

OptoNano Microsphere-assisted Microscope

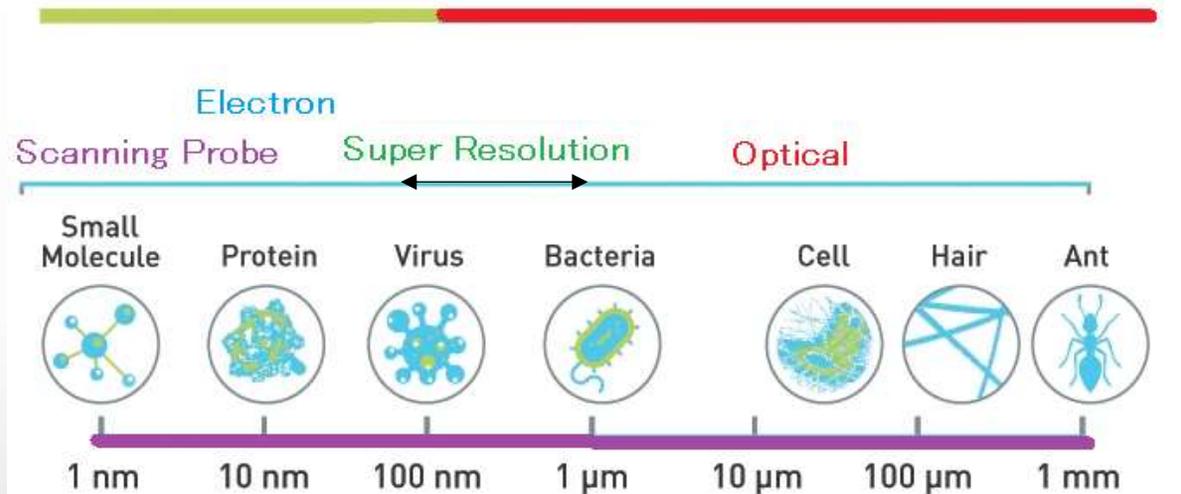
November 2020

Outline

- Microscope types and resolution
- Diffraction limit and Super-resolution microscopes
- Optical Microsphere Microscopy and the OptoNano
- OptoNano Details and Specifications

Overview of Microscope Resolution

The Limitations of Resolution



Scanning Tunneling
(STM: 0.01-10nm)

Transmission Electron (TEM: 0.01nm-100μm)

Atomic Force
(AFM: 1-10nm)

Scanning Electron (SEM: 0.4nm-1mm)

Compound Light (CLM: 200nm-10mm)

Human Eye
>200μm

The Super Resolution Microscope

Abbe's Resolution Limitation

Spatial resolution is limited by optical diffraction:

$$d = \frac{0.61\lambda}{n \sin \theta} \sim 0.61\lambda$$

Where: d - smallest feature size; λ - wavelength of optical light; n - refractive index of medium; θ - incident angle.

(The best resolution is roughly a half of the wavelength of the light used)

Super-resolution microscopy is a series of techniques in optical microscopy that allow such images to have resolutions better than those imposed by the diffraction limit.

Examples of Super-Resolution Techniques

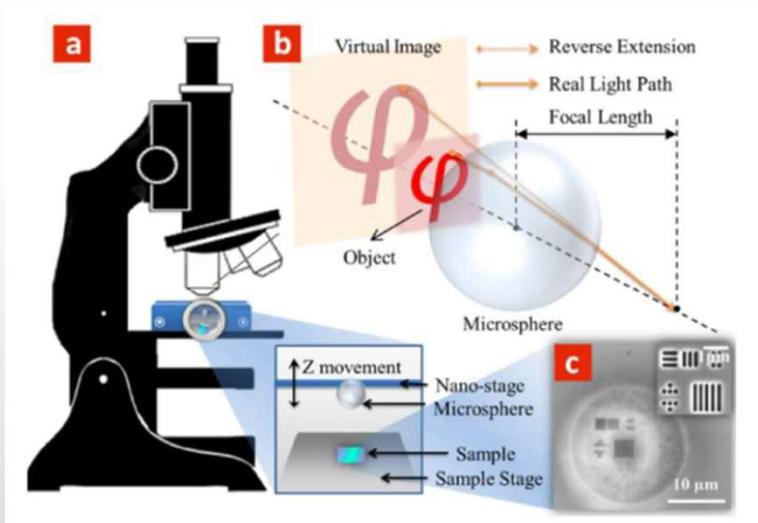
- Standard Fluorescence
- Structured Illumination (SIM)
- Stimulated Emission/Depletion (STED)
- Photo-Activated Localization (PALM)
- Stochastic Optical Reconstruction (STORM)
- Near Field Scanning (NSOM)

Comparison of Super Resolution Microscope Technologies

Microscope Type	Resolution	Requires Fluorescence	Sample Preparation	Image Acquisition Time	Complex Image Processing	Working Distance
<i>Rubric</i>	<i>Less is Better</i>	<i>None is Better</i>	<i>None is Better</i>	<i>Less is Better</i>	<i>Less is Better</i>	<i>Longer is Better</i>
Standard Fluorescence	250 nm	Yes	Yes	Very Fast (Real Time)	None	0.1-2.75 mm
Structured Illumination (SIM)	100 nm	Yes	Yes	Fast (Seconds)	Yes (Fourier Transform)	0.1-0.2 mm
Stimulated Emission/Depletion (STED)	30-70 nm	Yes	Yes	Fast (Seconds)	None	~0.10 mm
Photo-Activated Localization (PALM)	10-55 nm	Yes	Yes	Moderate (Minutes)	Yes(Centroid Identification)	~0.10 mm
Stochastic Optical Reconstruction (STORM)	10-55 nm	Yes	No	Moderate (Minutes)	Yes(Centroid Identification)	~0.10 mm
Near Field Scanning (NSOM)	10 nm	No	Yes	Long (0.5 Hour)	None	~0.00002 mm

Enter Optical Microsphere Microscopy

An optical microsphere enables a conventional optical microscope to visualize tiny features beyond the diffraction limit.



- A optical microsphere is located on the optical axis between the objective and the sample.
- The imaging process can be divided into two steps:
 1. The microsphere manipulates the light from the sample and forms a virtual image.
 2. The conventional optical microscope captures this virtual image.

For example, if the microsphere can provide a virtual image with a magnification of 4X, it means a 100-nm feature in the sample becomes 400 nm as a virtual image. $100 \text{ nm (original feature)} < 200 \text{ nm (diffraction limit threshold)} < 400 \text{ nm (virtual image)}$. Therefore you have a conventional optical microscope that can resolve sub-diffraction features by way of a magnified virtual image.

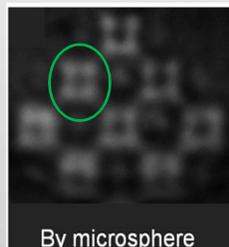
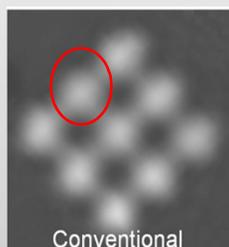
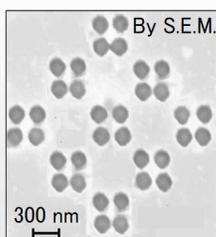


Revisit The Super Resolution Microscope Comparison

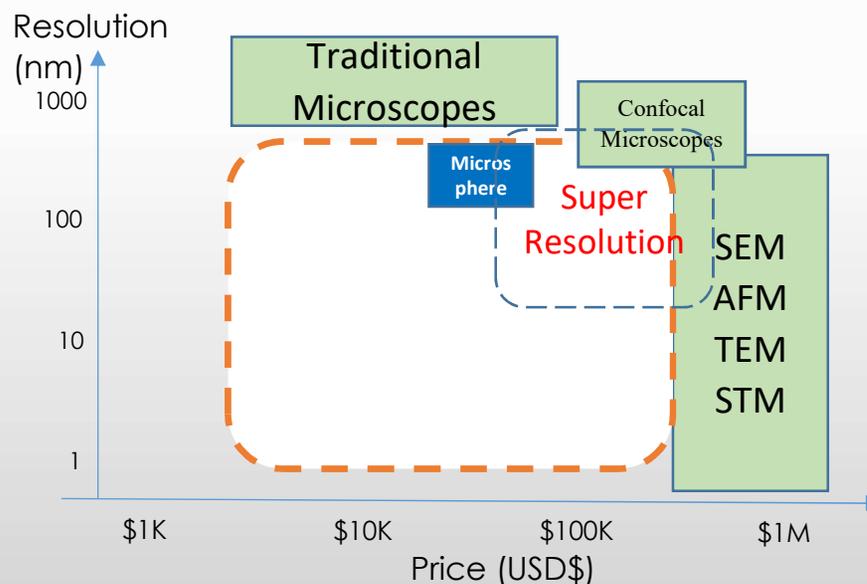
Microscope Type	Resolution	Requires Fluorescence	Sample Preparation	Image Acquisition Time	Complex Image Processing	Working Distance
<i>Rubric</i>	<i>Less is Better</i>	<i>None is Better</i>	<i>None is Better</i>	<i>Less is Better</i>	<i>Less is Better</i>	<i>Longer is Better</i>
Standard Fluorescence	250 nm	Yes	Yes	Very Fast (Real Time)	None	0.1-2.75 mm
Optical Microsphere(OM)	137-200 nm	No	No	Very Fast (Real Time)	None	~0.003 mm
Structured Illumination (SIM)	100 nm	Yes	Yes	Fast (Seconds)	Yes (Fourier Transform)	0.1-0.2 mm
Stimulated Emission/Depletion (STED)	30-70 nm	Yes	Yes	Fast (Seconds)	None	~0.10 mm
Photo-Activated Localization (PALM)	10-55 nm	Yes	Yes	Moderate (Minutes)	Yes(Centroid Identification)	~0.10 mm
Stochastic Optical Reconstruction (STORM)	10-55 nm	Yes	No	Moderate (Minutes)	Yes(Centroid Identification)	~0.10 mm
Near Field Scanning (NSOM)	10 nm	No	Yes	Long (0.5 Hour)	None	~0.00002 mm

Review of Optical Microsphere(OM) Advantages

- No Sample Preparation Needed
- Fluorescence Not Required
- Provides Real-Time Images
- Generally Lower Cost
- Improved Resolution



Price vs. Resolution



The OptoNano Super-Resolution Microscope

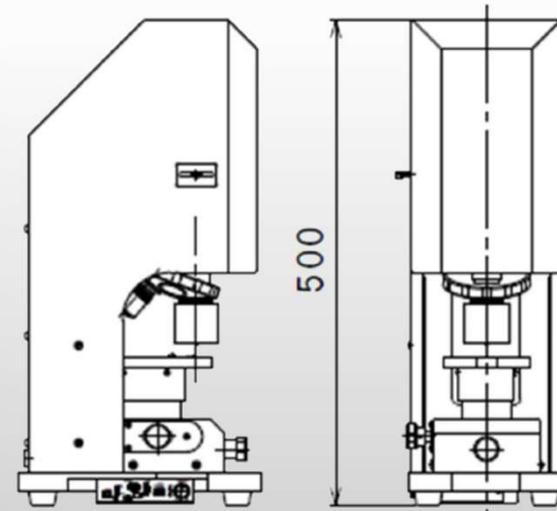
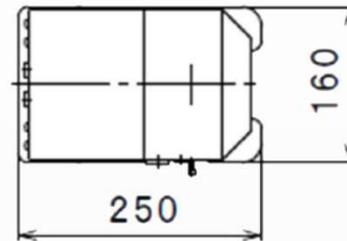
- The OptoNano works exactly like an optical microscope in terms of operation, features and function. It can be ordered with objective lenses from 5x to 100x.
- An additional Microsphere-Assisted Objective Lens is included that provides you with ability to see below 200nm.
- Additional Features:
 1. Able to do auto focus on sample base on different objective selected.
 2. The software and motor stages give it the capability to stitch together 100 images into a single image.
 3. Have measurement software up to accuracy of ~35nm



Product Images and Drawing

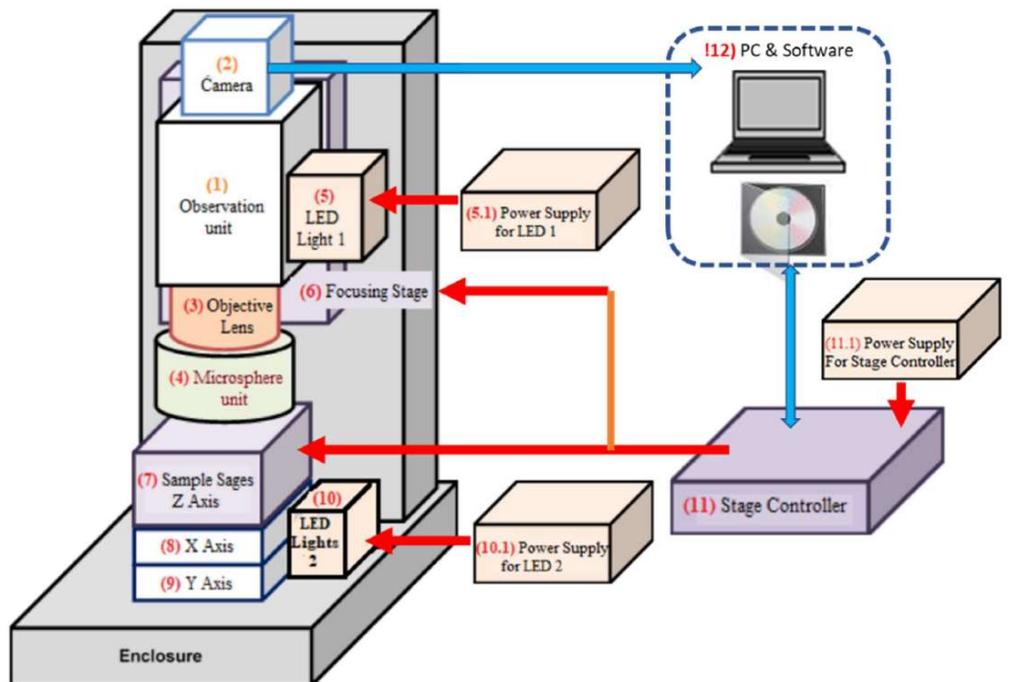


Side View (Cover Removed)



Dimensions in mm

OptoNano Block Diagram

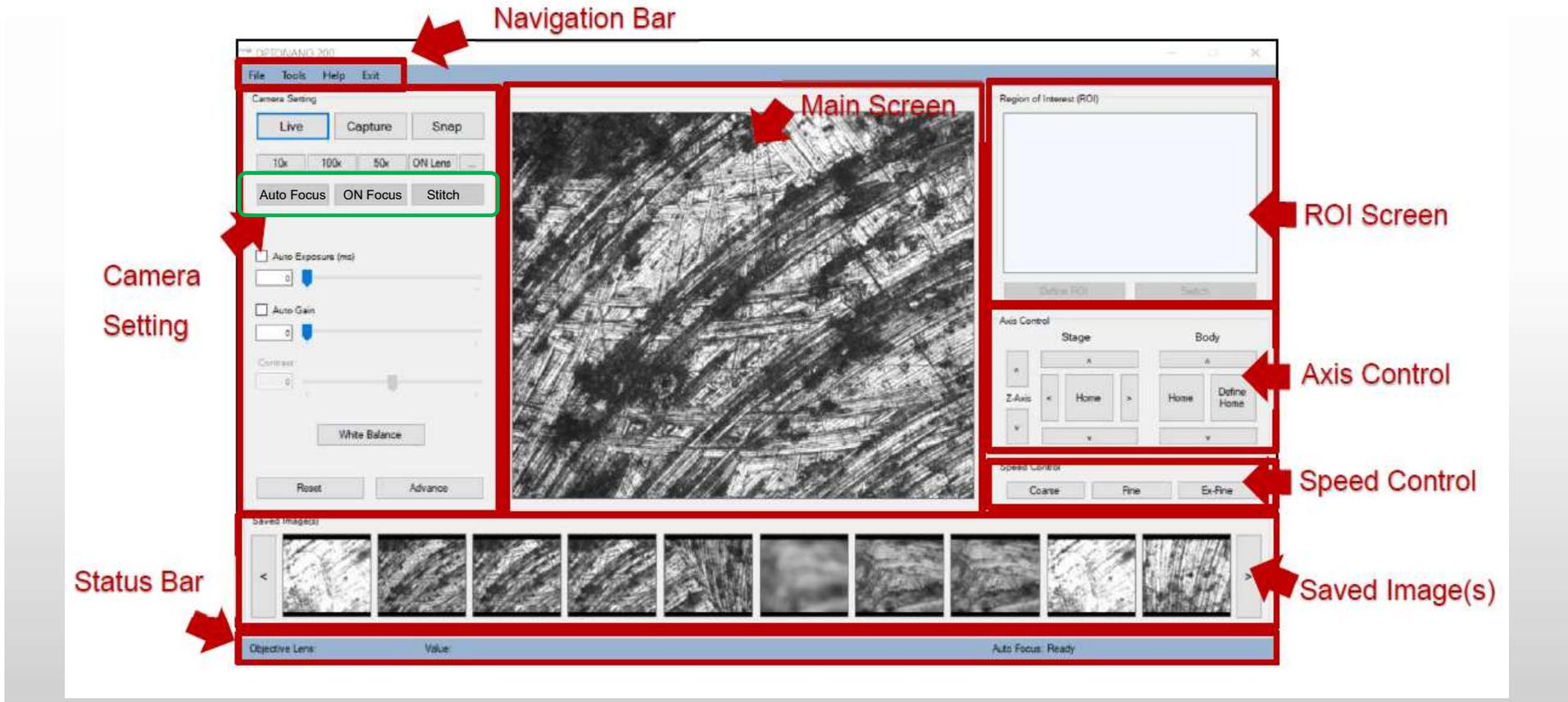


- ① Observation Unit
- ② Camera (Powered by PC)
- ③ Objective Lens
- ④ Microsphere Unit
- ⑤ Reflective LED lights & Power supply
- ⑥ Focusing Stage Z axis
- ⑦ Sample Stage Z axis
- ⑧ Sample Stage X axis
- ⑨ Sample Stage Y axis
- ⑩ Transmission LED lights & Power supply
- ⑪ 4 Axis Stage controller & Power supply
- ⑫ PC & Software

OptoNano Microscope Specifications

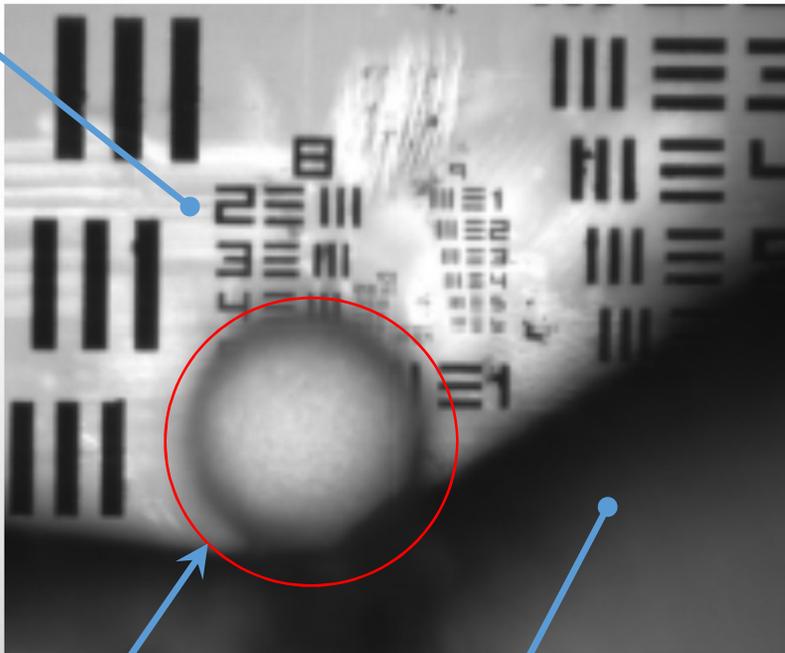
Observation Conditions	Air	
Illumination wavelength	460 nm (Blue)	~ 700 nm (white)
Lens	10X, OptoNano200 Lens	
Working Distance	0 to 3 μ m	
Limit of Resolution	\leq 200 nm	
– Effective Resolution	~ 137 nm	~ 154 nm
Lens Turret	4 holes Manual Revolving (spaced at 90 degree intervals)	

Software User Interface



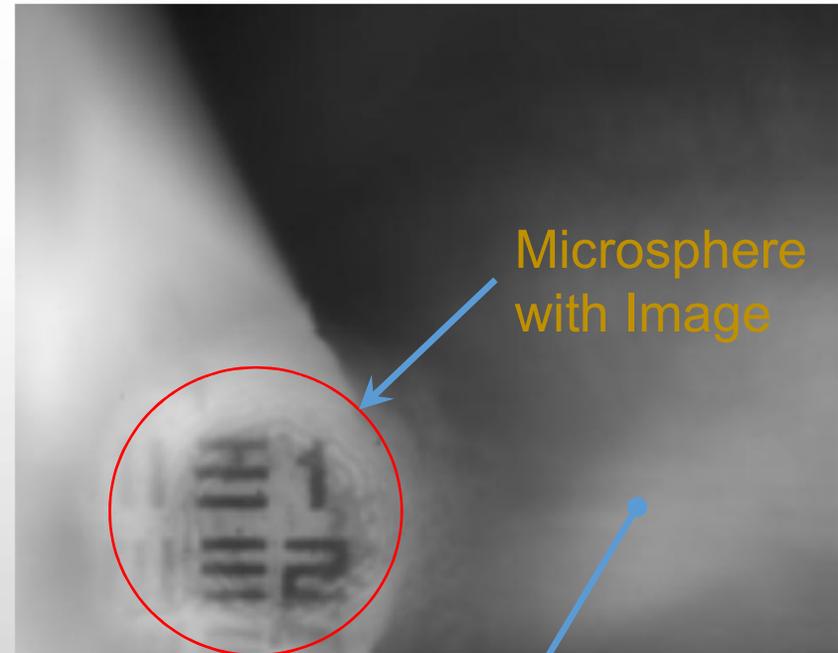
The OptoNano Objective Showing Microsphere

Resolution
Test
Target



Microsphere
(Pre Focus)

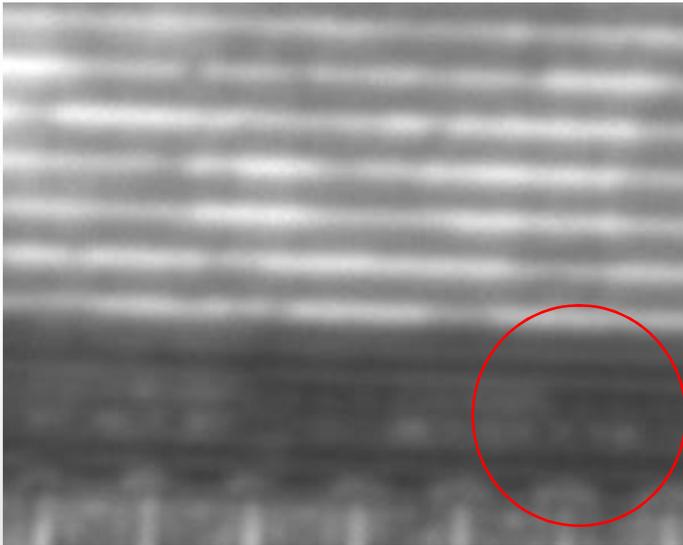
Microsphere
(Support Structure)



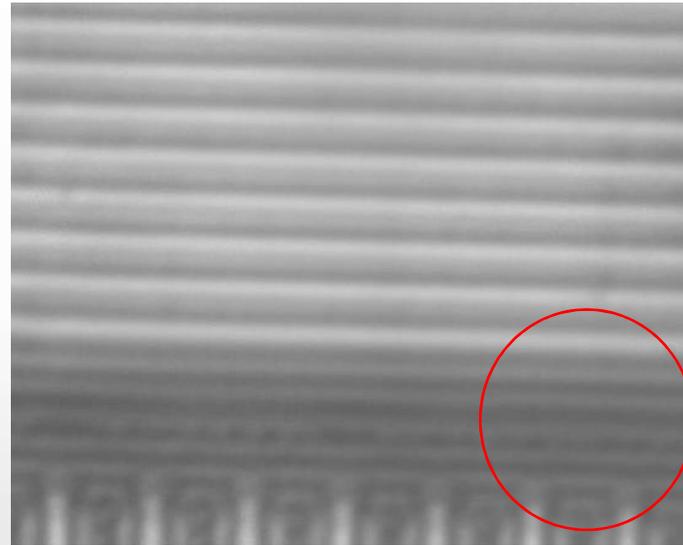
Microsphere
with Image

Microsphere
(Support Structure)

Comparison of Images



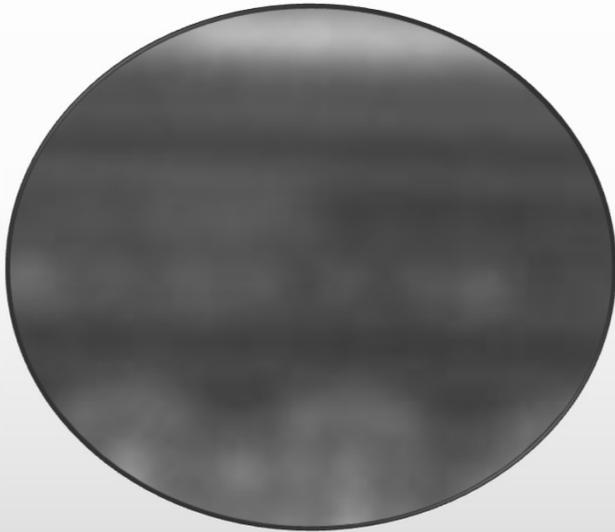
State-of-the-art best-of-class Image
(From world leading microscopy company)



OptoNano Microsphere-assisted Image

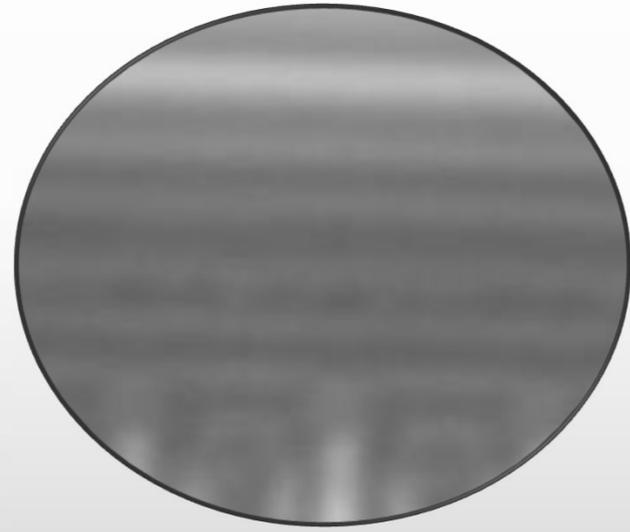
Zoom in on selected regions

Line pairs are not visible



State-of-the-art best-of-class Image
(From world leading microscopy company)

Line pairs are visible



OptoNano Microsphere-assisted Image

Recap

- Many types of microscopes, differentiated by resolution
- For light microscopes, diffraction limits best resolution to about half the light's wavelength
- Super resolution microscopes break through the diffraction barrier using different techniques
- Microsphere microscopes provide super resolution capability with advantages over other super resolution devices.
- The OptoNano is a microsphere-assisted super resolution microscope now being released by OptoSigma.

Now let's take a look at an OptoNano up close and see a actual demonstration!