Light Microscopy Research Group (LMRG)

Study #2 Results

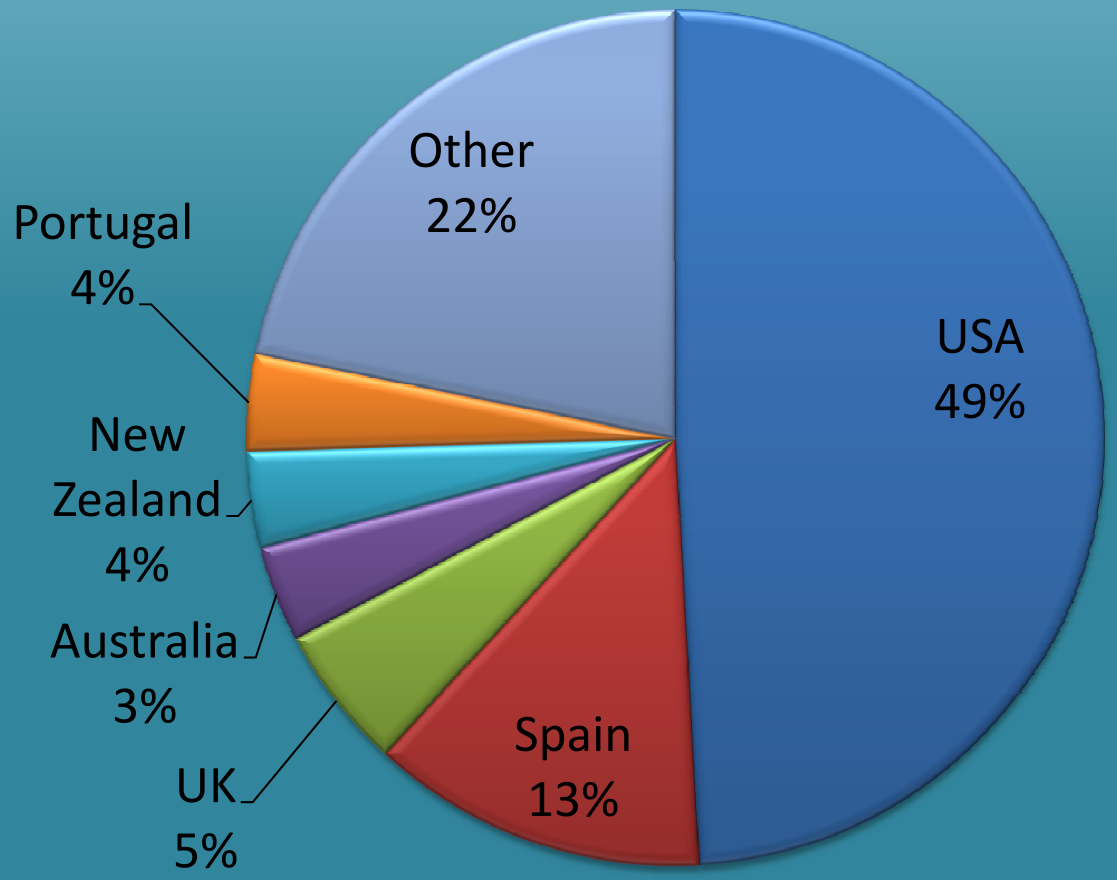
Claire M. Brown

LMRG Chair

Study Participants

- Responses from:
  - 124 laboratories
  - 24 countries
- Data Received from:
  - 55 laboratories
  - 18 countries
- 44% Response Rate

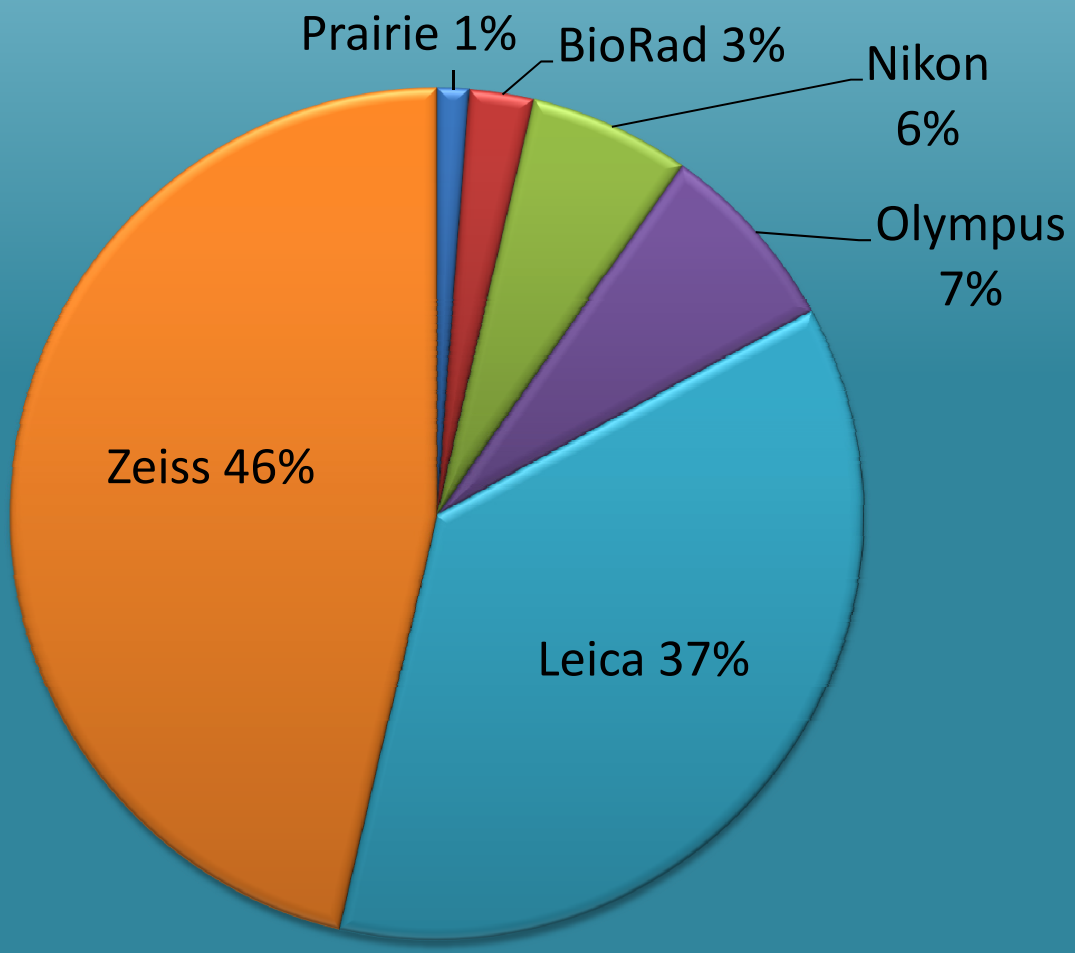
Study Participants
18 Countries



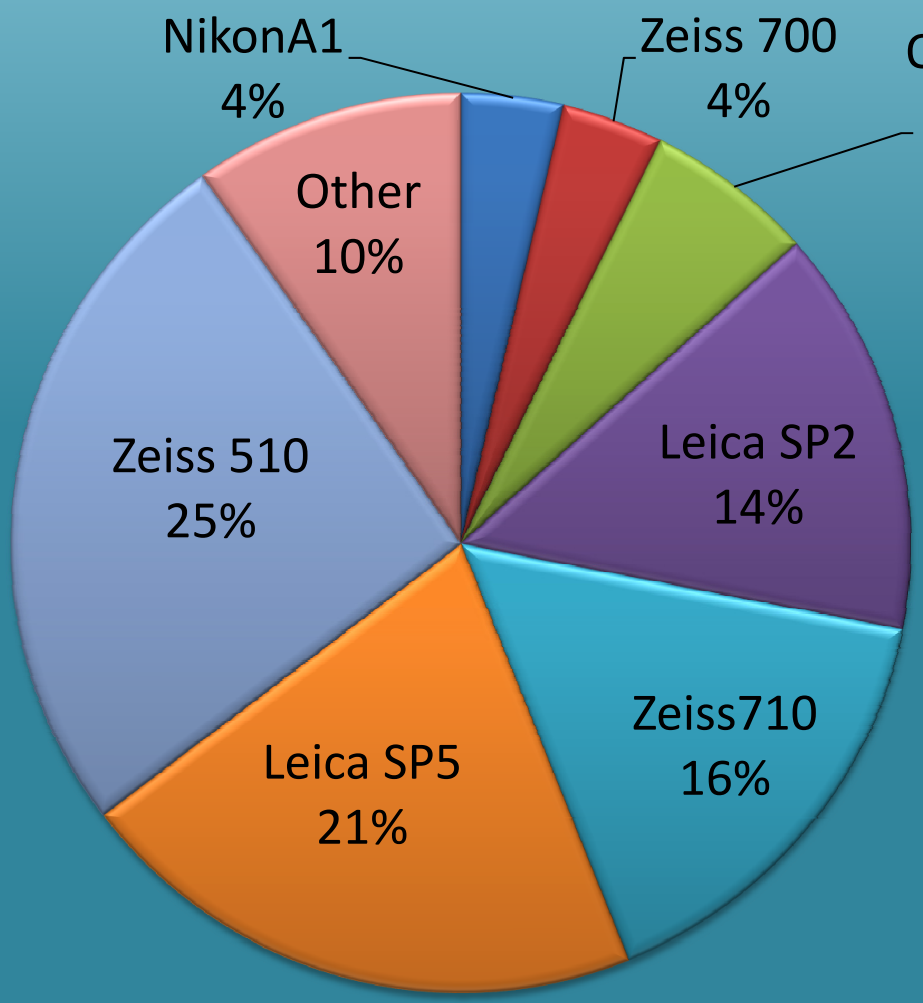
Other Countries:

Canada
Belgium
Finland
France
Germany
Israel
Italia
Norway
Singapore
Sweden
Switzerland
The Netherlands

Company Representation



Microscope Model



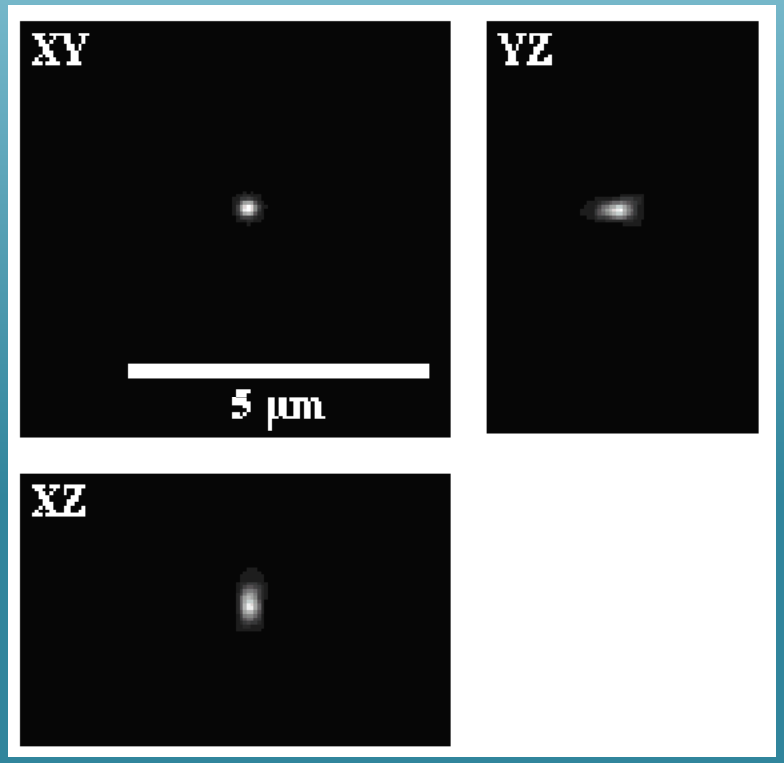
Other Models:

BioRad Radiance 2000
Leica SP1
Nikon A1R
Olympus FV500
Prairie Ultima-IV
Zeiss 780

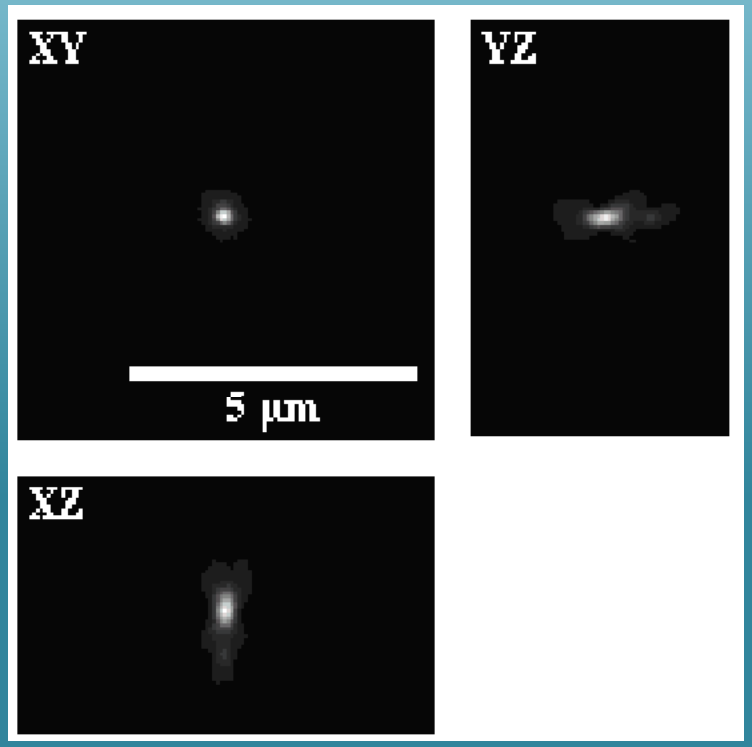
Point Spread Functions (PSF)

- 49 Participants
- 140 lenses!
- 290 point spread functions

Good PSFs

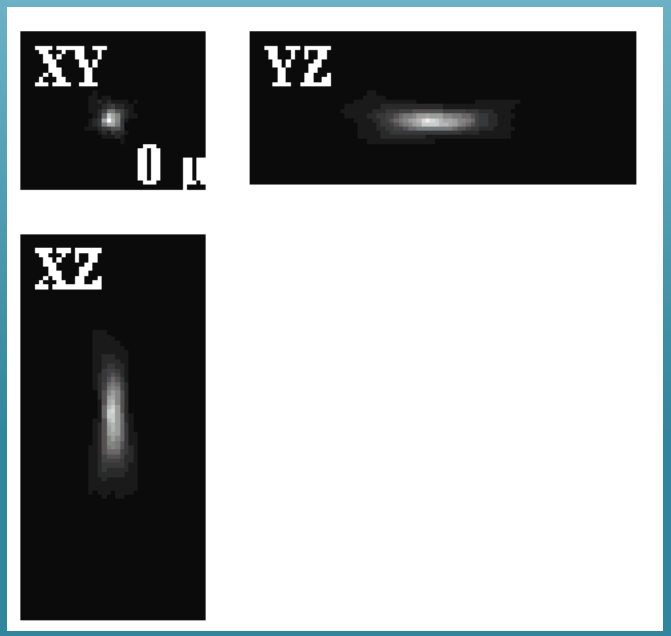


63x Oil/1.4 NA
Pinhole 1 Airy Unit

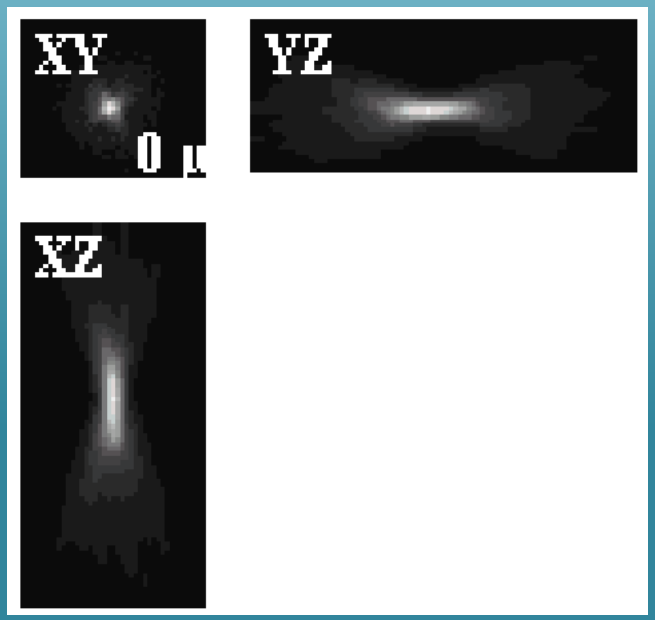


63x Oil/1.4 NA
Pinhole 5 Airy Unit

Good PSFs

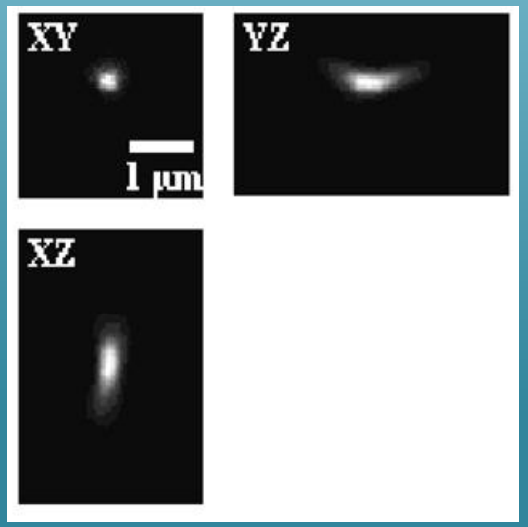


20x Water/0.7 NA
Pinhole 1 Airy Unit

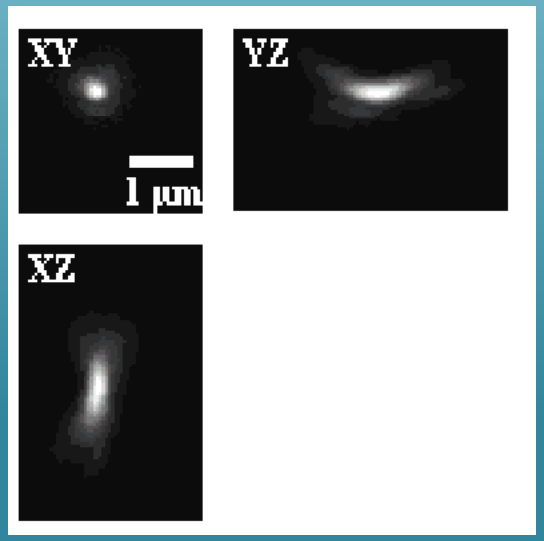


20x Water/0.7 NA
Pinhole 5 Airy Unit

Aberrations - Coma

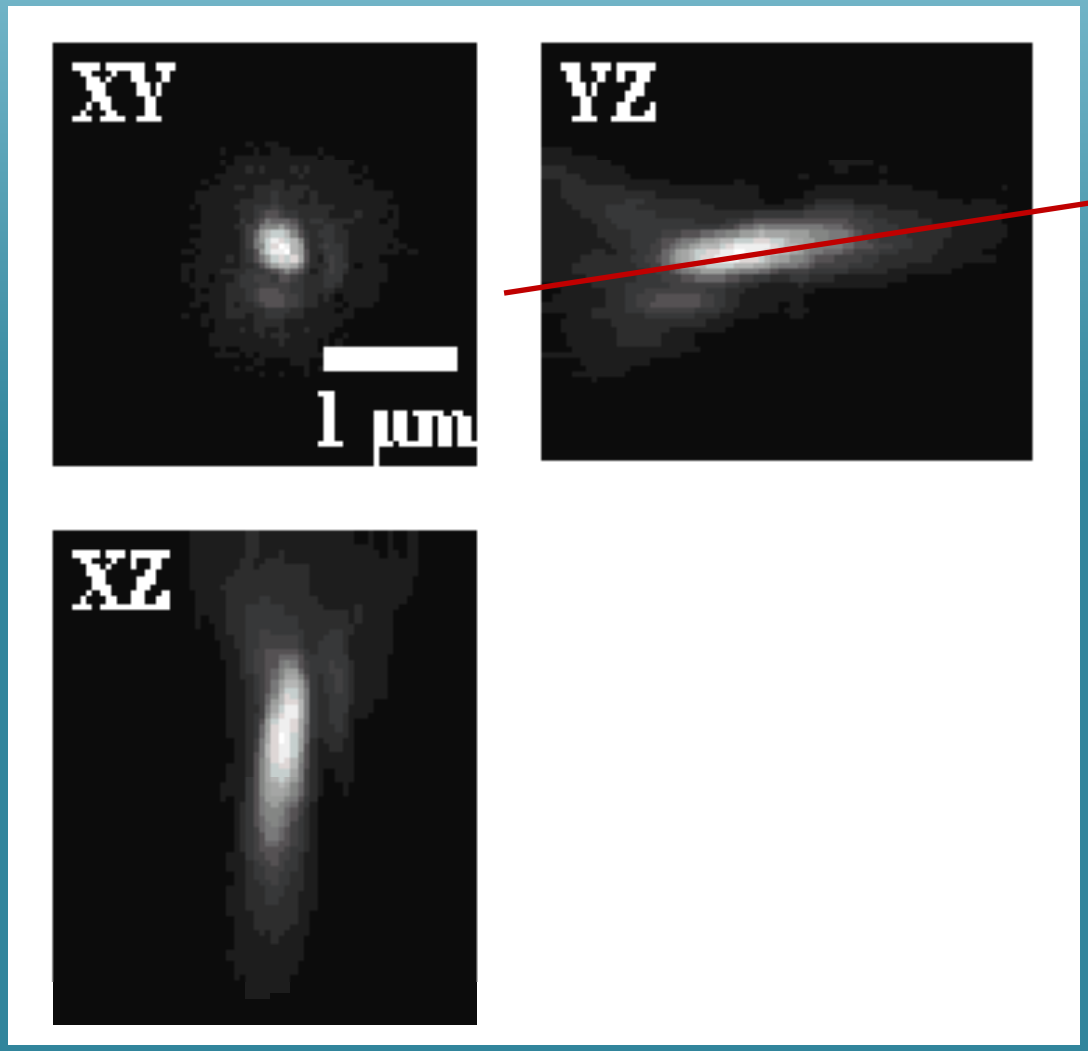


63x Oil/1.4 NA
Pinhole 1 Airy Unit

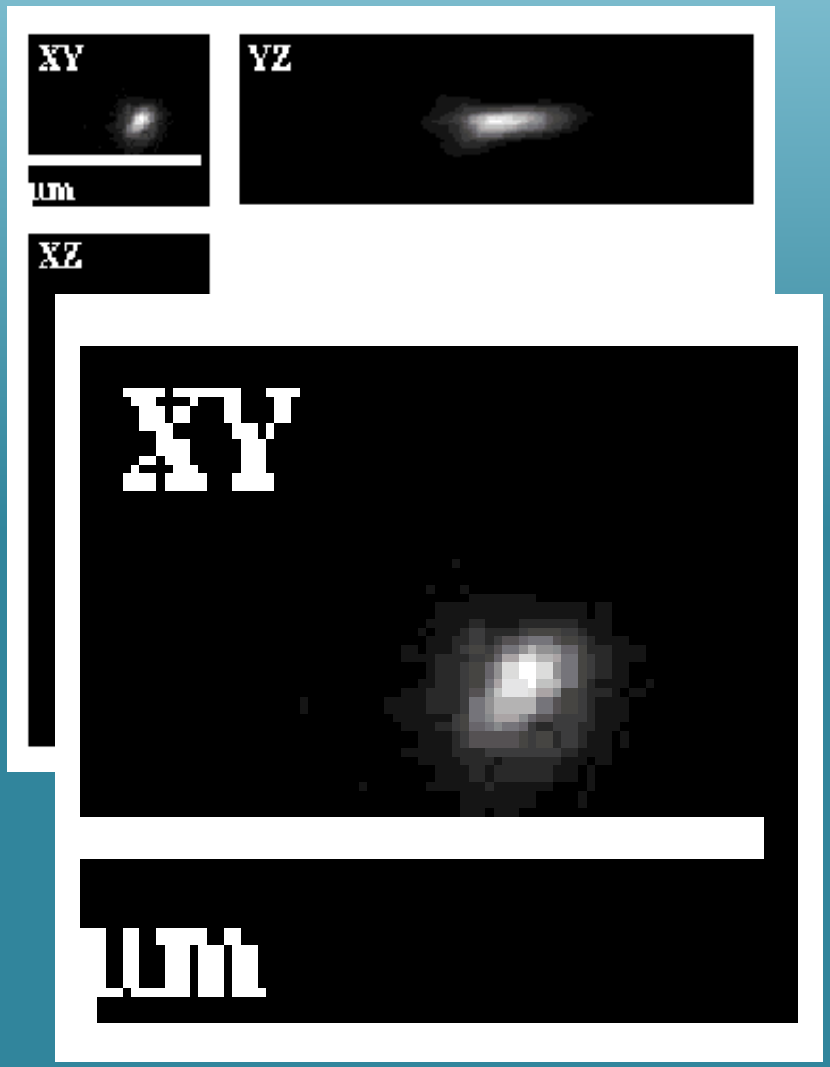


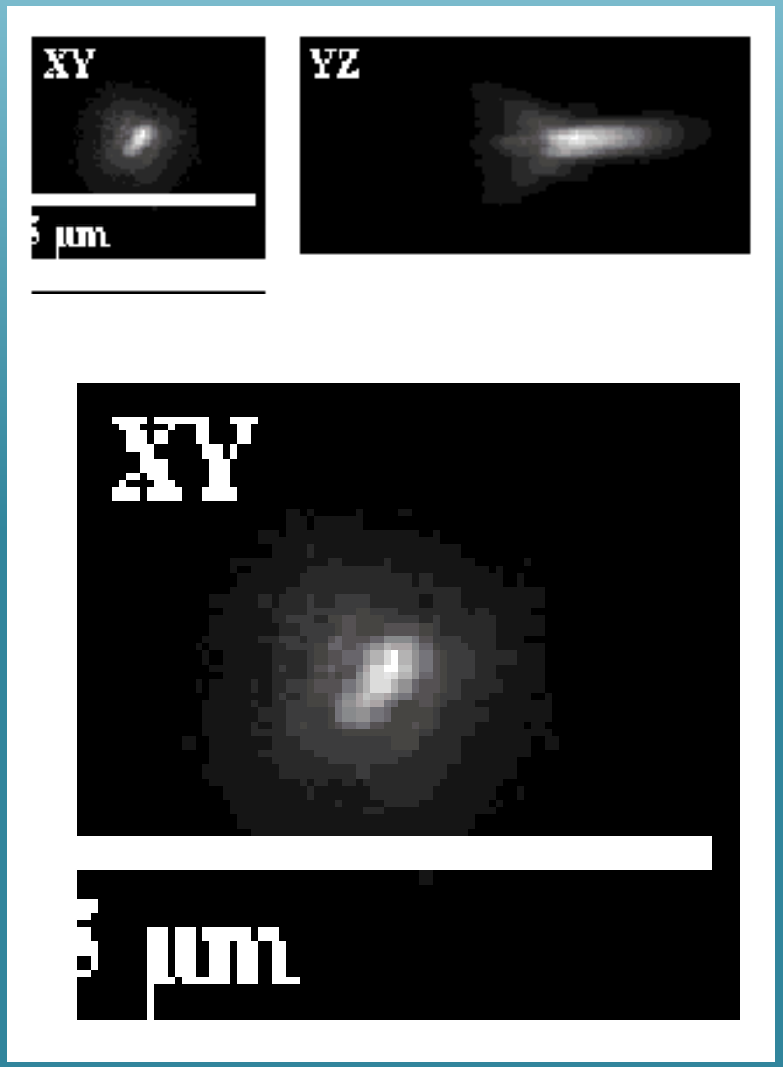
63x Oil/1.4 NA
Pinhole 5 Airy Unit

Aberrations – Stage Drift

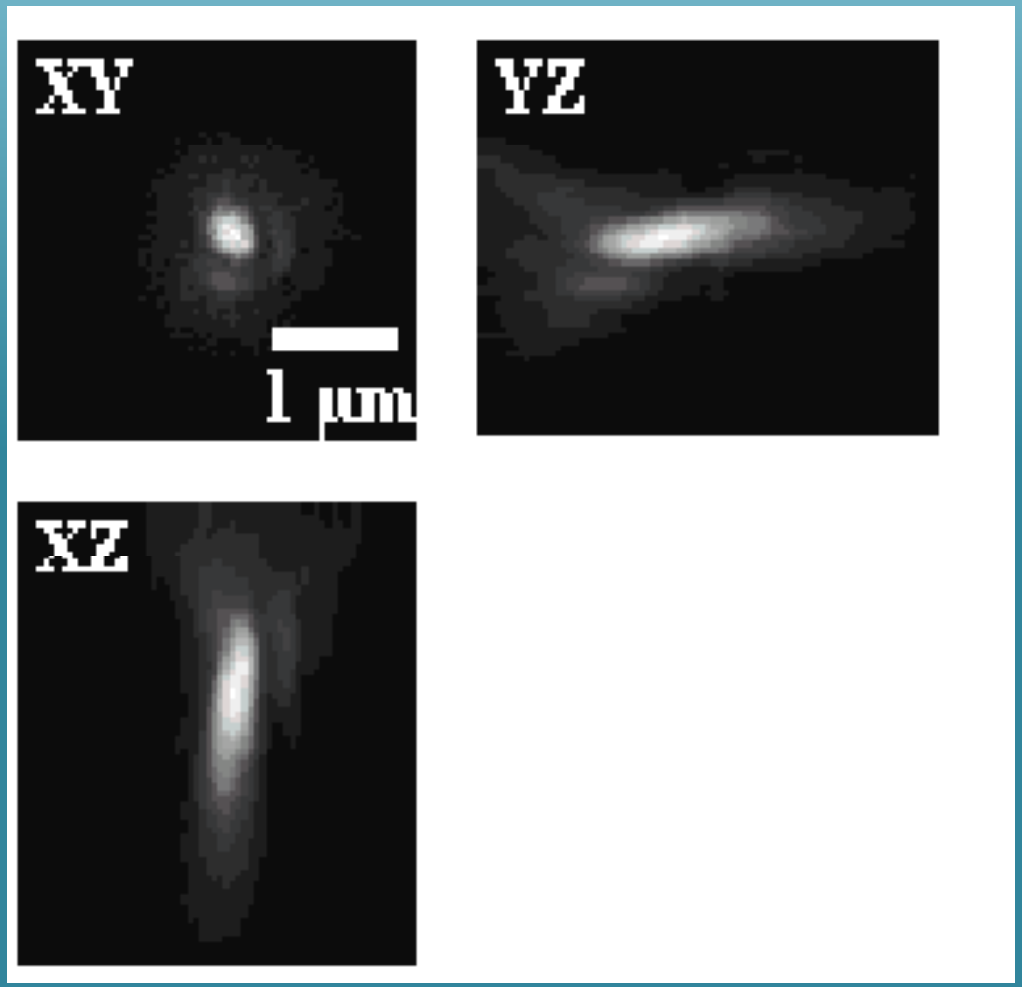


Aberrations – DIC Optics In Place

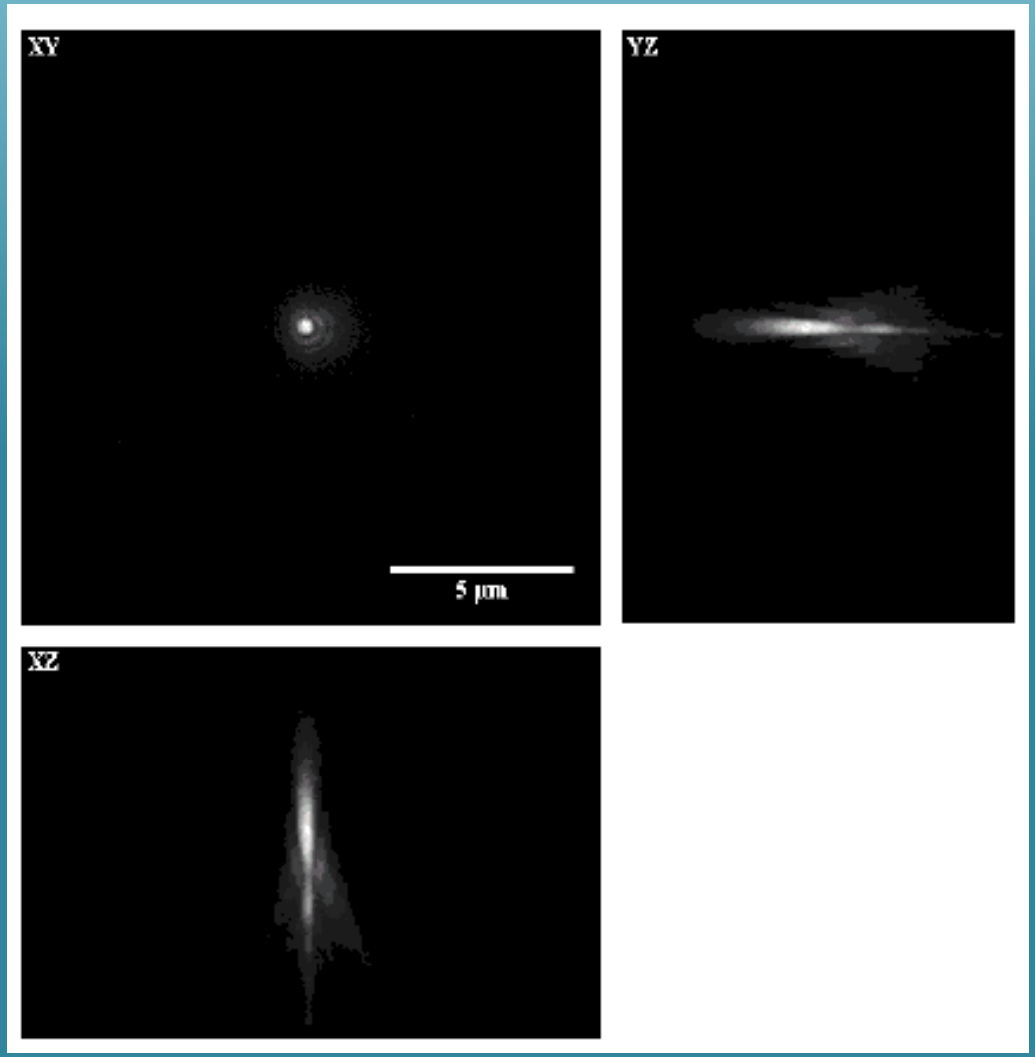




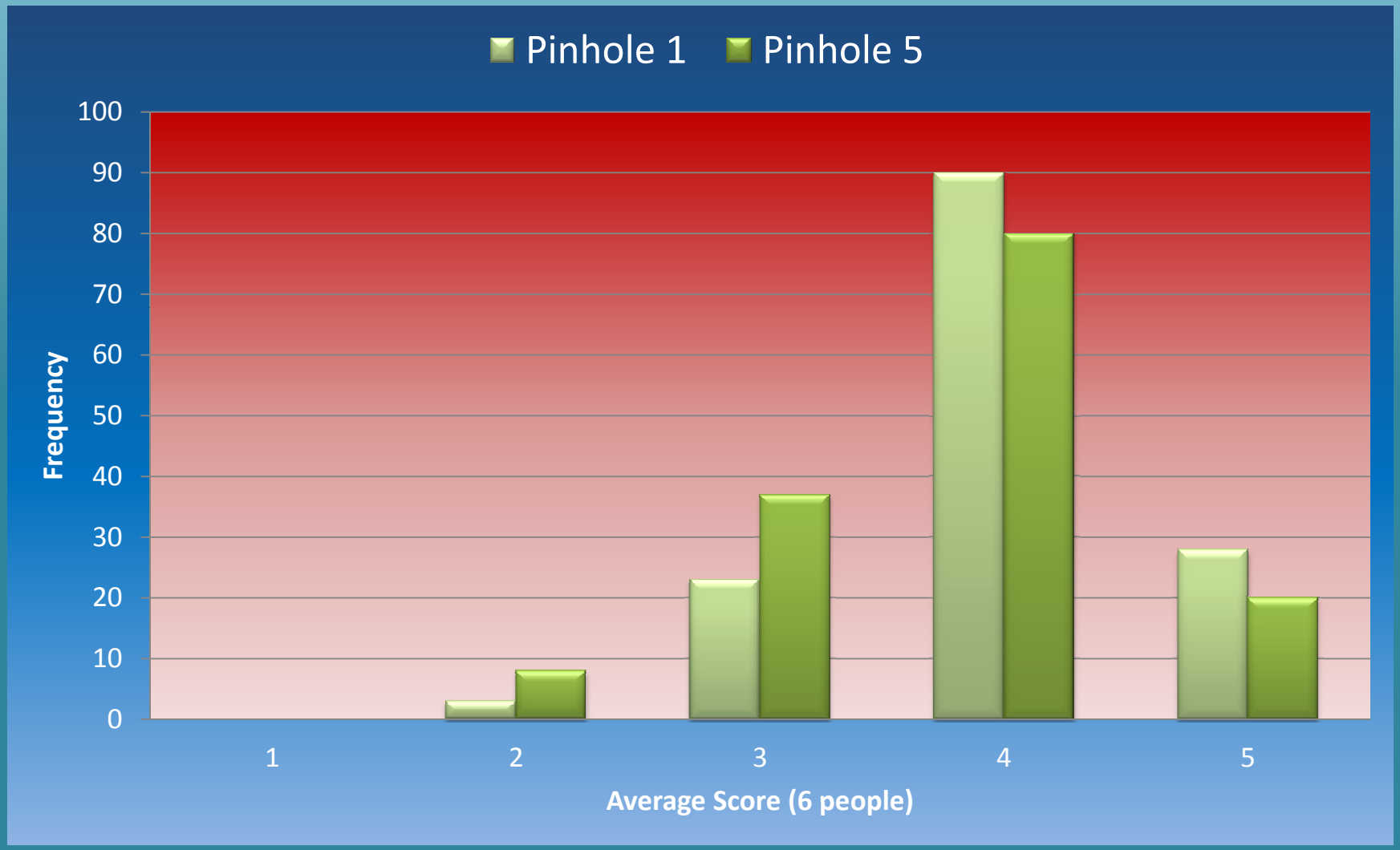
Aberrations – Spherical Aberrations



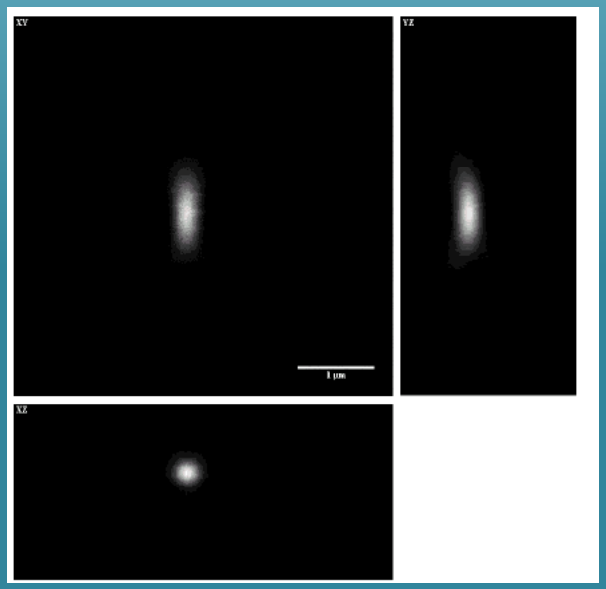
PSF Multi-photon



Scoring of All Data



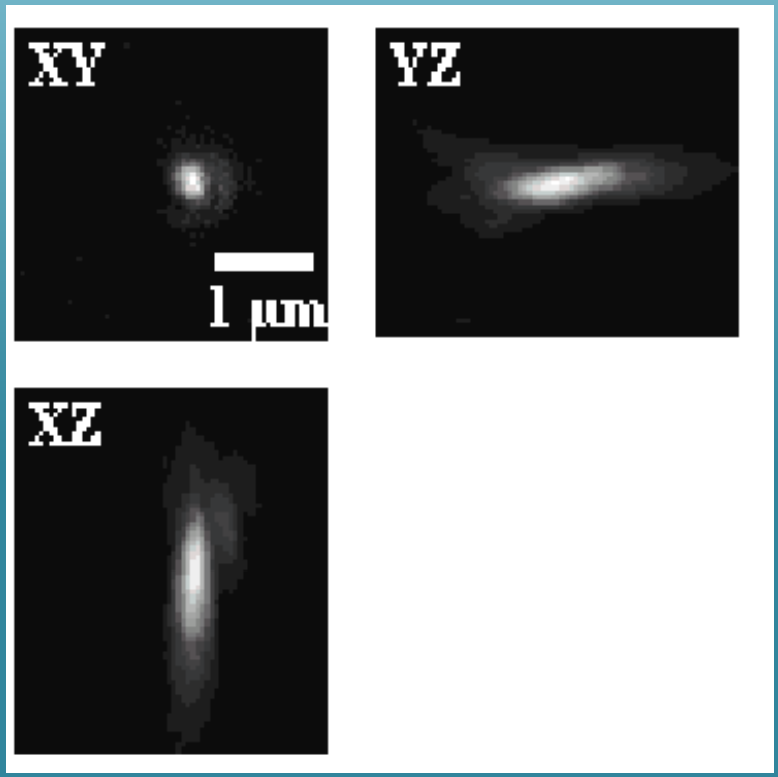
Open Pinhole Shows Defects



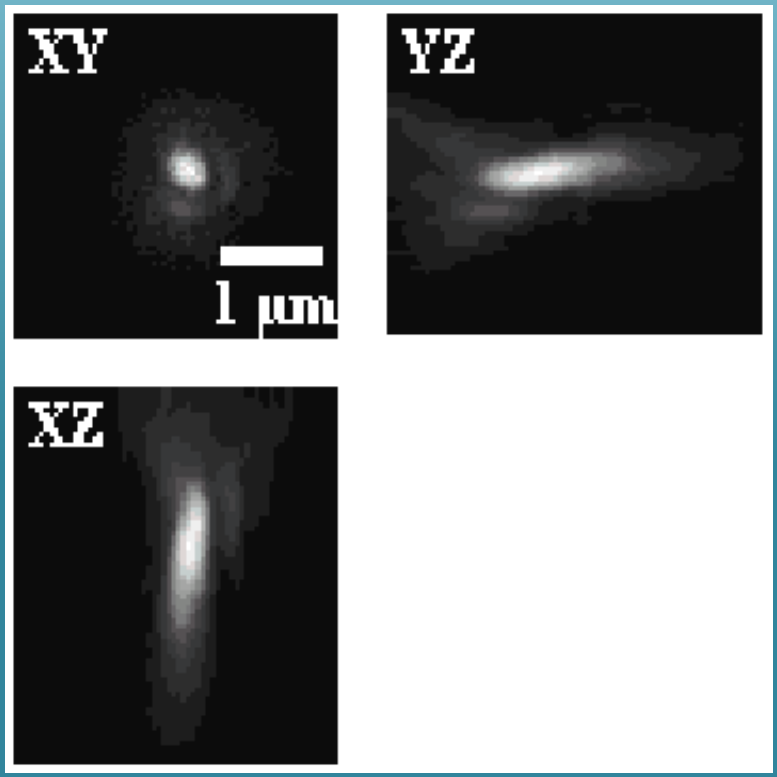
63x Oil 1.4 NA
Pinhole 1 Airy Unit

63x Oil 1.4 NA
Pinhole 5 Airy Unit

Open Pinhole Shows Defects

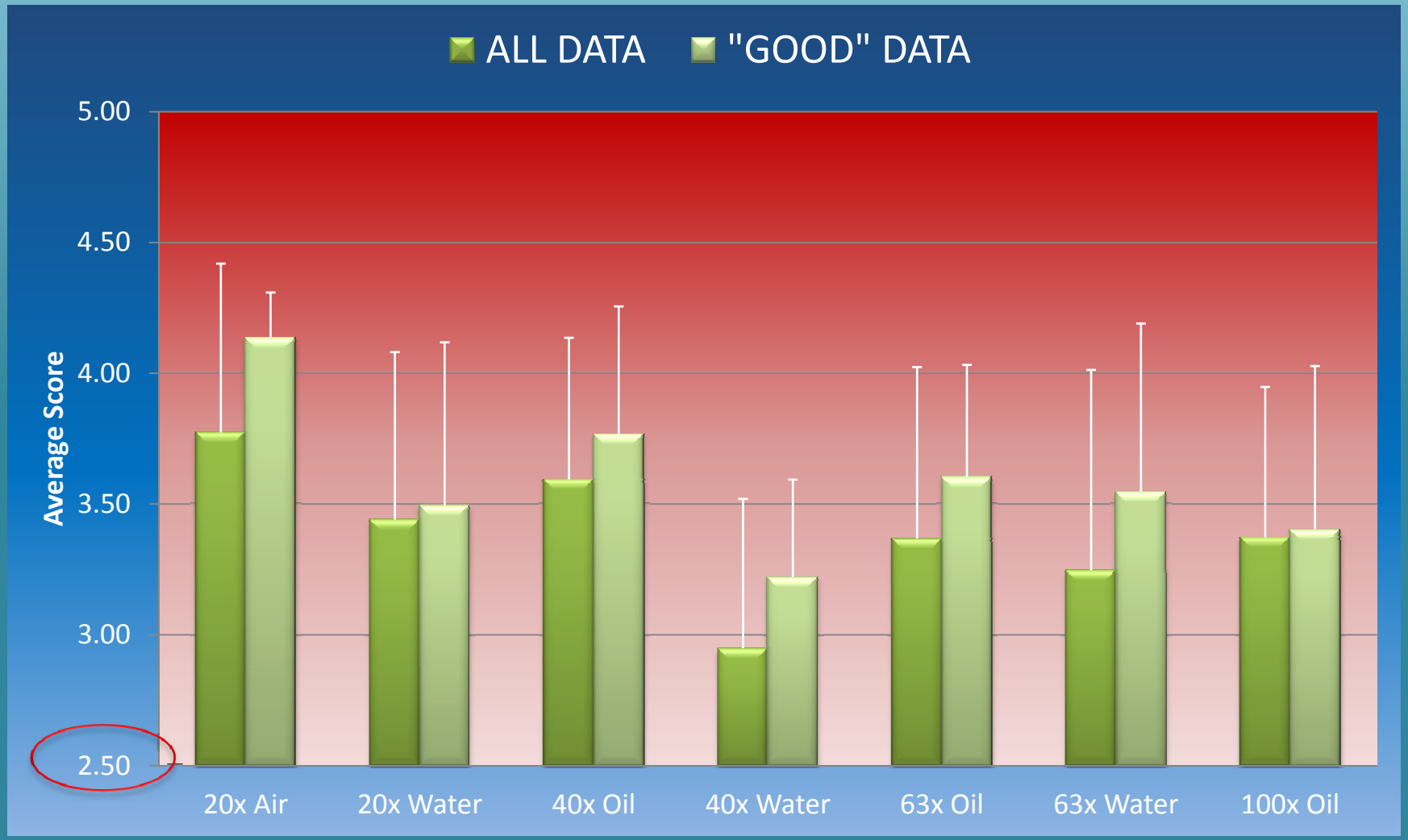


63x Water 1.2 NA
Pinhole 1 Airy Unit



63x Water 1.2 NA
Pinhole 5 Airy Unit

Scoring versus Lens Type

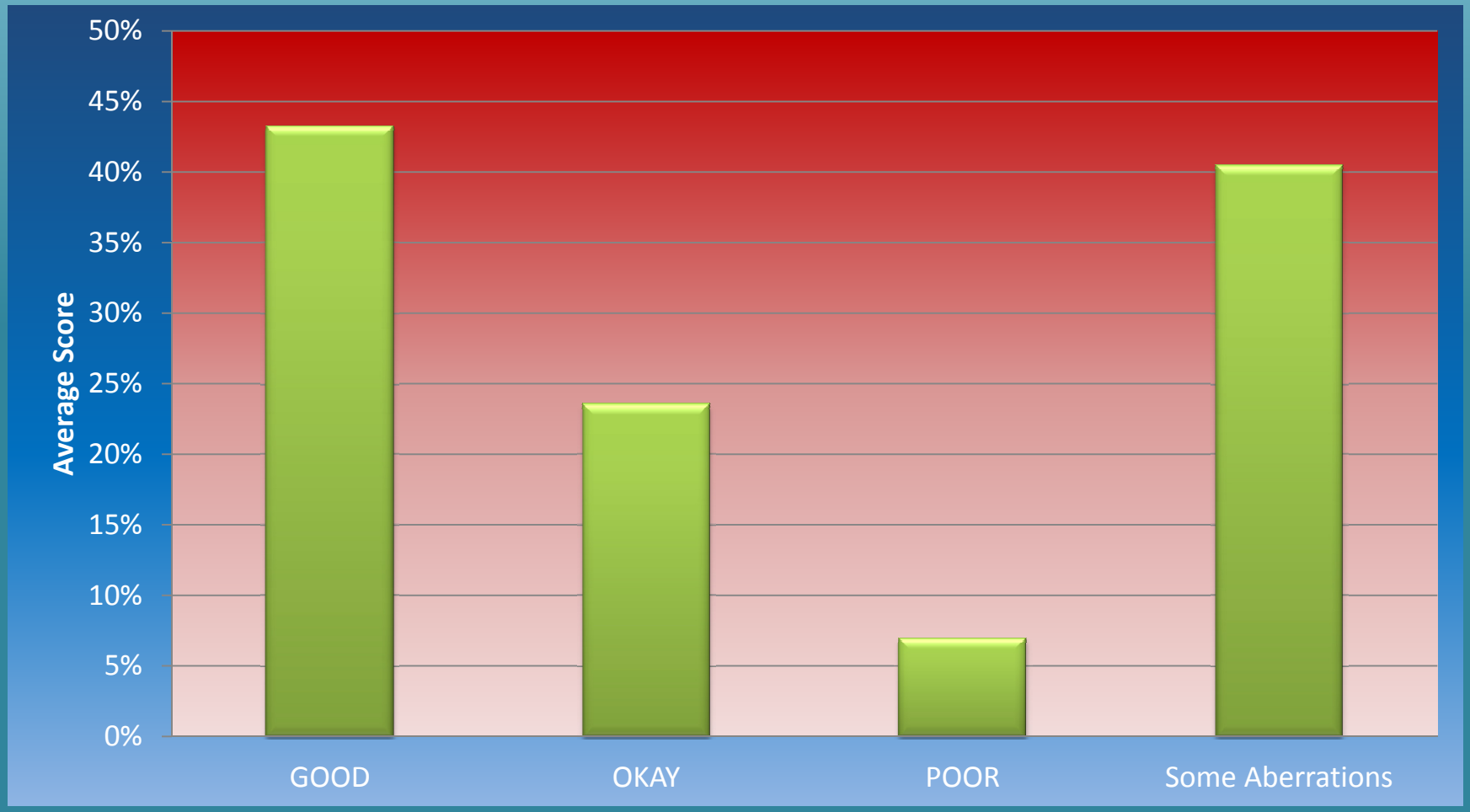


Water Lenses Have lower Scores. “GOOD” data has lower Standard Deviation.

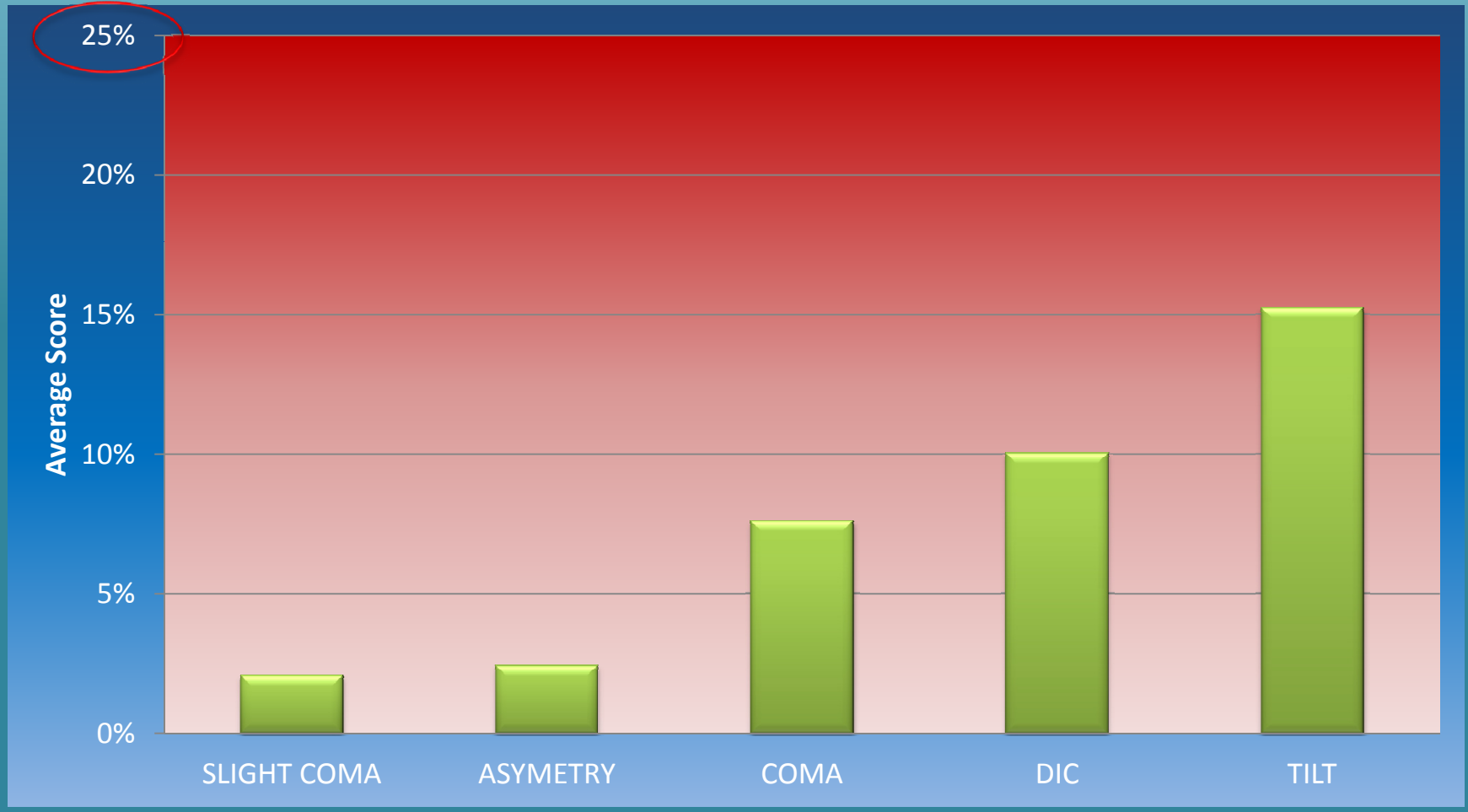
PSF Quality

67% of PSFs Good or Okay

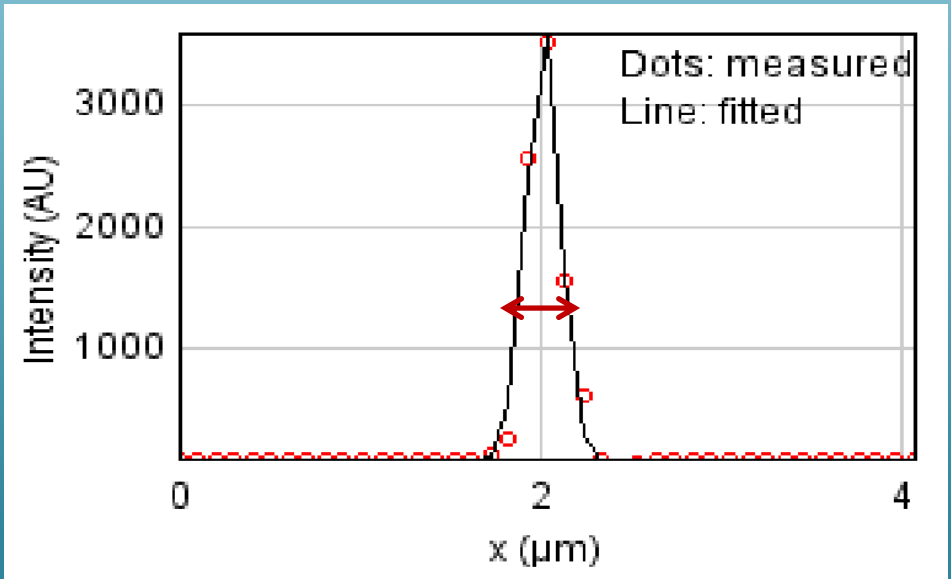
40% with some kind of aberration



Aberrations

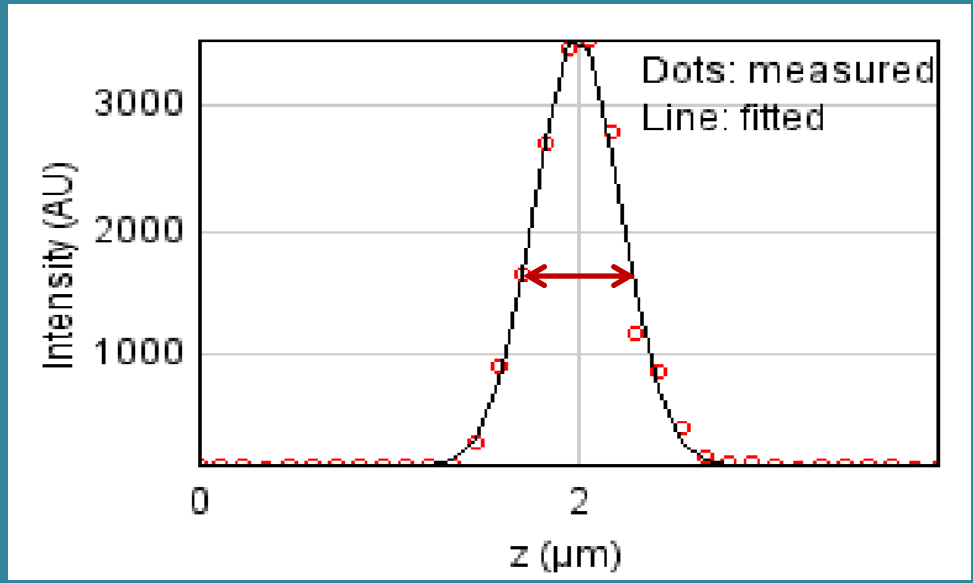


PSF Resolution

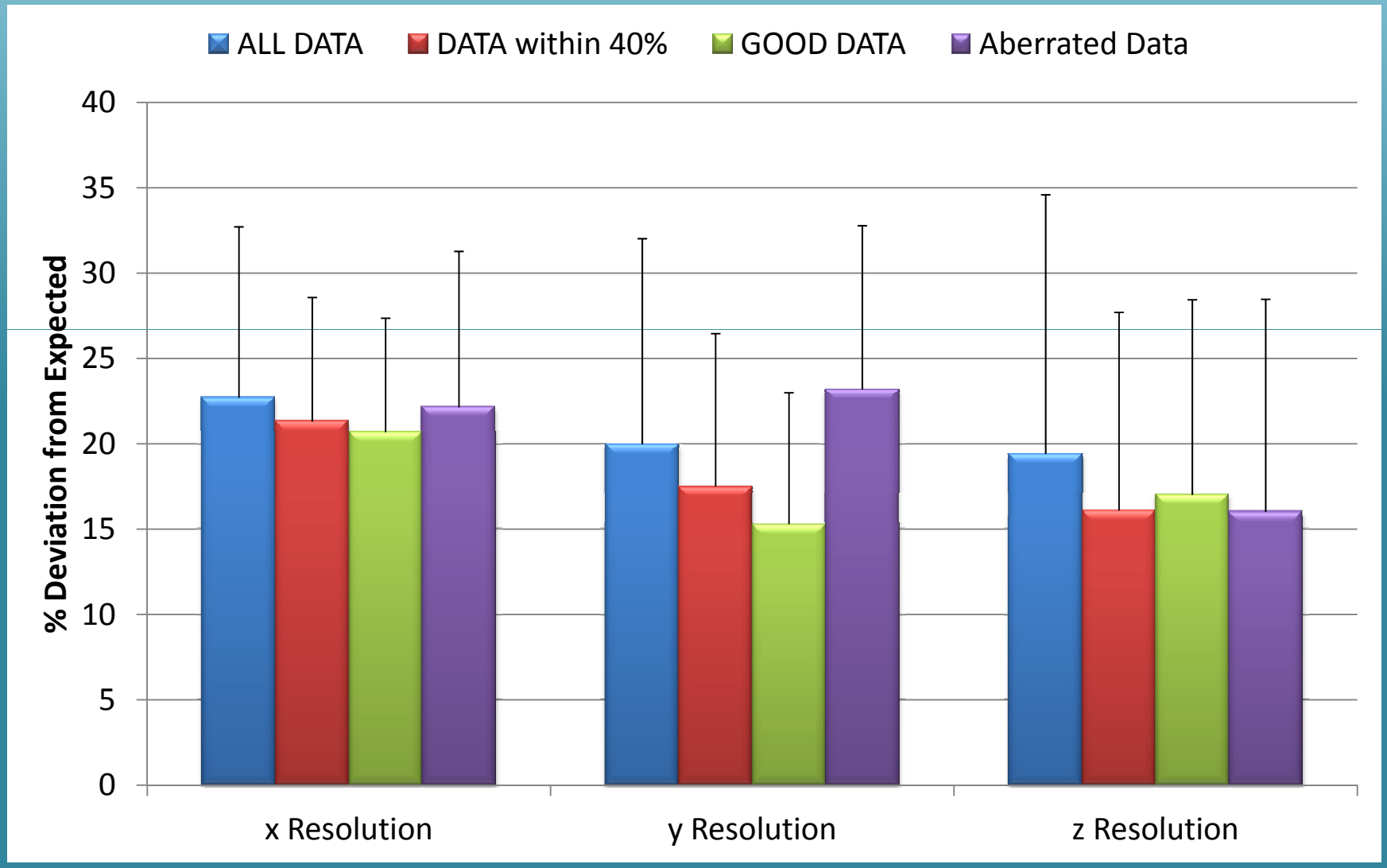


Gaussian fit and FWHM determined for x and y-axis.

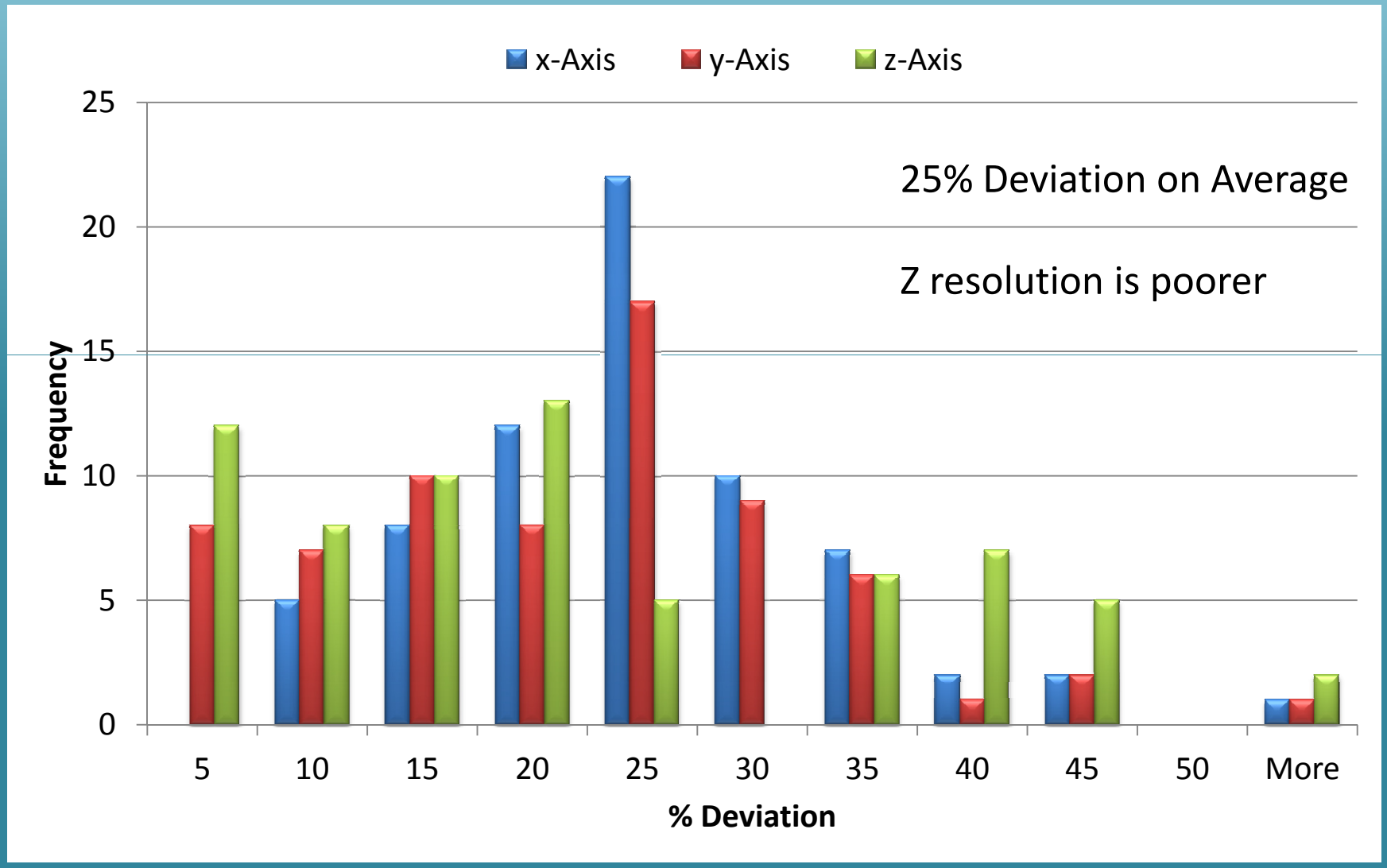
Gaussian fit and FWHM determined for z-axis.



PSF Resolution 63x Oil/1.4 NA

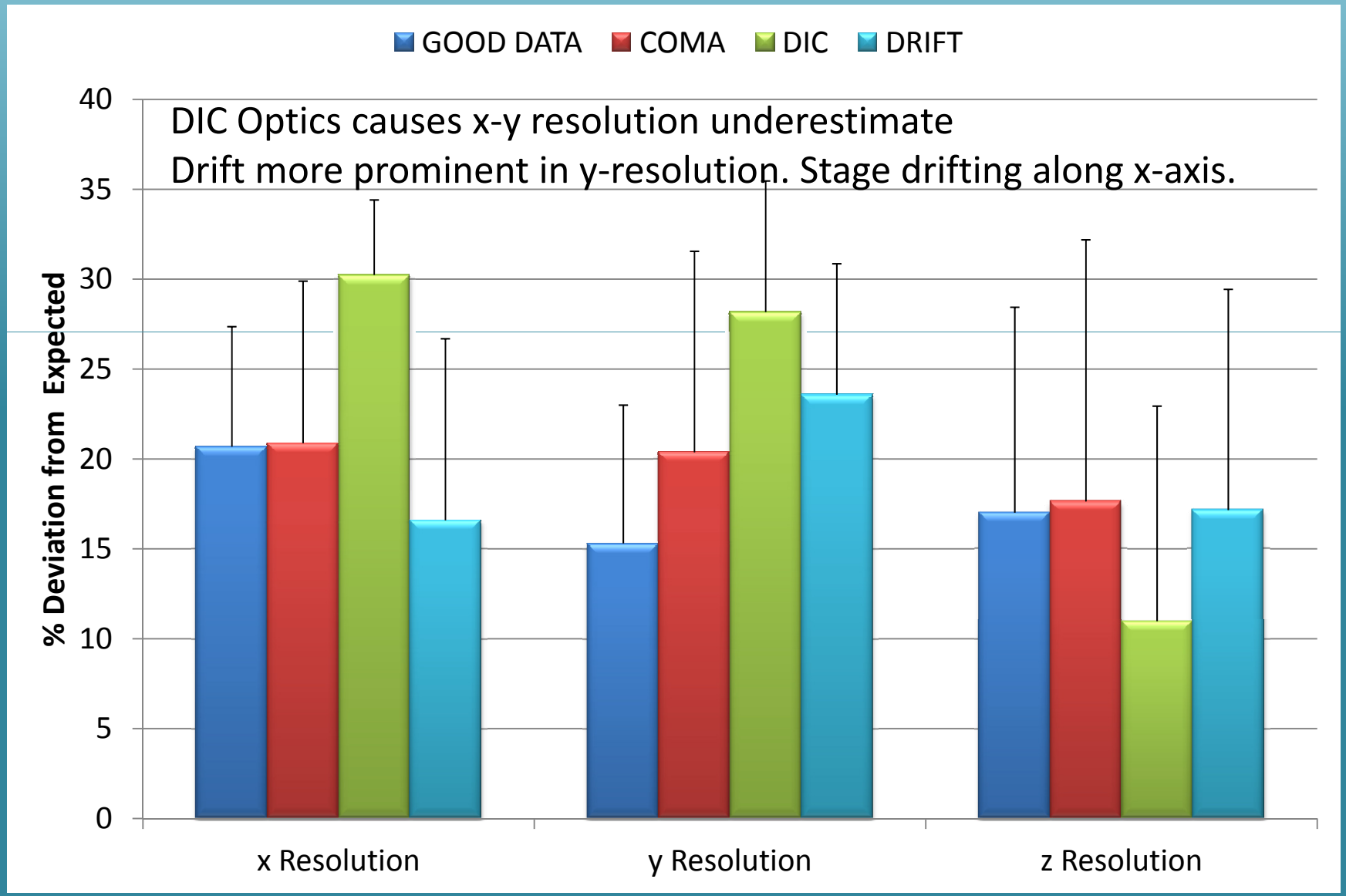


PSF Resolution 63x Oil/1.4 NA

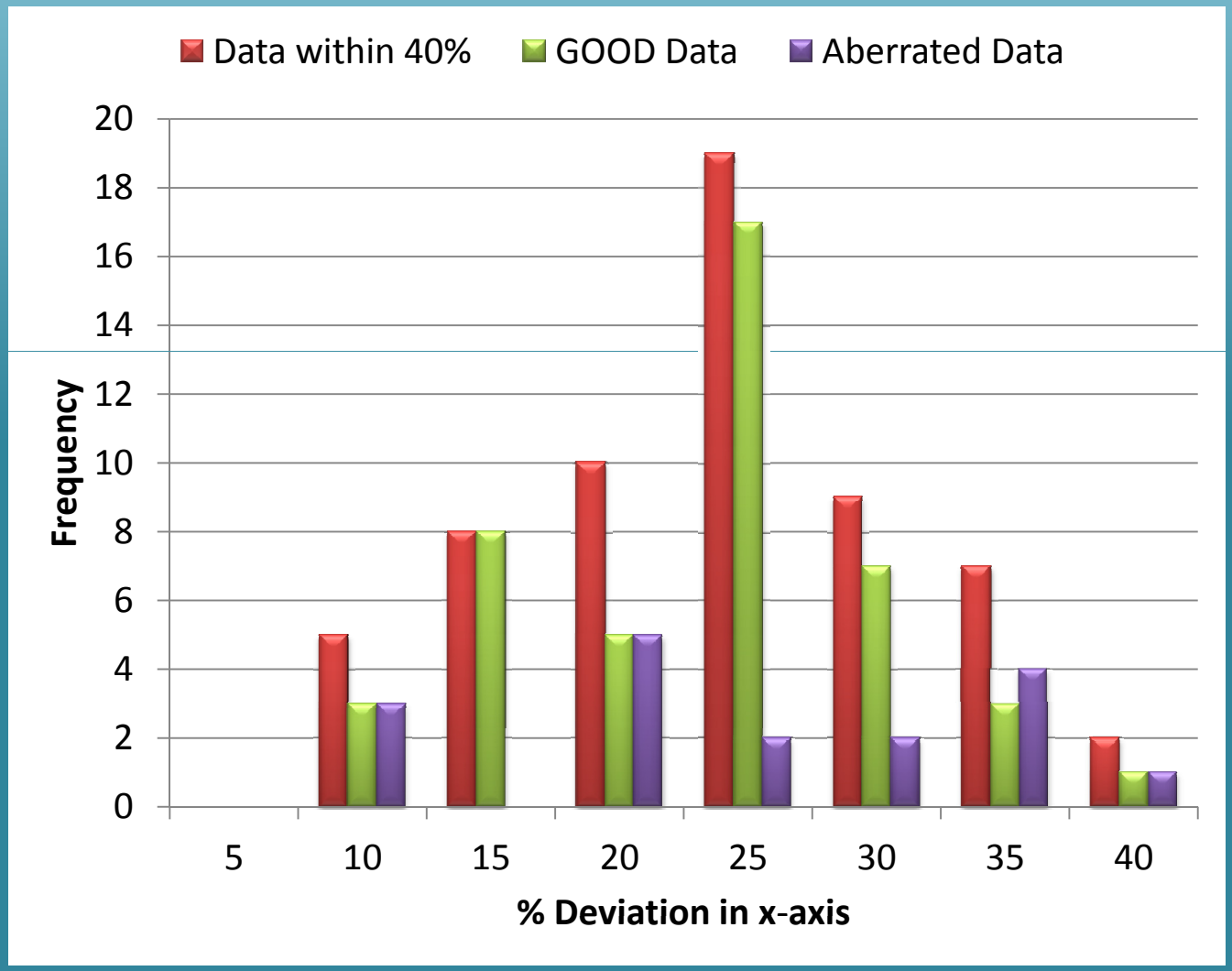


13% of data outside of 40% Deviation

PSF Resolution Data – 63x Oil 1.4NA



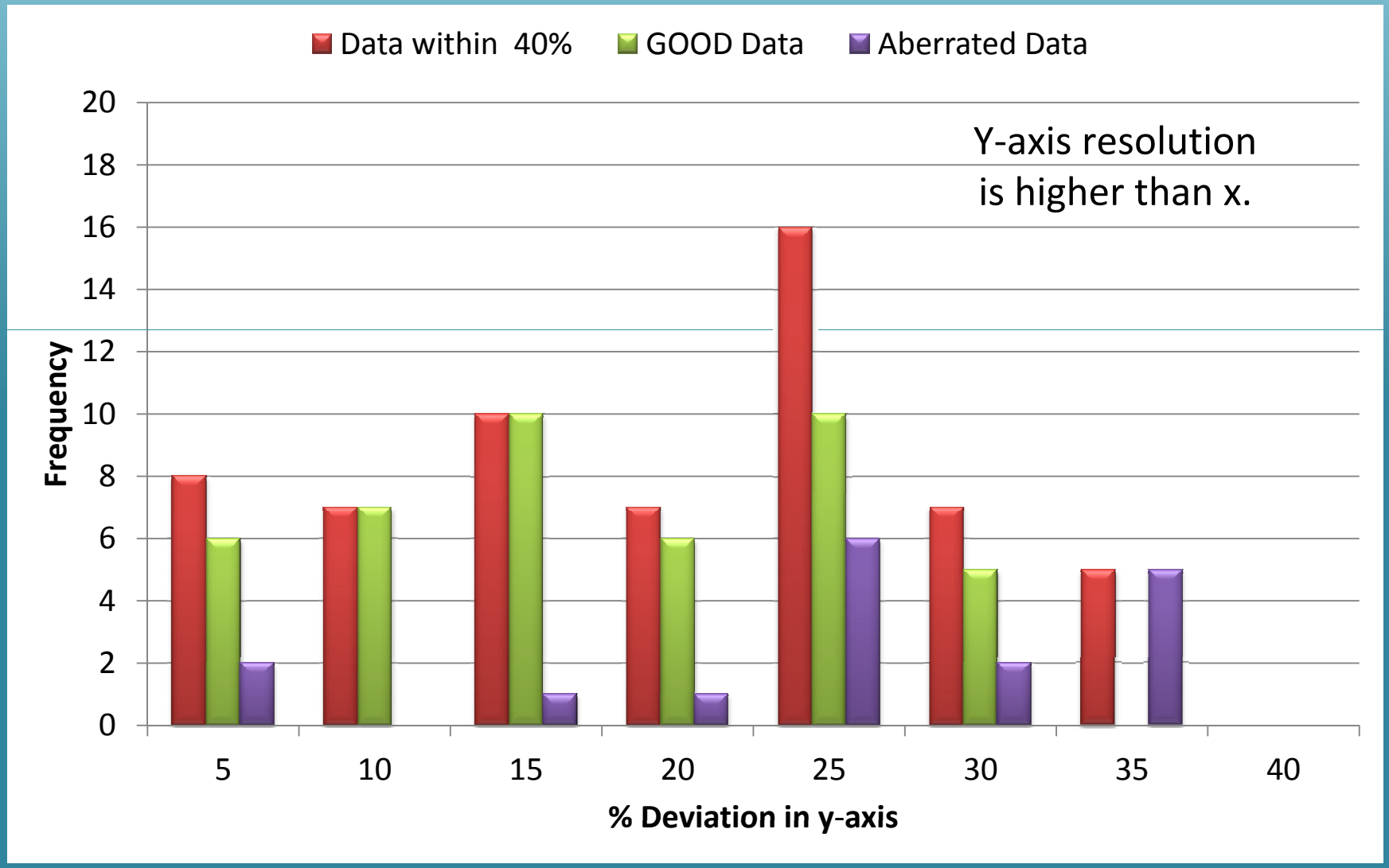
PSF Resolution 63x Oil/1.4 NA – x-axis



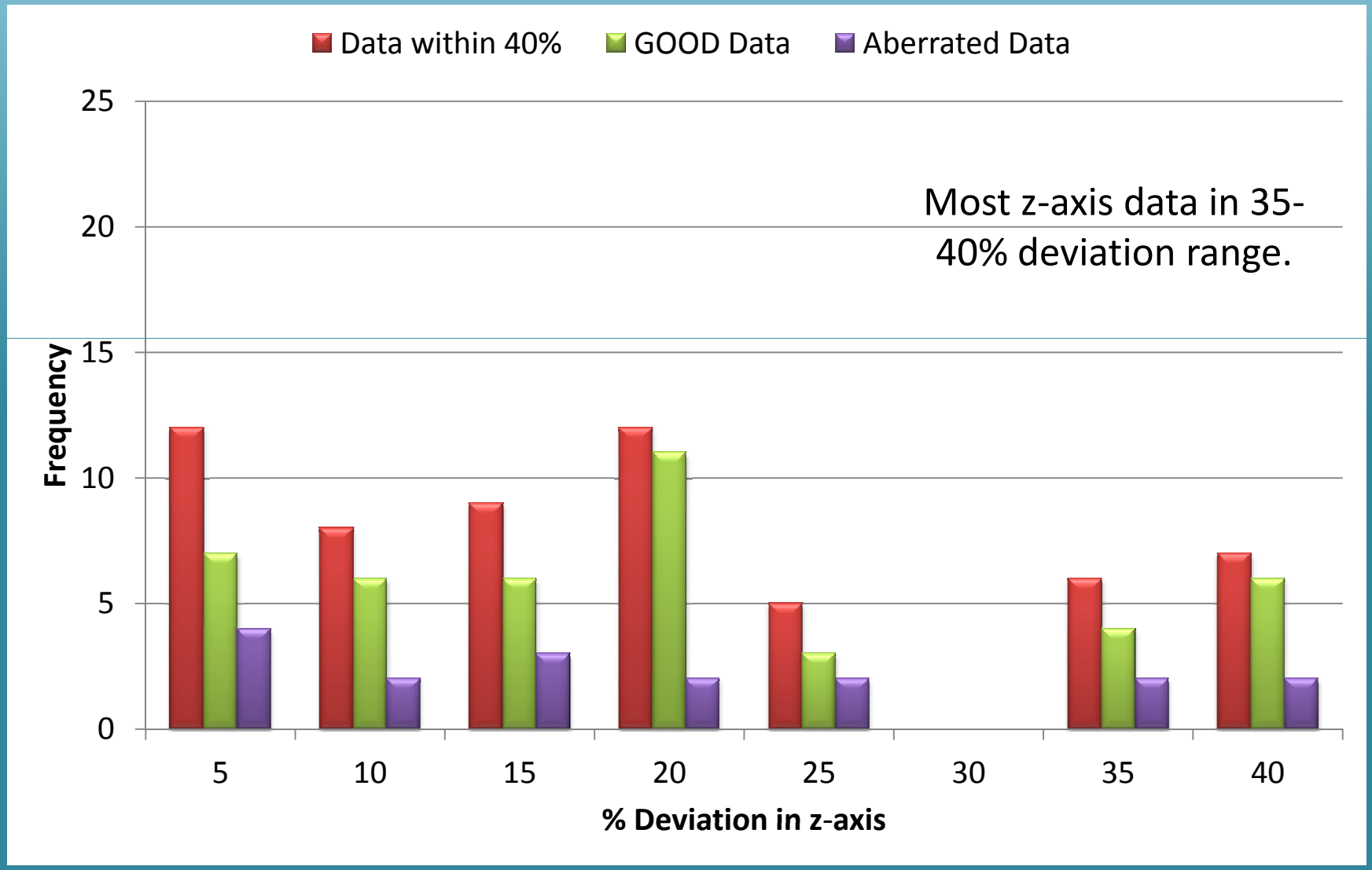
Visual scoring has little correlation with resolution measurements.

Aberrated data has higher deviation from actual resolution.

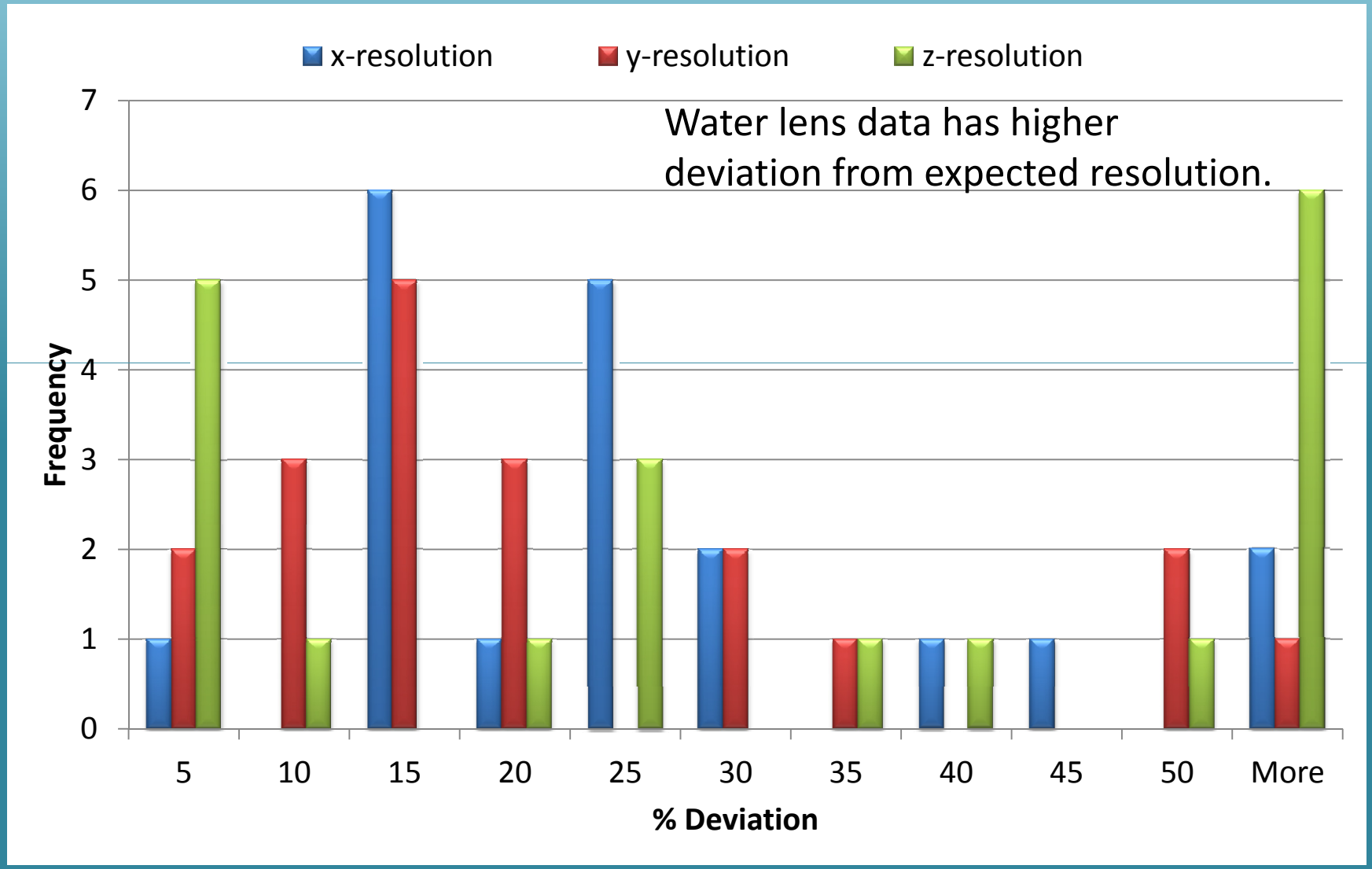
PSF Resolution 63x Oil/1.4 NA – y-axis



PSF Resolution 63x Oil/1.4 NA – z-axis



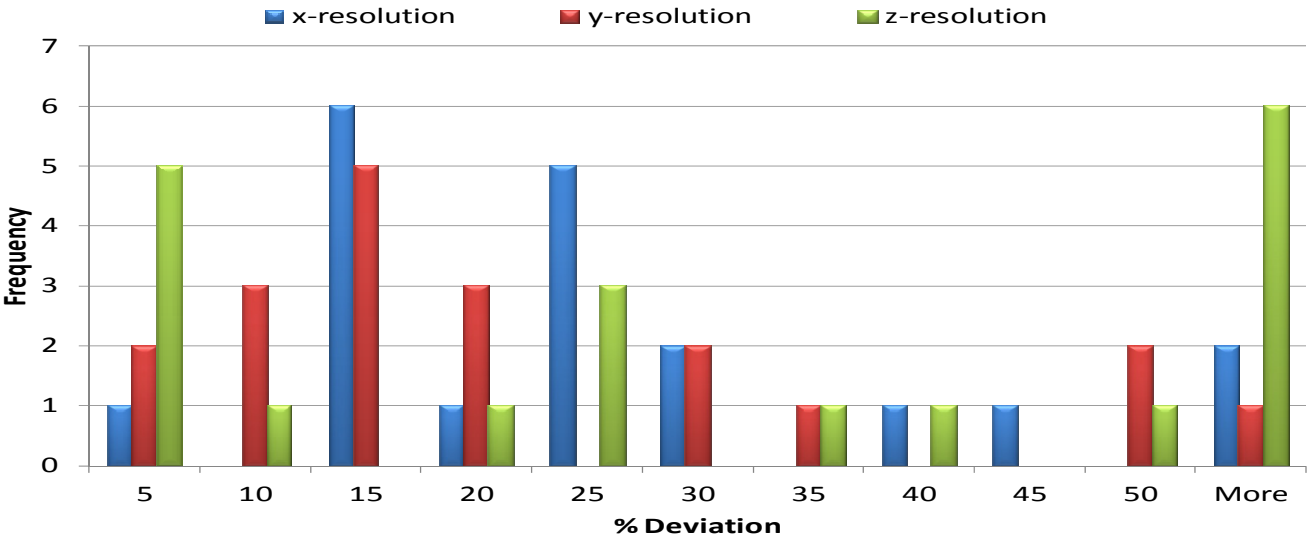
PSF Resolution 63x Water/0.9-1.4 NA



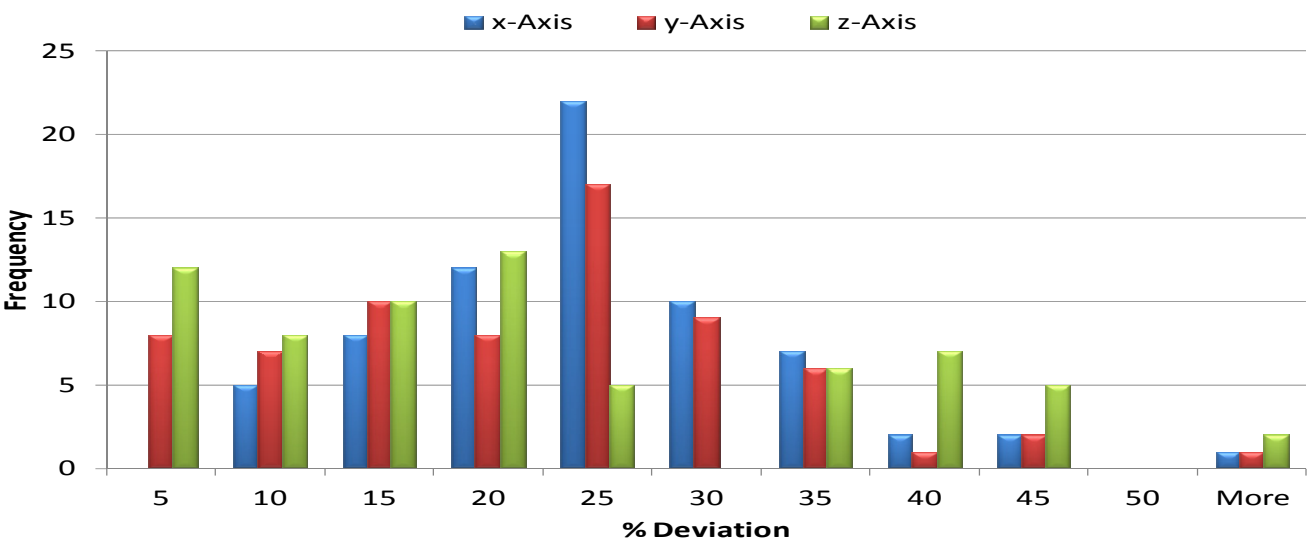
42% of data outside of 40% Deviation

Oil or Water Lens?

63 Water

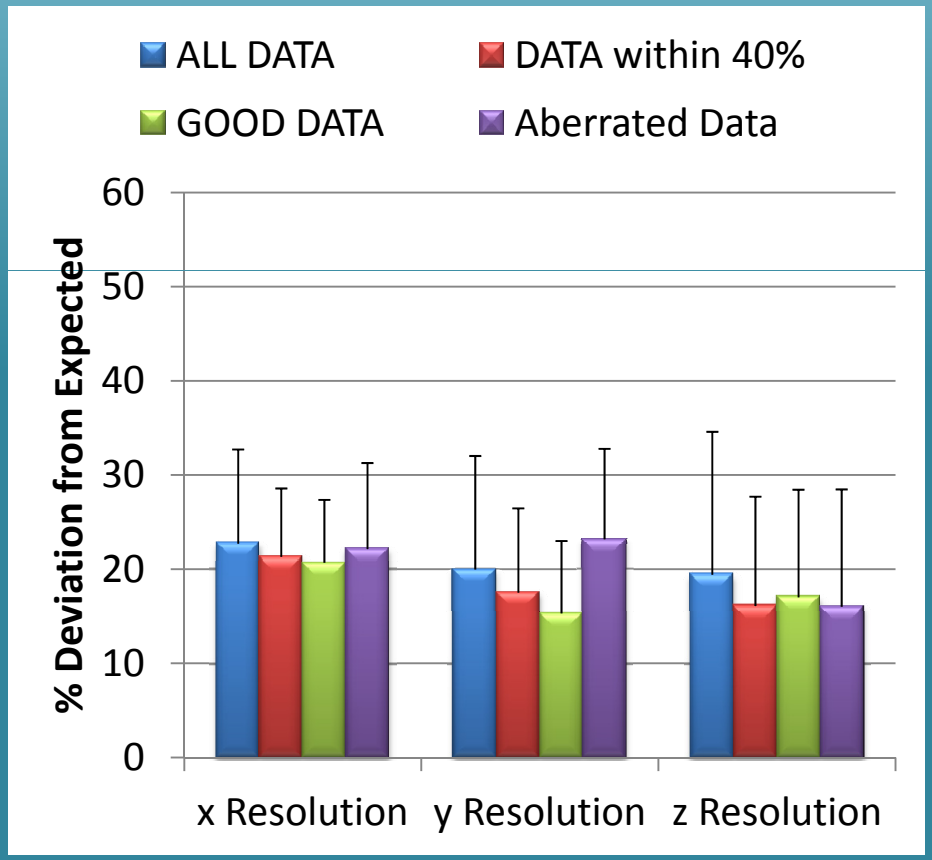


63 Oil

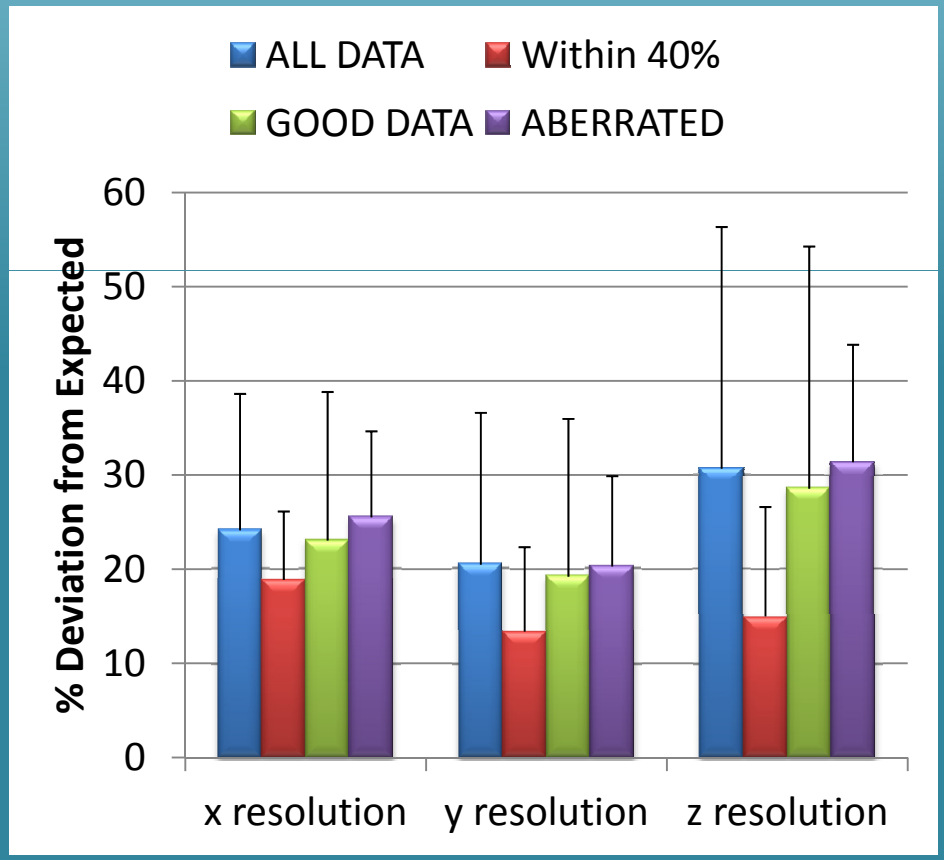


Oil or Water Lens?

63 Oil

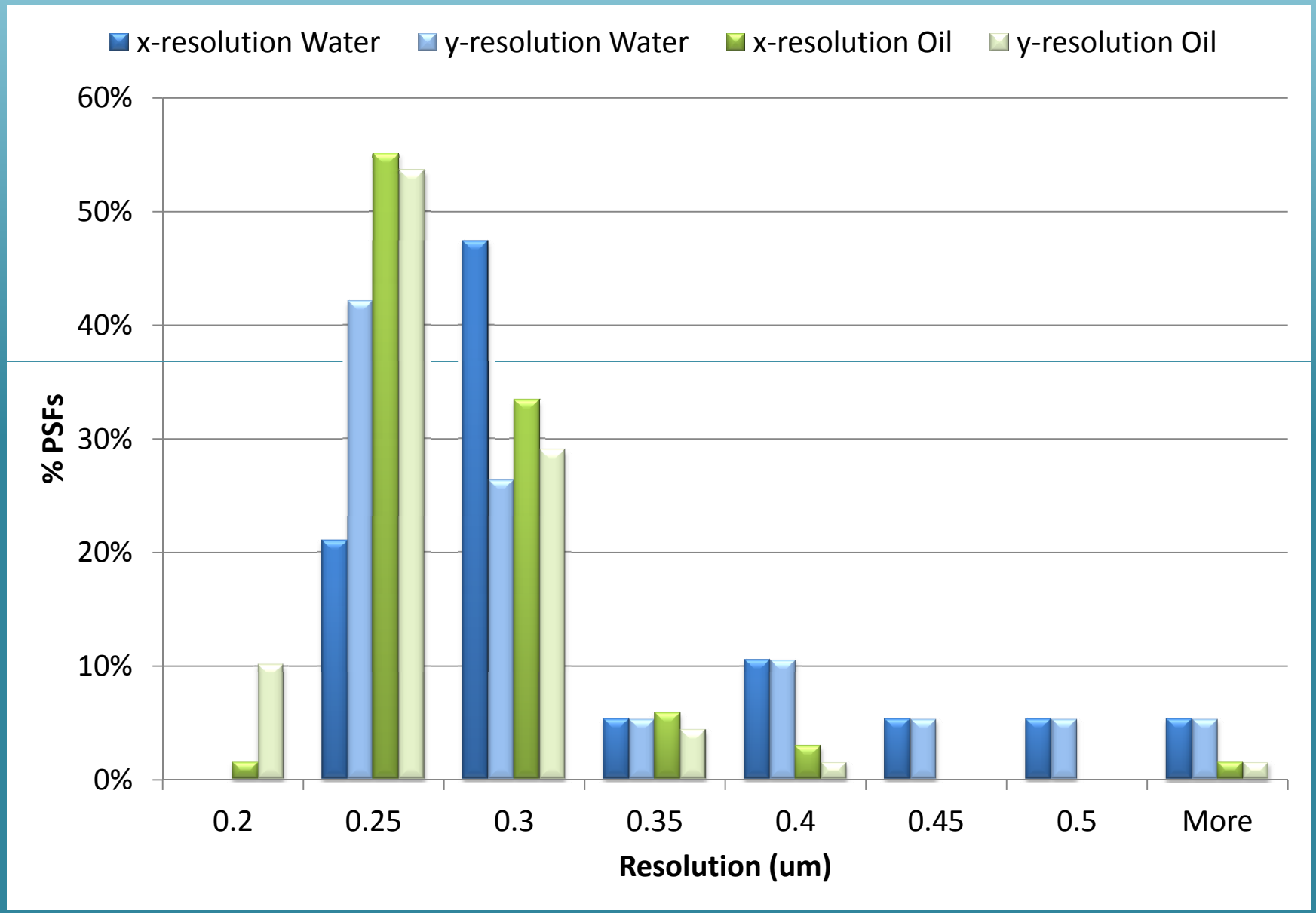


63 Water

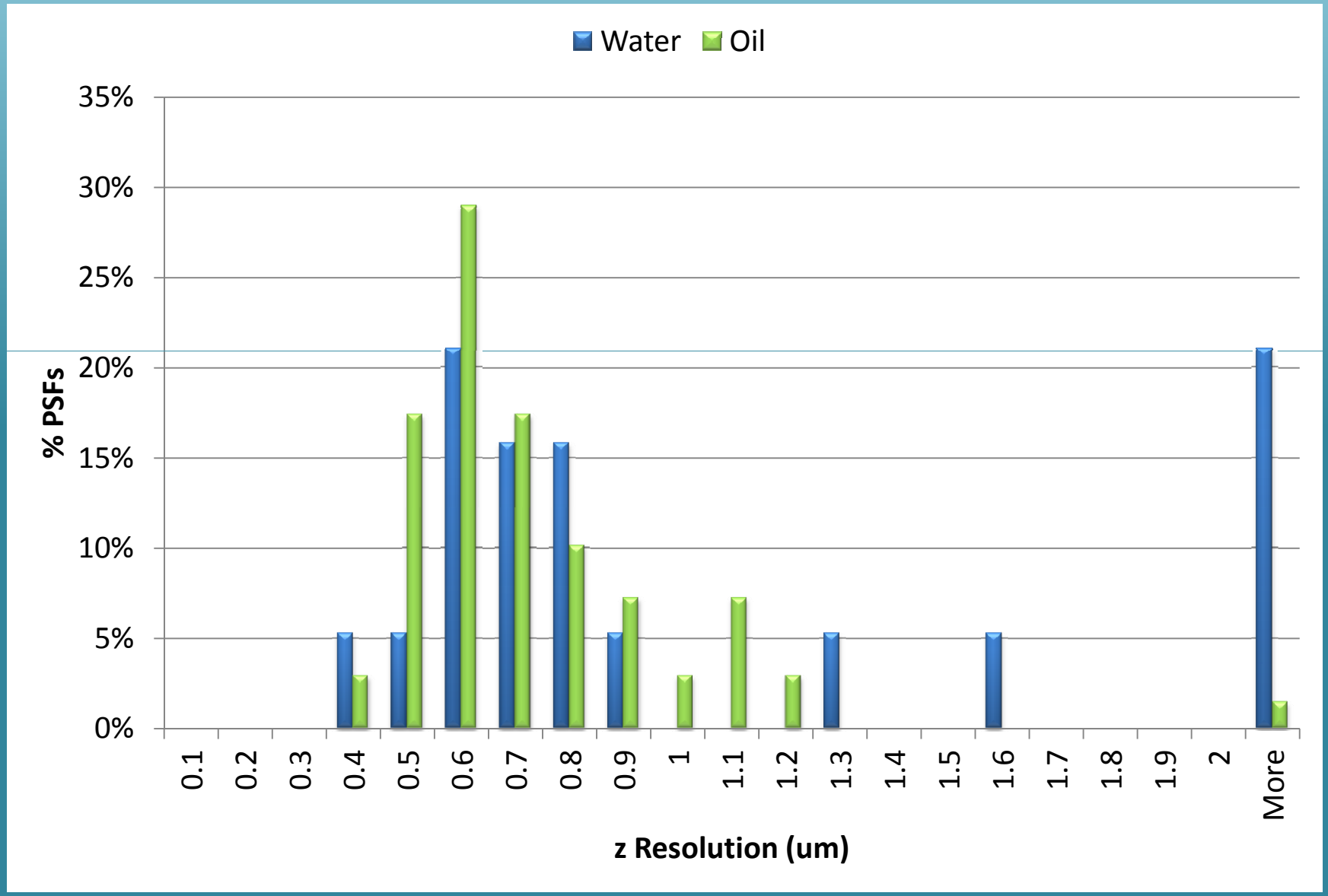


Z-resolution with water lenses is poorer than with oil lenses.

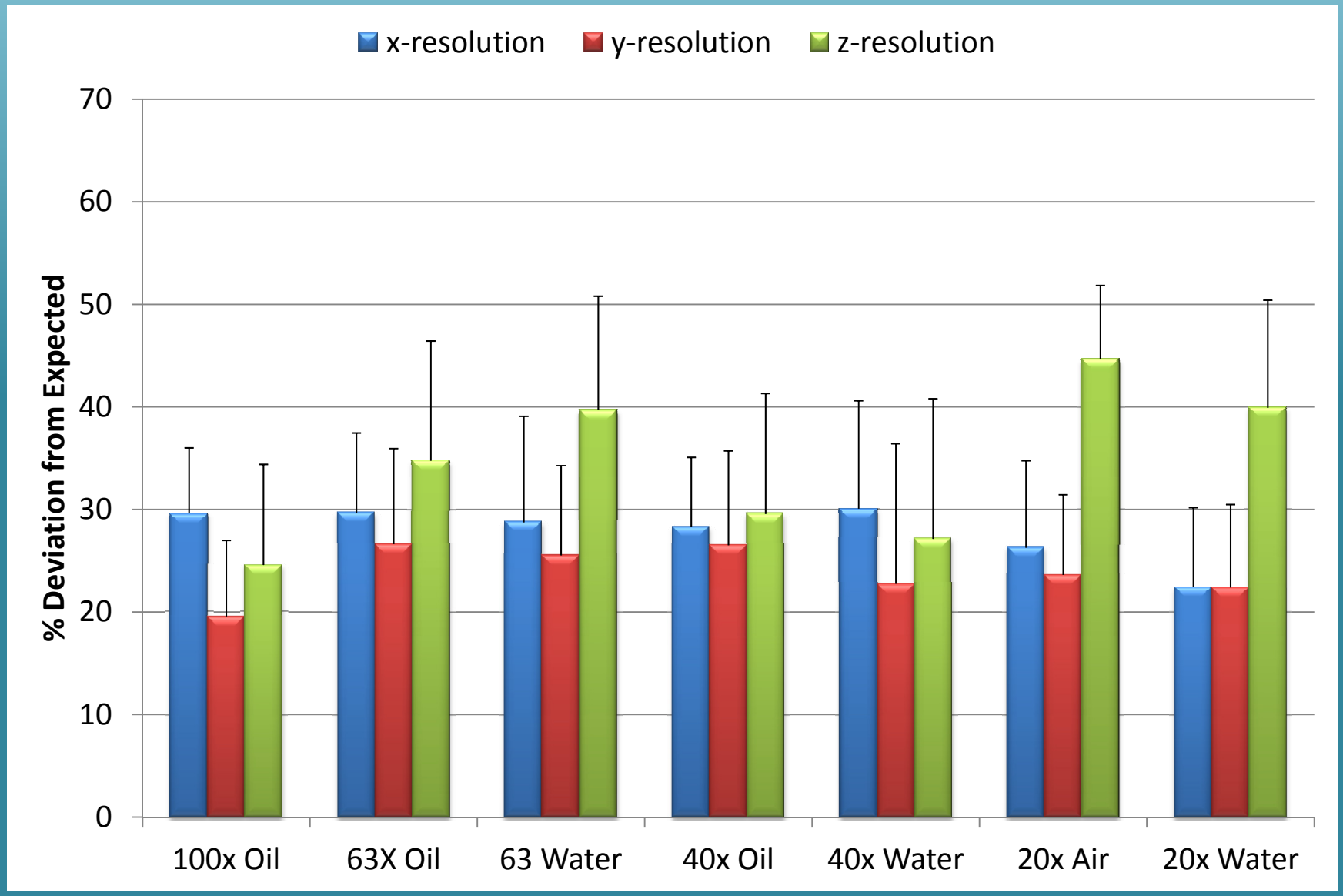
Oil or Water Lens? X-Y resolution



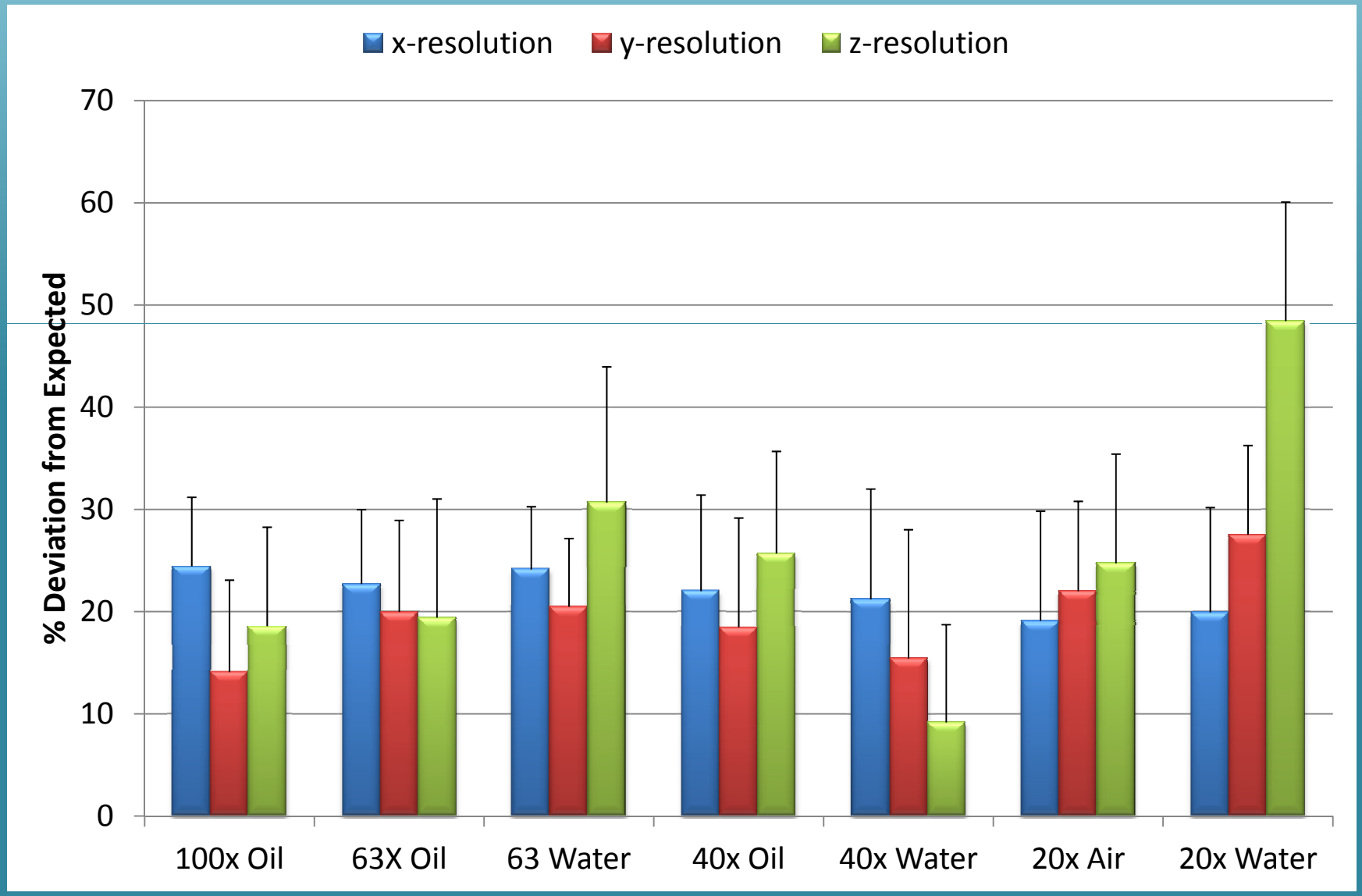
Oil or Water Lens? Z-resolution



Resolution All Lenses 5 Airy Units



Resolution All Lenses 1 Airy Unit



PSF Study

- Quality of PSFs was quite good.
- Visual scoring very subjective.
- Outliers were typically due to errors such as DIC optics in place.
- y-resolution is better than x.
- Water lenses performed most poorly.
- If water lenses are to be used the correction collar must be properly adjusted.

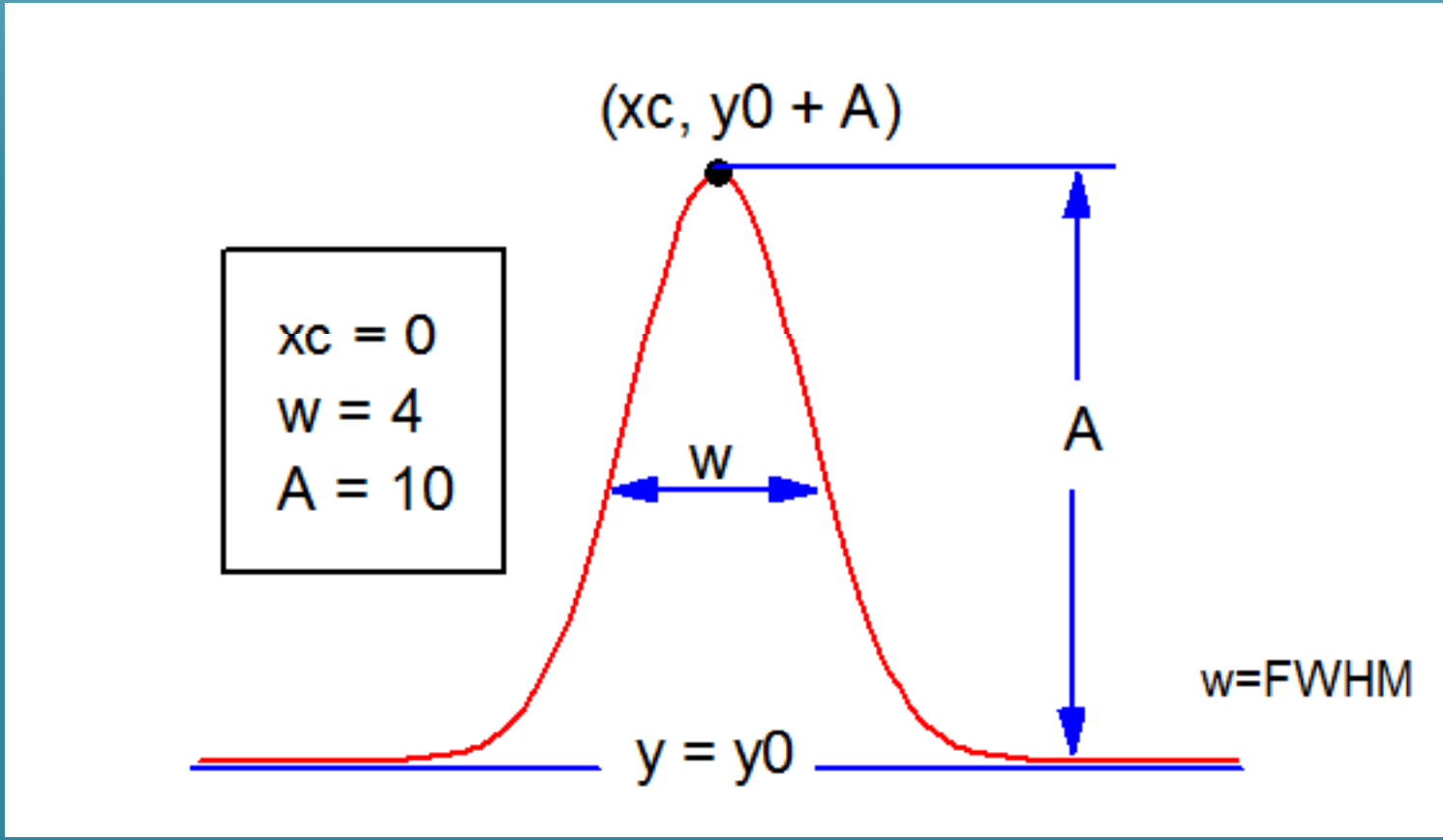
Spectral Accuracy

- 42 Participants
- Four different confocal platforms
- Olympus and Nikon users could not perform the test due to laser reflection blocking that cannot be deactivated within the systems.

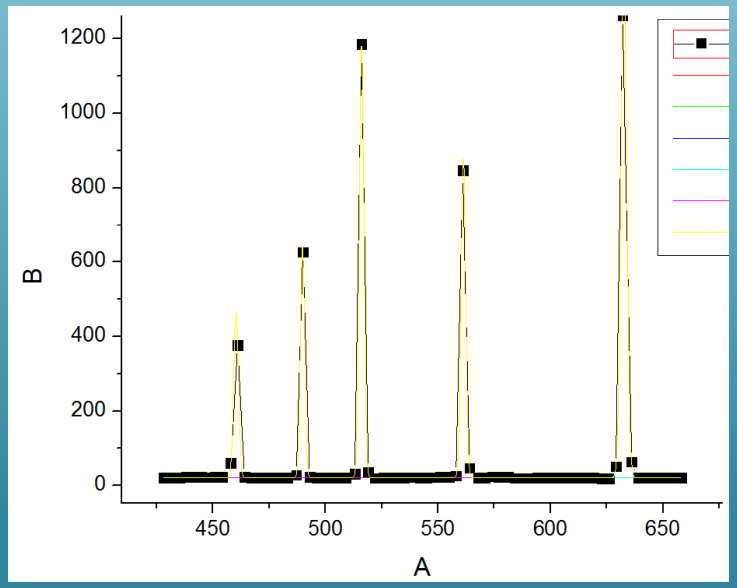
Spectral Accuracy

Origin Software
Non-linear multi-peak Gaussian fit
Max iteration: 400

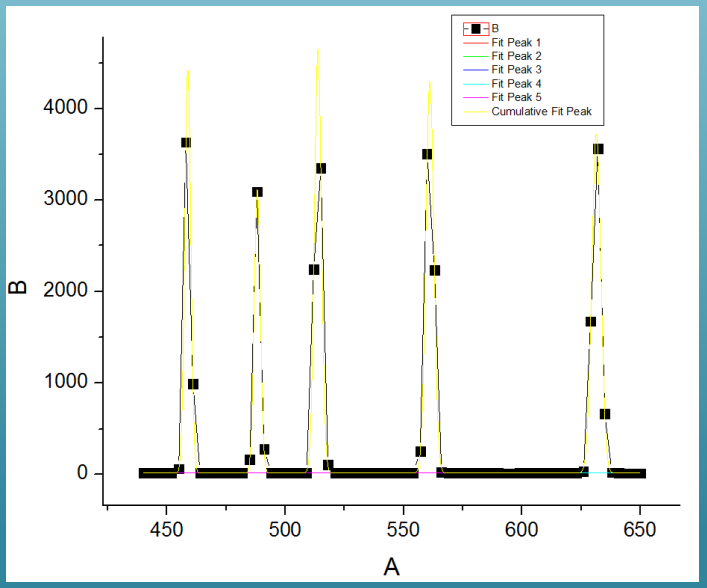
Wavelength of Peak Intensity
Precision = FWHM



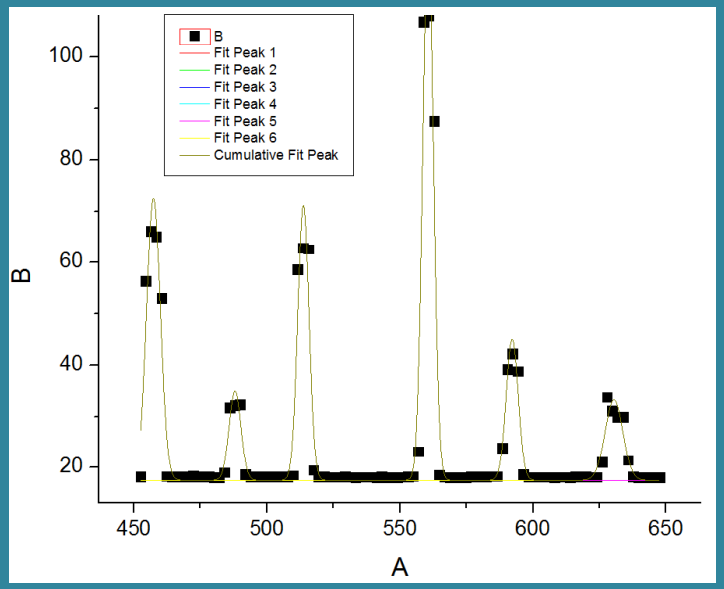
Spectral Accuracy



Zeiss 510

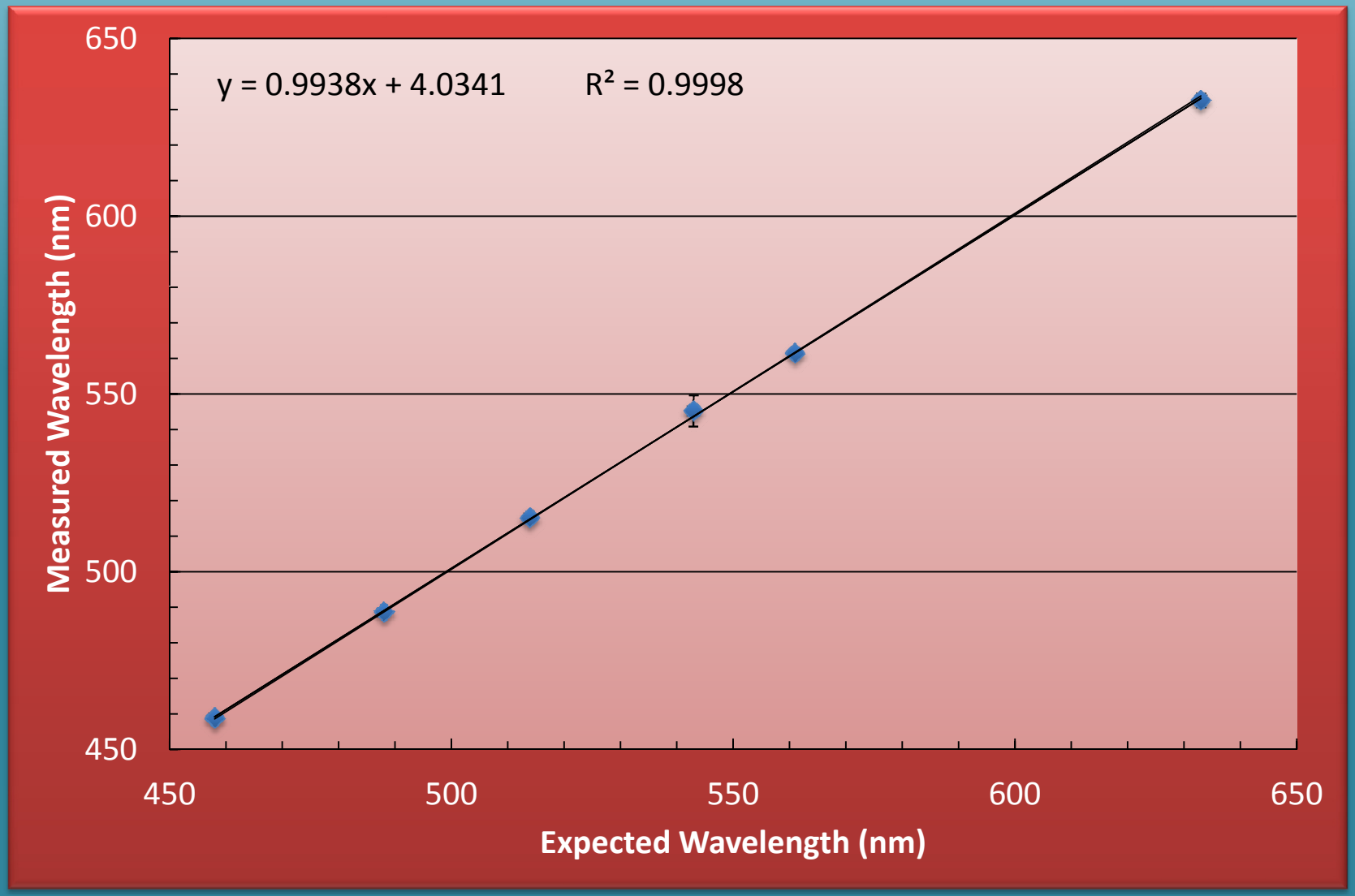


Zeiss 710

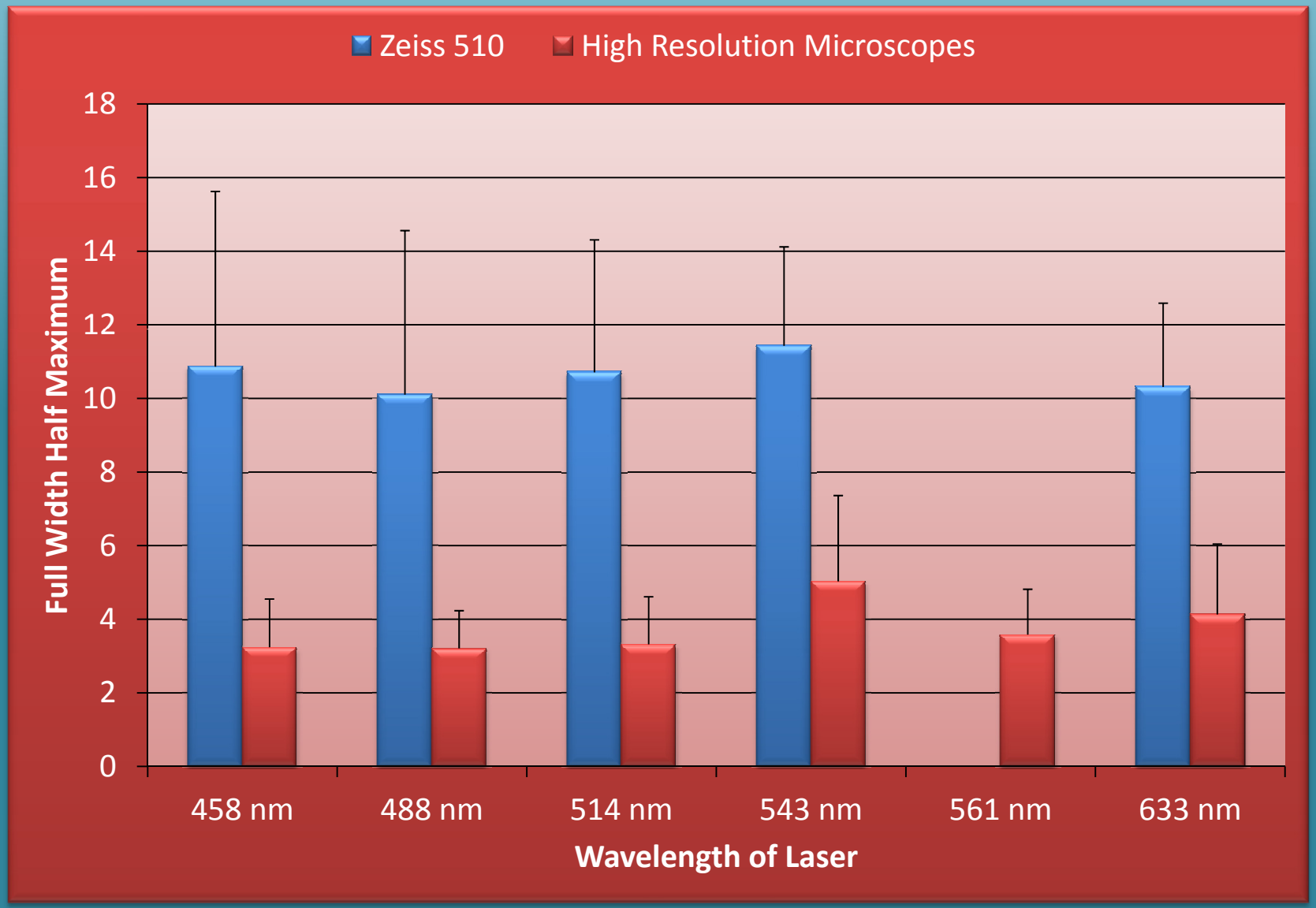


Leica SP2

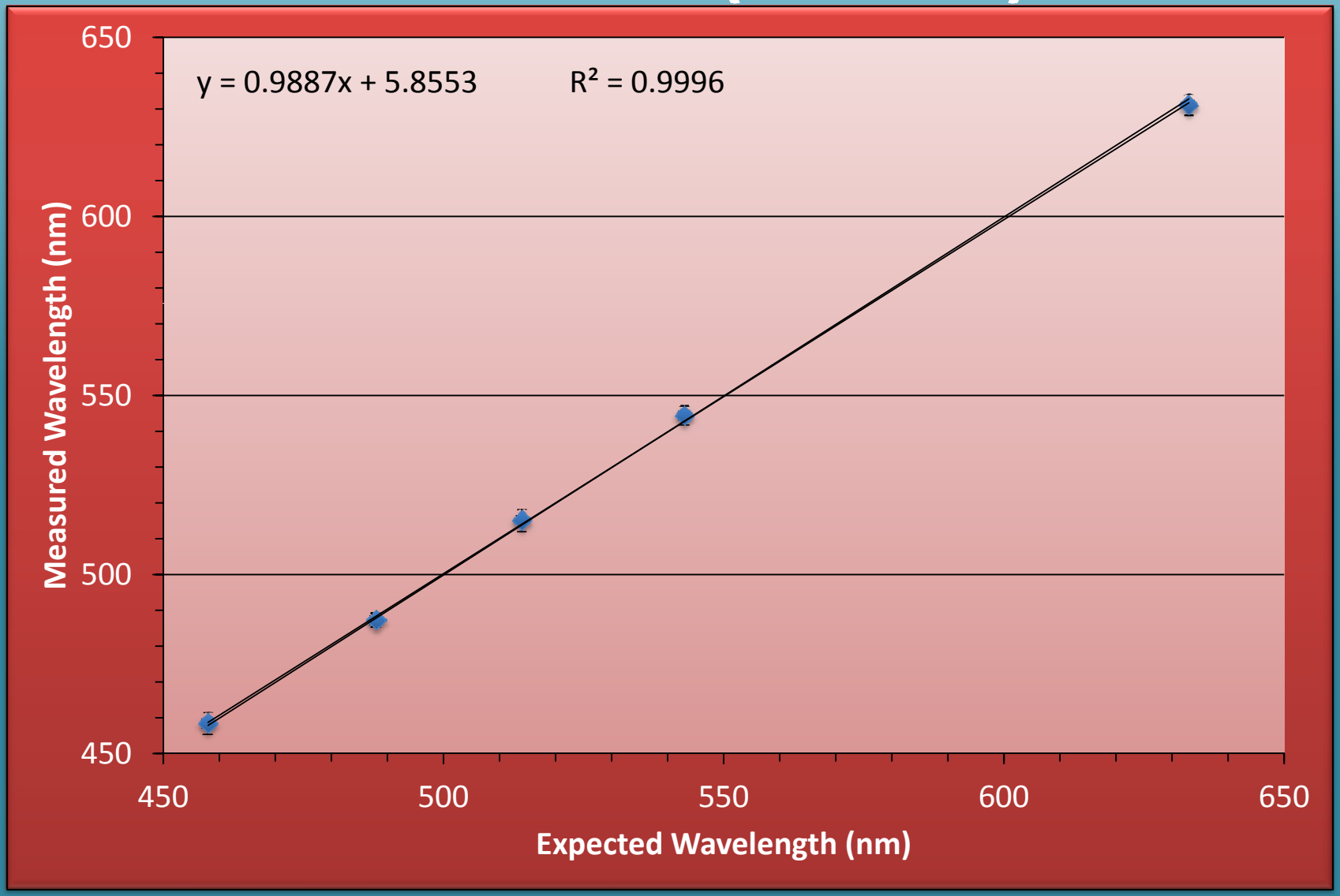
Spectral Accuracy – High Resolution Instruments (~ 3 nm)



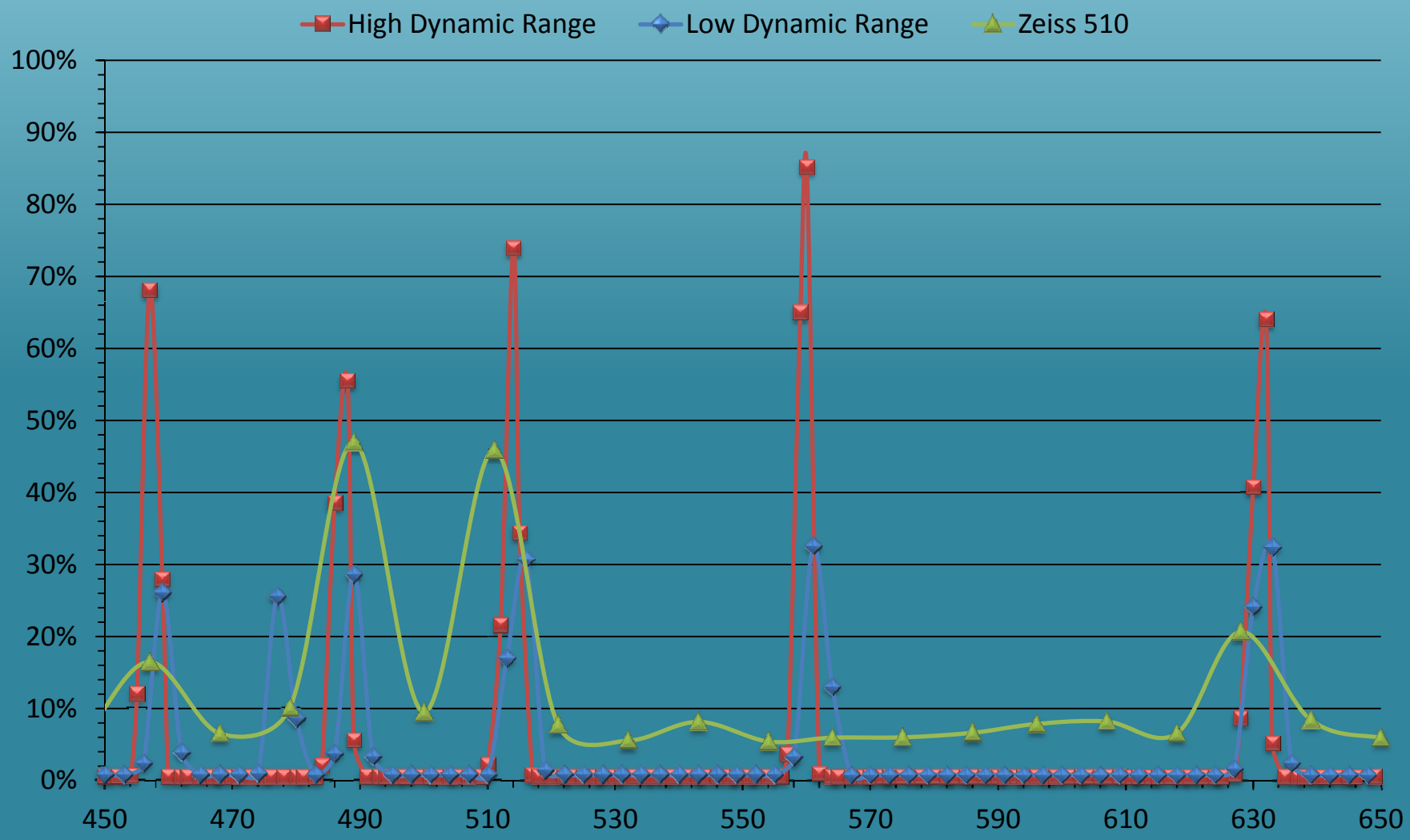
Spectral Accuracy - FWHM



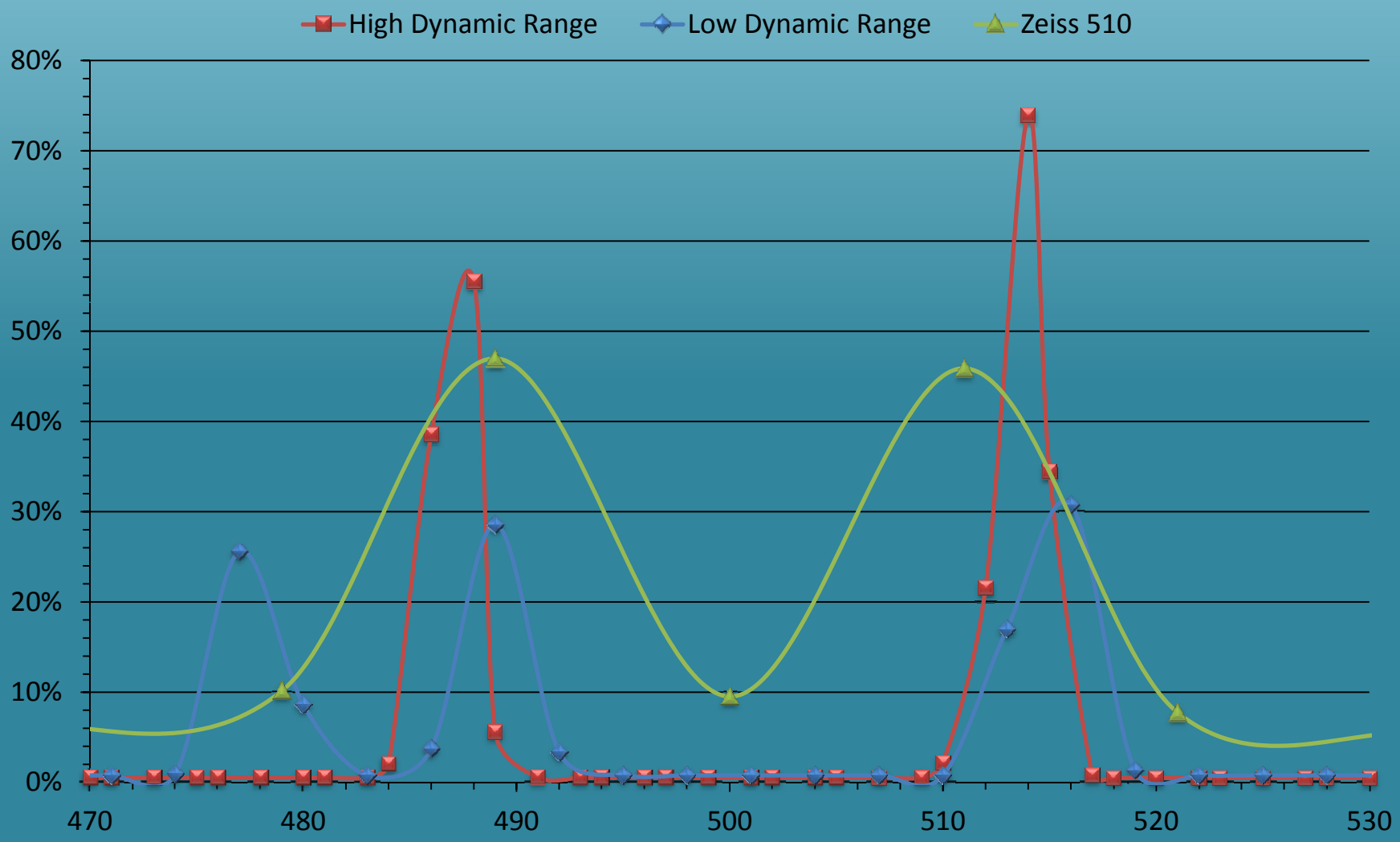
Spectral Accuracy – Low Resolution Instrument (~10 nm)



Spectral Accuracy



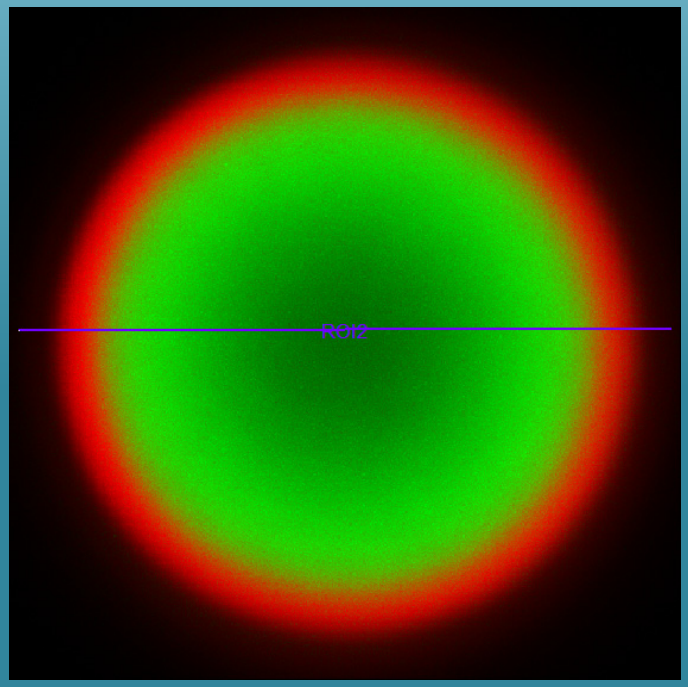
Spectral Accuracy

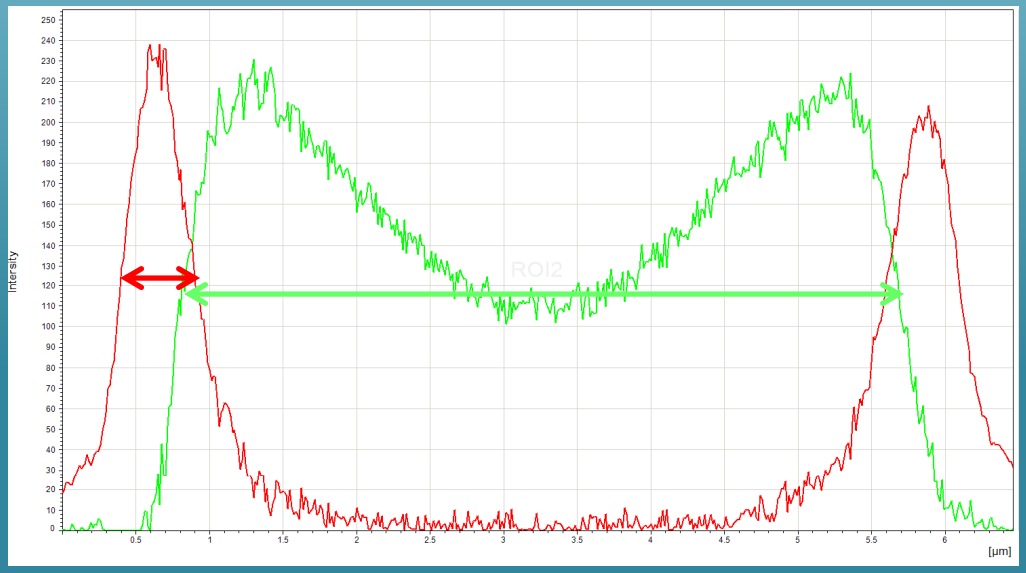


Spectral Un-mixing

- 27 Participants
- Two platforms
- Only companies that allowed input of known dye spectra were used due to difficulty having affordable access to single dye control samples.

Spectral Un-mixing





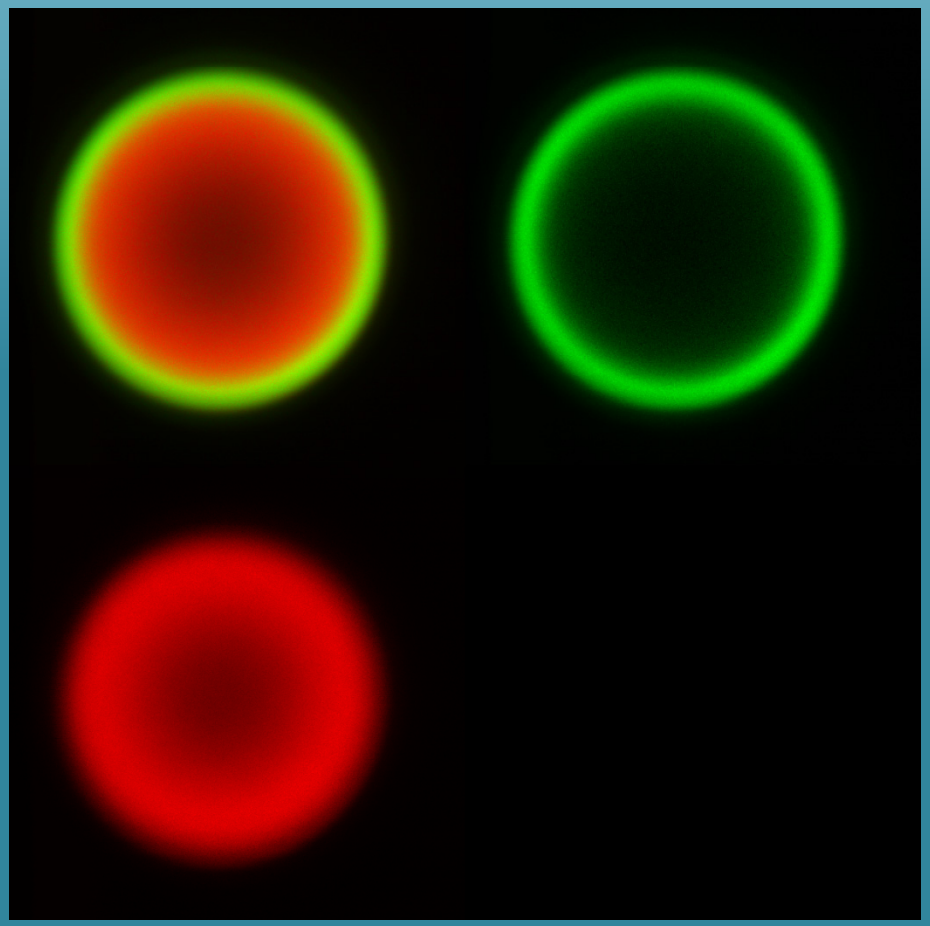
Ring = 0.52 µm

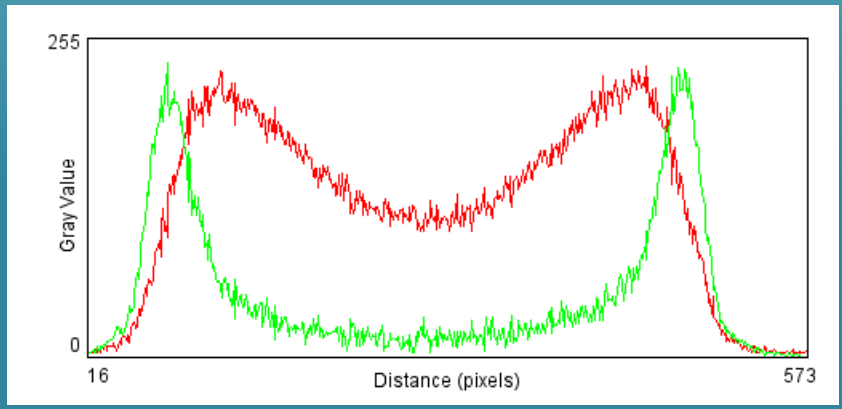
Core = 4.98 µm

Ratio = 10.51

Molecular Probes Specifications
Core = 5.5 µm
Ring = 0.5 µm
Ratio = 11

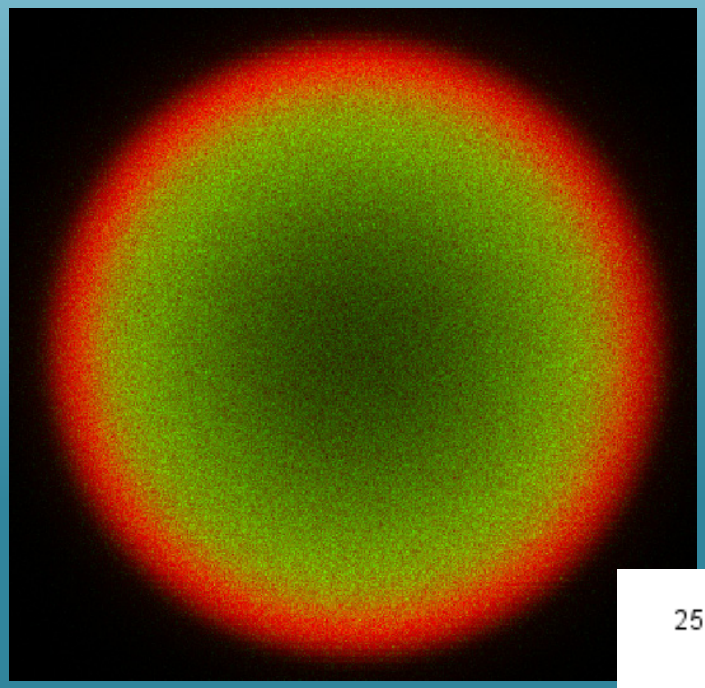
Spectral Un-mixing





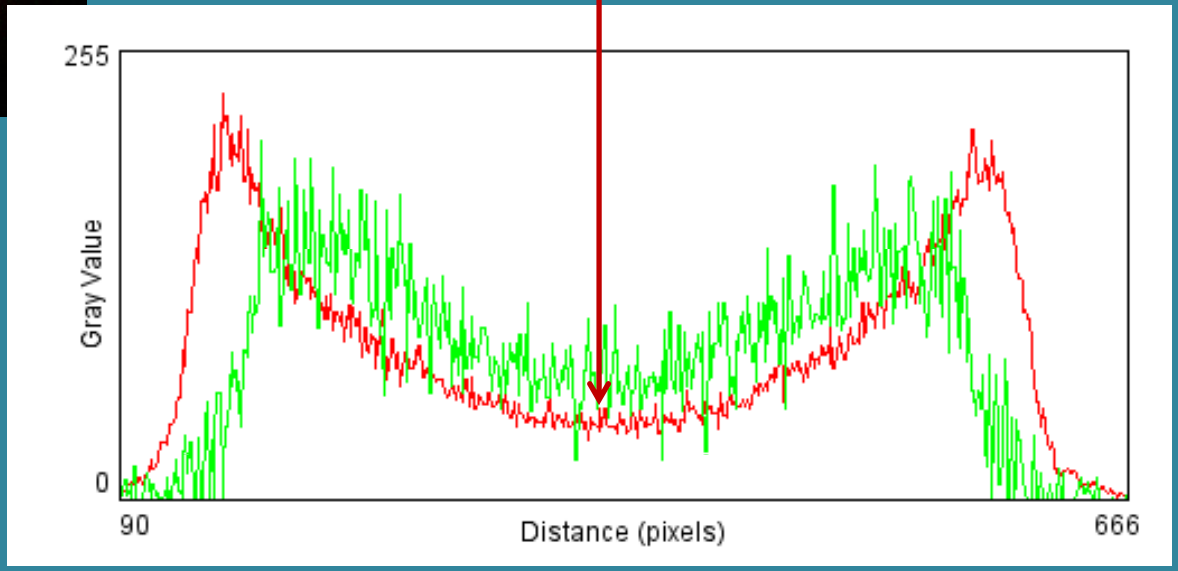
Ratio = 10.10

Spectral Un-mixing



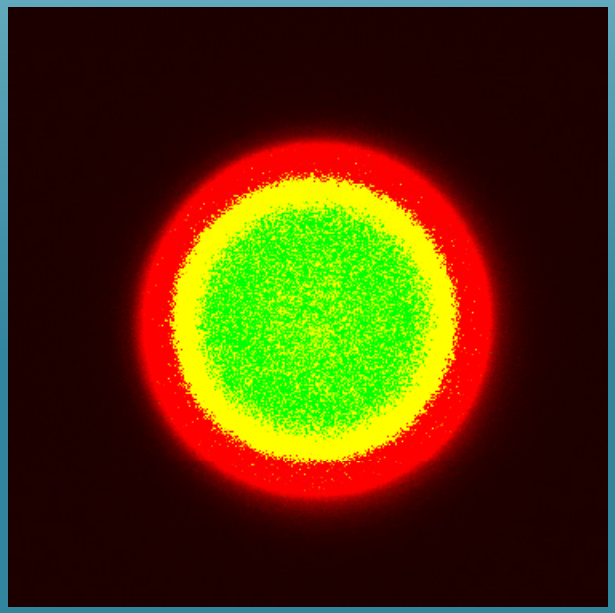
The core is showing up in the ring.

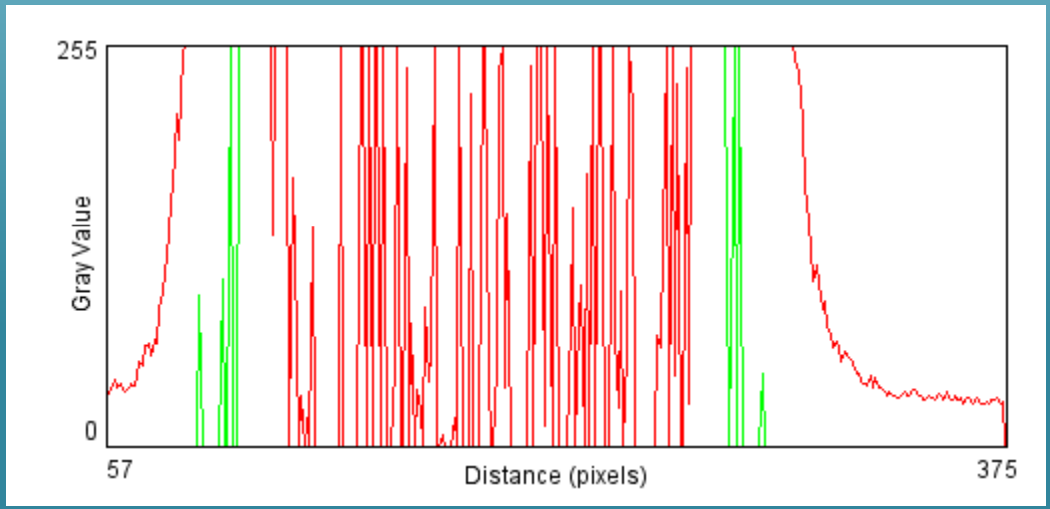
Poor Separation



Ratio = 13.81

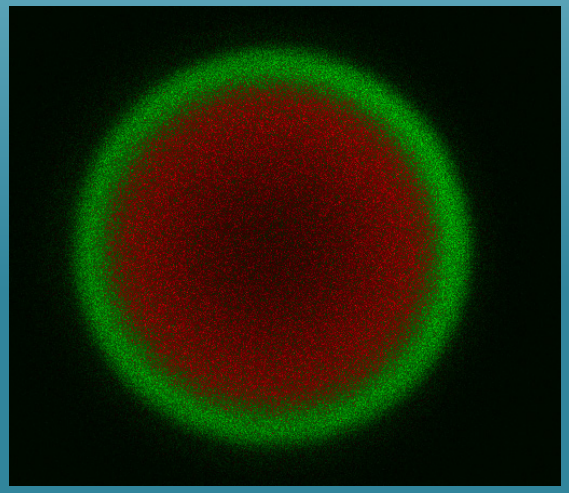
Spectral Unmixing

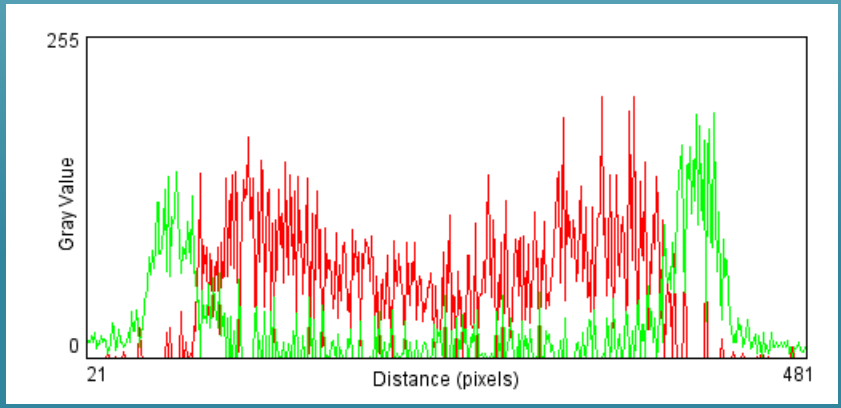




Ratio = 22.64

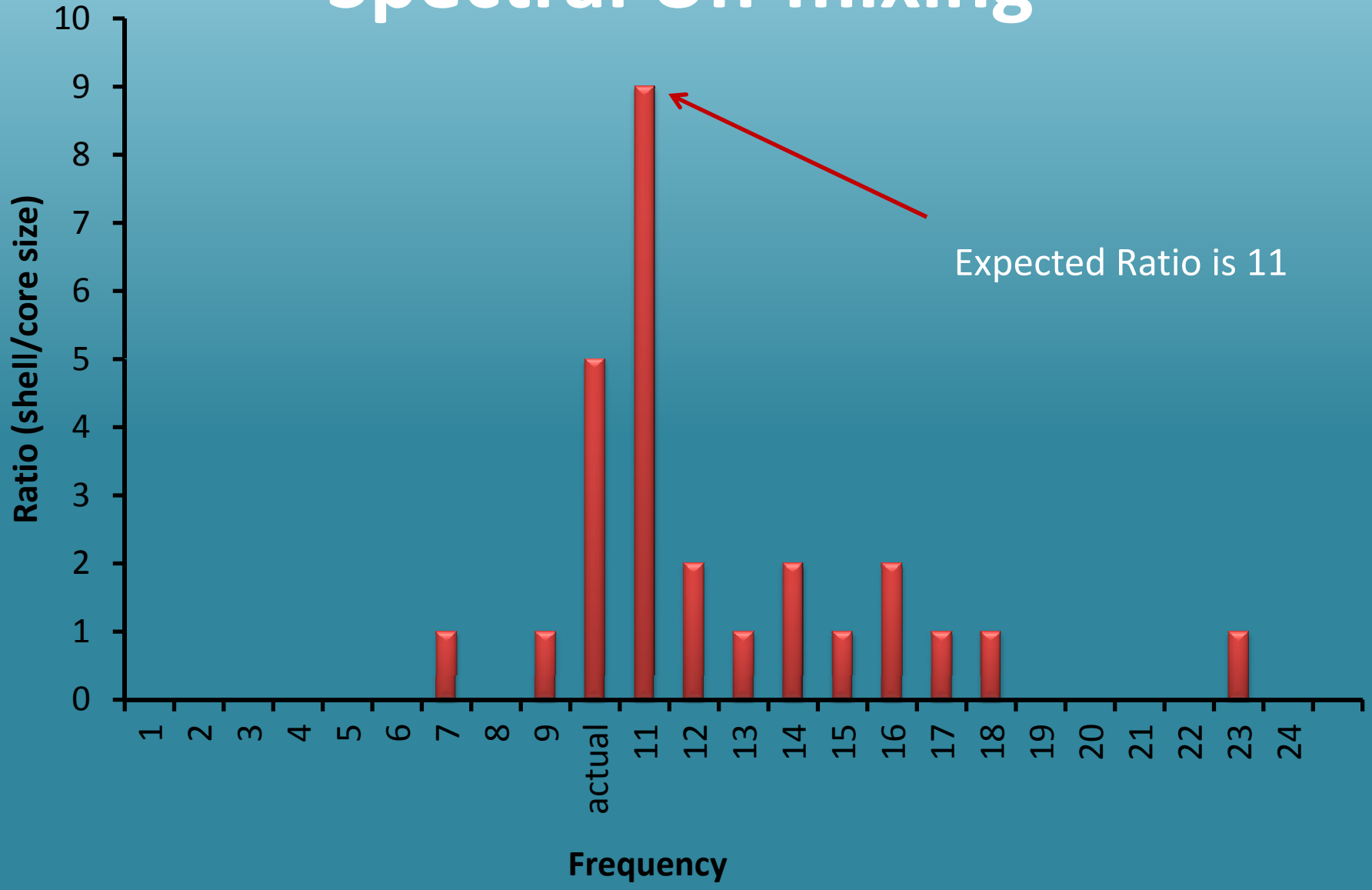
Spectral Un-Mixing



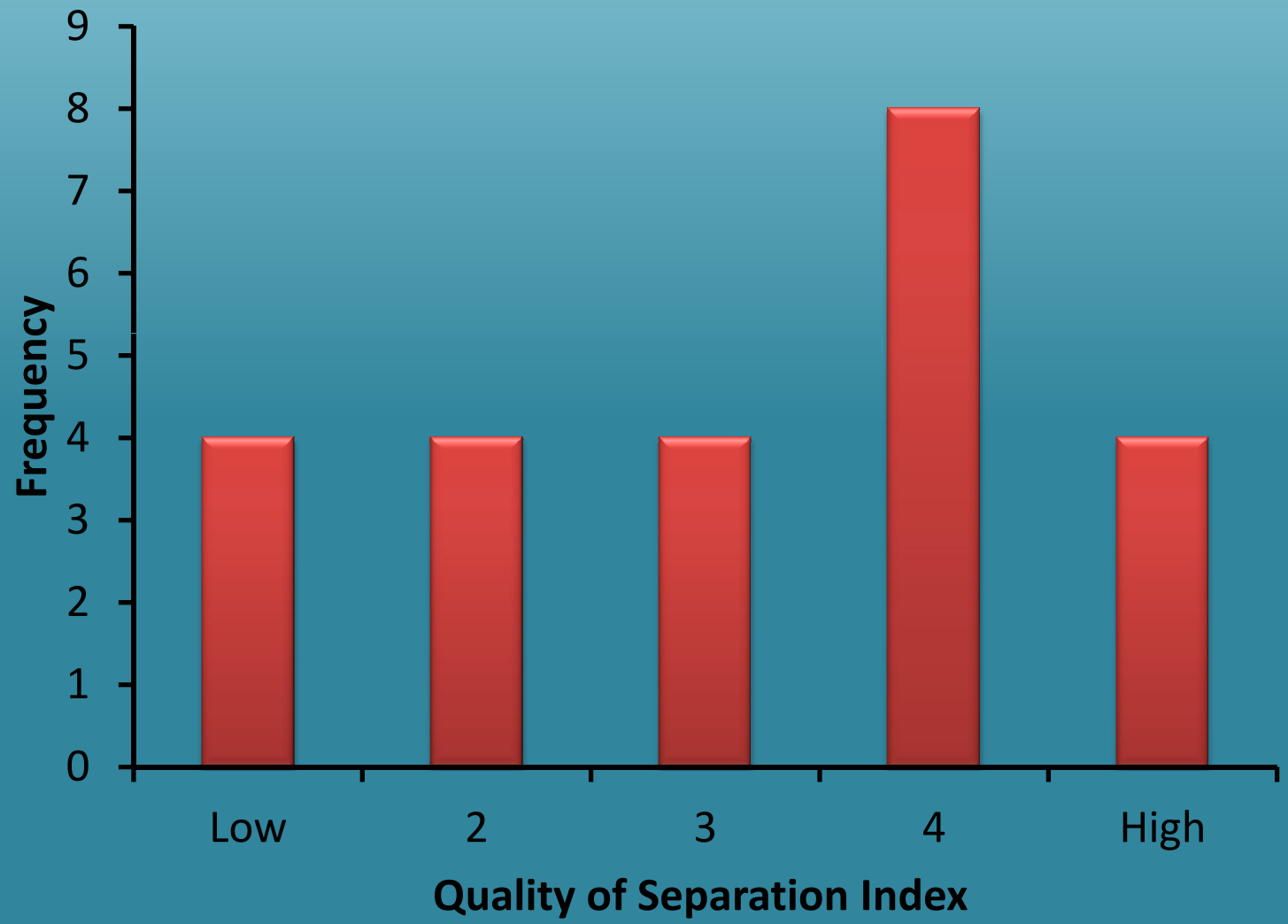


Ratio = 10.61

Spectral Un-mixing



Spectral Un-mixing



Spectral Summary

- Spectral accuracy is very good even for low resolution systems.
- Spectral unmixing is good in general. Most issues were due to data that was not collected properly or reference spectra that were not well measured.

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Blood Center of Wisconsin

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Marc Thibault

Ecole Polytechnique, Montreal

Thank You to Our Participants!

Name	Country	Name2	Country2	Name3	Country3
Cameron Nowell	Australia	Juan Luis Ribas	Spain	Doug Taatjes	USA
Justin Ross	Australia	Julien Colombelli	Spain	Fred Indig	USA
Chris Guerin	Belgium	Manel Bosch	Spain	G. Esteban Fernandez	USA
James Jonkman	Canada	Sylvie le Guyader	Sweden	Gabriel Gaidosh	USA
Kimmo Tanhuanpää	Finland	Justine Kusch	Switzerland	Lauren Ehrlich	USA
Francois Waharte	France	Lauran Oomen	The Netherlands	Lu Hilenski	USA
Ralf Zenke	Germany	Allison van de Meene	UK	Sarah Swanson	USA
Aryeh Weiss	Israel	Ann Wheeler	UK	Sean Wilson	USA
Domenico Marzulli	Italia	David Johnston	UK	Stanislav Vitha	USA
Jacqueline Ross	New Zealand	Noriko Kane-Goldsmith	USA	Stephen Lentz	USA
Lloyd Donaldson	New Zealand	Carol Norris	USA	Steven Hoffman	USA
Hege Avsnes Dale	Norway	Caroline Miller	USA	Susan Garfield	USA
Paula Sampaio	Portugal	David Burk	USA	Wai Chan	USA
Pedro Almada	Portugal	Will Yutzy	USA	Yan Deng	USA
Meredith Calvert	Singapore	Alan Siegel	USA	Zhengmei Mao	USA
Alberto Hernández Cano	Spain	Anda Cornea	USA	Anne-Marie Girard	USA
Carlos Sanchez Martin	Spain	Arvydas Matiukas	USA	Carol Bayles	USA
Elena Rebollo	Spain	Brian Armstrong	USA		
Giovanna Expósito Romero	Spain	Damir Sudar	USA		