoom to zoom in image

Find Z slice with most in focus position

Plug Ins → Transform → Transform J → Transform Rotate → Rotate Y axis 90 degrees

Plug Ins → Transform J → Transform J Scale → Factor 10 scale

Draw line through a bead

Analyze → Plot profile

Click "List" in graph

Check for Max value. Also if min values on either side are not one you need to subtract that to get your value

Take half of max value. If above you had to subtract background you must add that number back on to get the value.

Find values for that number in Plot Values

Subtract those numbers

This is your Axial Resolution

Axial Resolution