

# OptoNano Microsphere-assisted Microscope

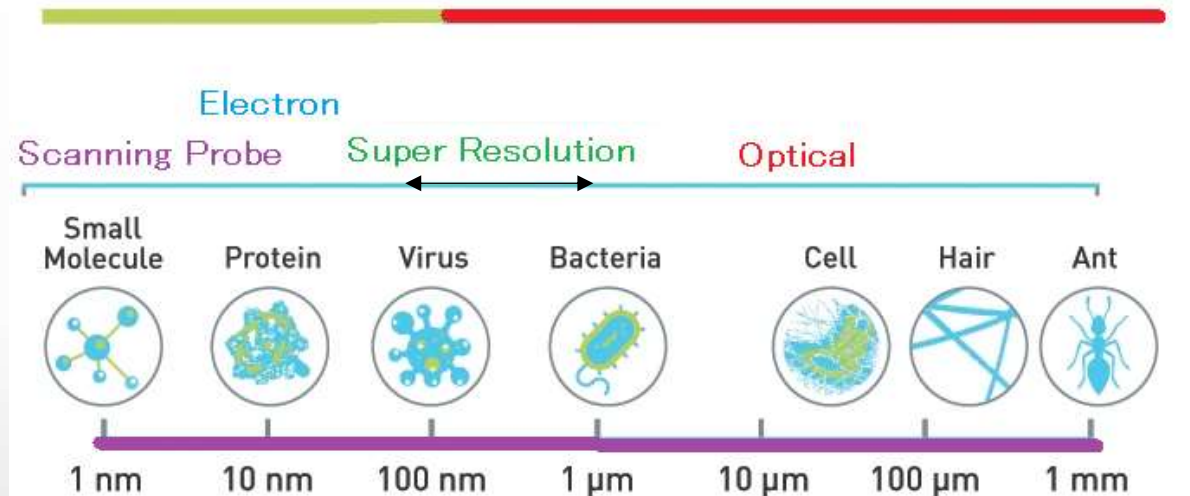
November 2020

# 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;  $\lambda$  - wavelength of optical light;  $n$  - refractive index of medium;  $\theta$  - 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

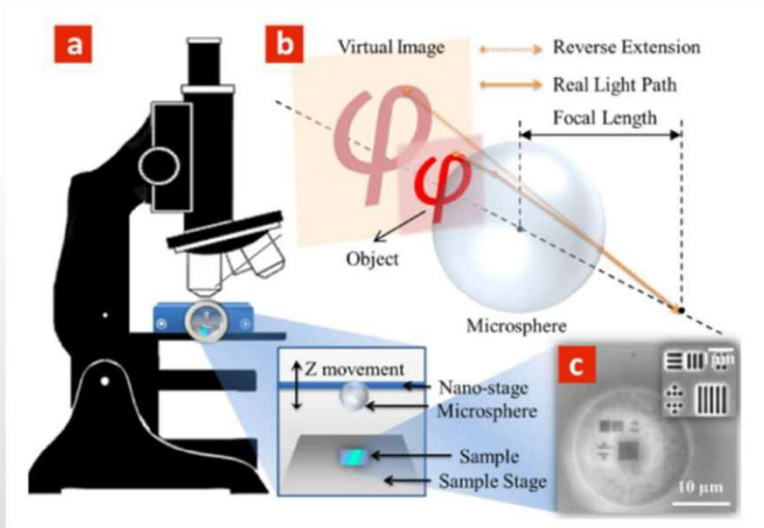
- Standard Fluorescence
- Structured Illumination (SIM)
- Stimulated Emission/Depletion (STED)
- Photo-Activated Localization (PALM)
- Stochastic Optical Reconstruction (STORM)
- Near Field Scanning (NSOM)

## Comparison of Super Resolution Microscope Technologies

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

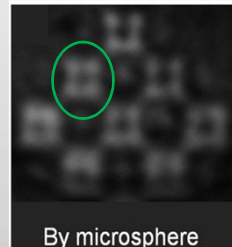
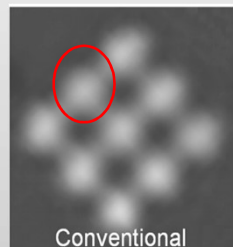
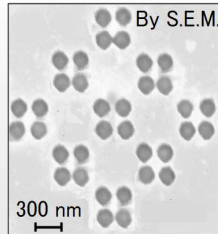
For example, if the microsphere can provide a virtual image with a magnification of 4X, it means a 100-nm feature in the sample becomes 400 nm as a virtual image. 100 nm (original feature) < 200 nm (diffraction limit threshold) < 400 nm (virtual image). Therefore you have a conventional optical microscope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

## Revisit The Super Resolution Microscope Comparison

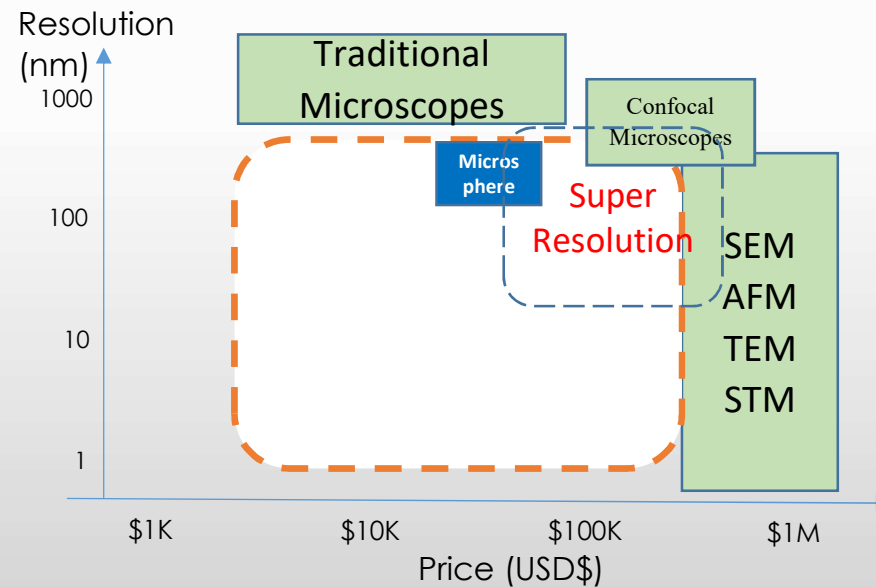
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<b>Standard Fluorescence</b>	250 nm	Yes	Yes	Very Fast (Real Time)	None	0.1-2.75 mm
<b>Optical Microsphere(OM)</b>	137-200 nm	No	No	Very Fast (Real Time)	None	~0.003 mm
<b>Structured Illumination (SIM)</b>	100 nm	Yes	Yes	Fast (Seconds)	Yes (Fourier Transform)	0.1-0.2 mm
<b>Stimulated Emission/Depletion (STED)</b>	30-70 nm	Yes	Yes	Fast (Seconds)	None	~0.10 mm
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<b>Near Field Scanning (NSOM)</b>	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

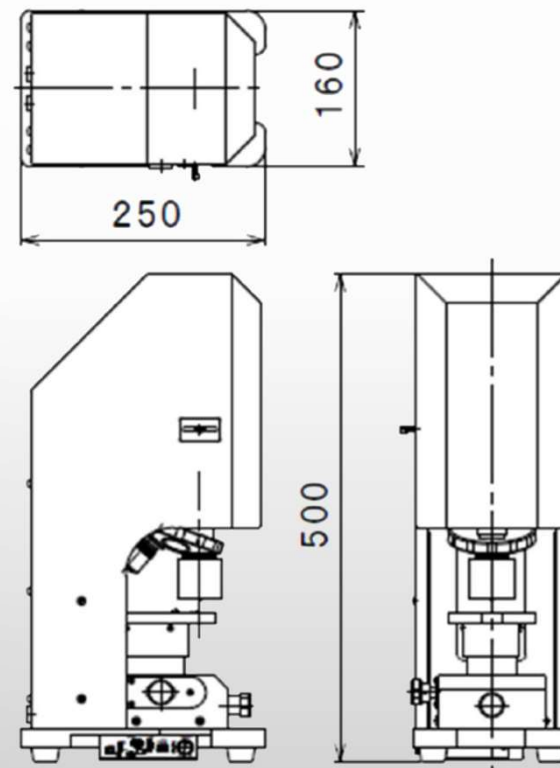
- The OptoNano works exactly like an optical microscope in terms of operation, features and function. It can be ordered with objective lenses from 5x to 100x.
- An additional Microsphere-Assisted Objective Lens is included that provides you with ability to see below 200nm.
- Additional Features:
  1. 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

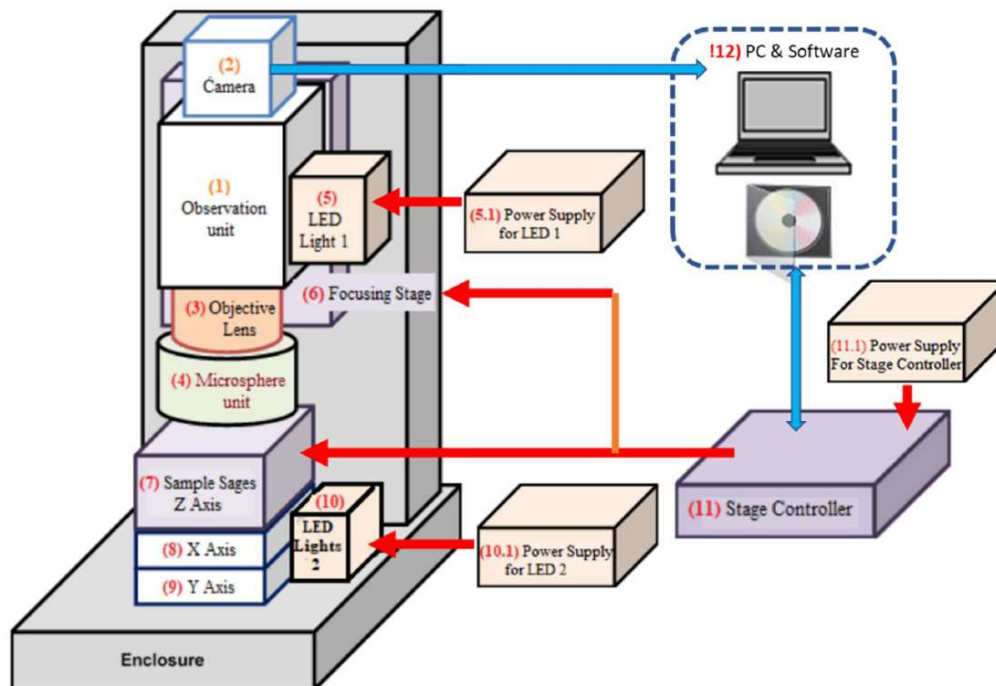


Side View (Cover Removed)



Dimensions in mm

# OptoNano Block Diagram

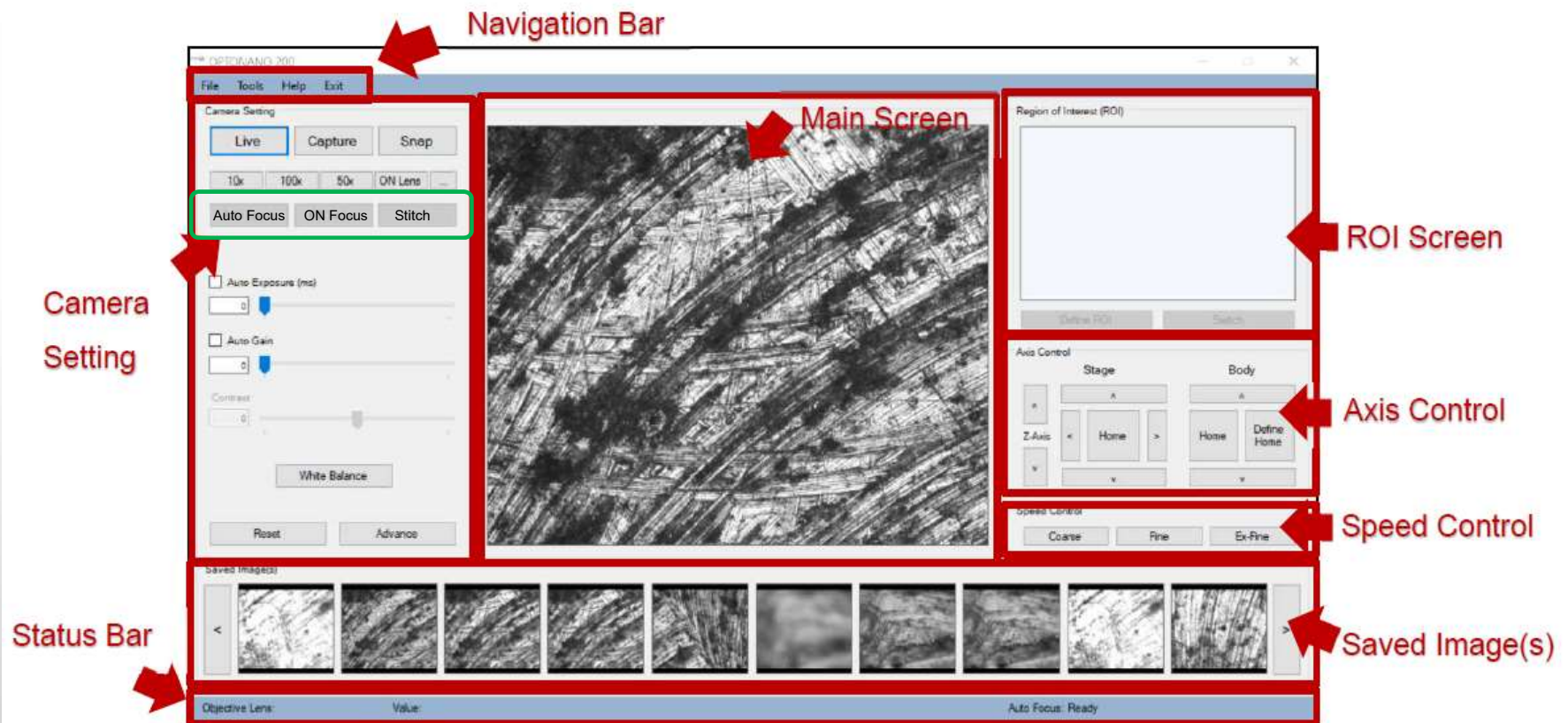


- ① Observation Unit
- ② Camera (Powered by PC)
- ③ Objective Lens
- ④ Microsphere Unit
- ⑤ Reflective LED lights & Power supply
- ⑥ Focusing Stage Z axis
- ⑦ Sample Stage Z axis
- ⑧ Sample Stage X axis
- ⑨ Sample Stage Y axis
- ⑩ Transmission LED lights & Power supply
- ⑪ 4 Axis Stage controller & Power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

# OptoNano Microscope Specifications

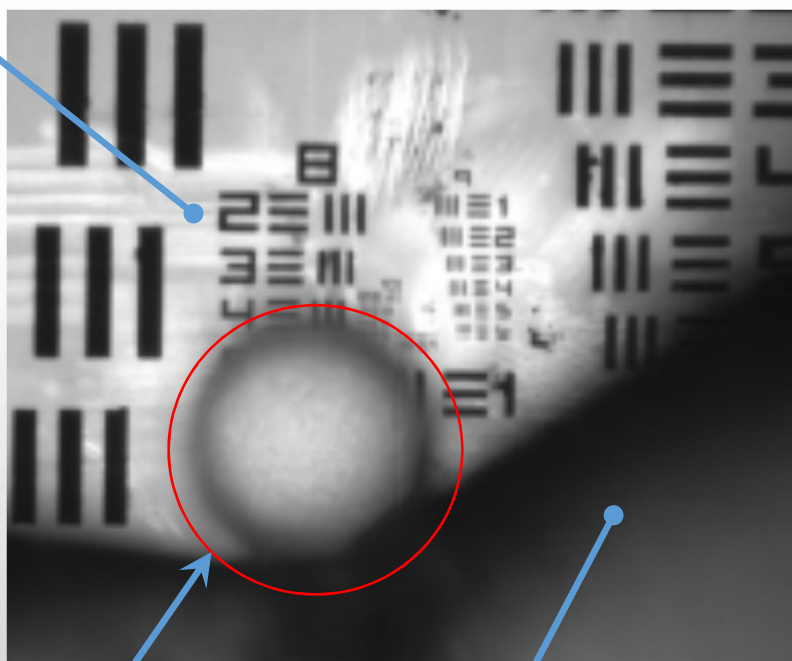
<b>Observation Conditions</b>	Air	
<b>Illumination wavelength</b>	460 nm (Blue)	~ 700 nm (white)
<b>Lens</b>	10X, OptoNano200 Lens	
<b>Working Distance</b>	0 to 3 $\mu$ m	
<b>Limit of Resolution</b>	$\leq$ 200 nm	
– Effective Resolution	~ 137 nm	~ 154 nm
<b>Lens Turret</b>	4 holes Manual Revolving (spaced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

# Software User Interface



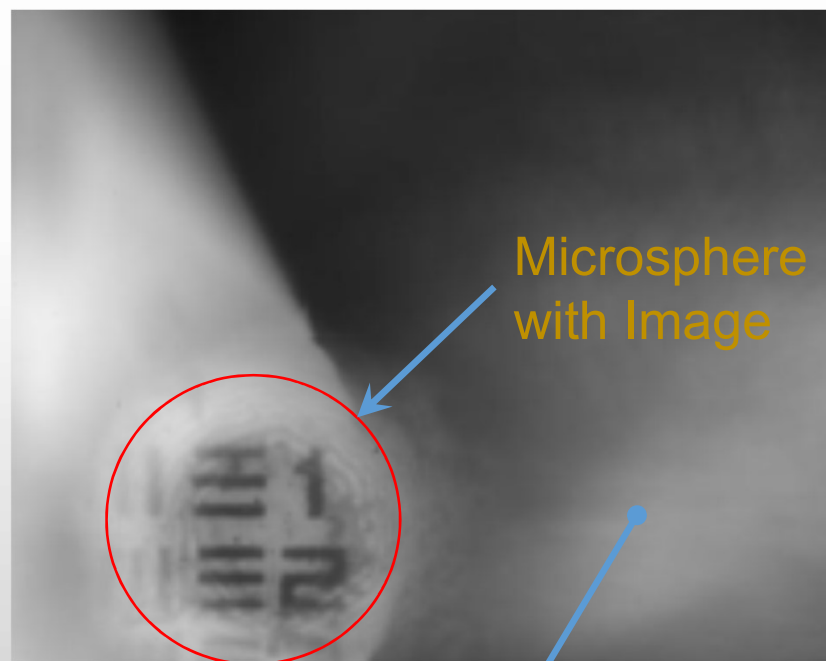
# The OptoNano Objective Showing Microsphere

Resolution  
Test  
Target



Microsphere  
(Pre Focus)

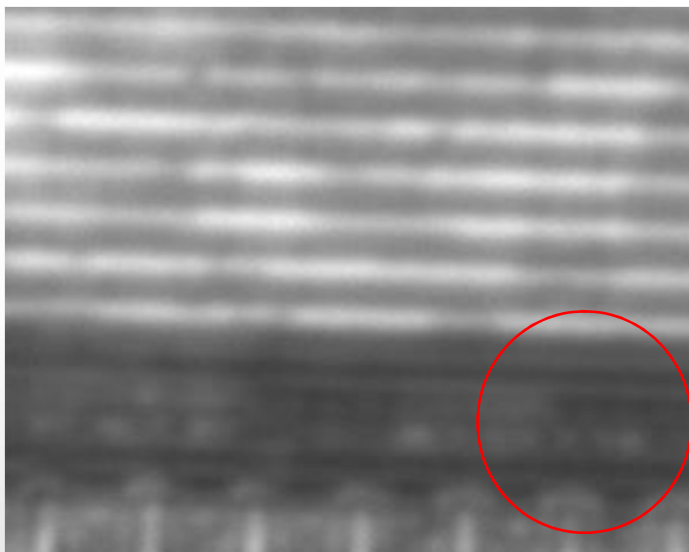
Microsphere  
(Support Structure)



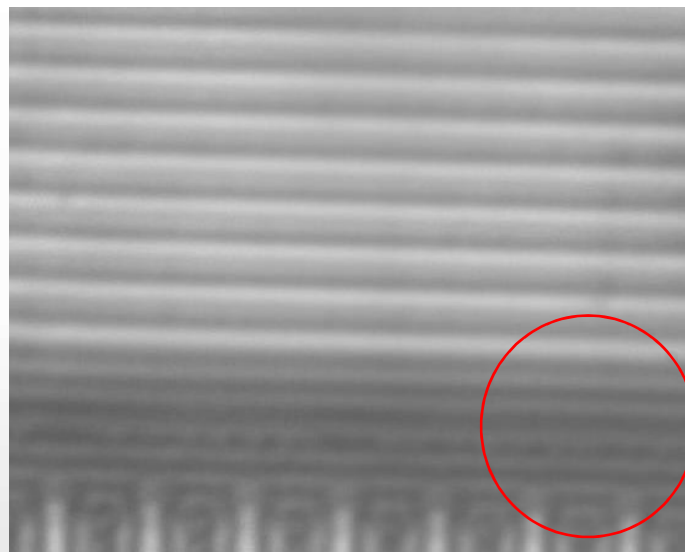
Microsphere  
with Image

Microsphere  
(Support Structure)

# Comparison of Images



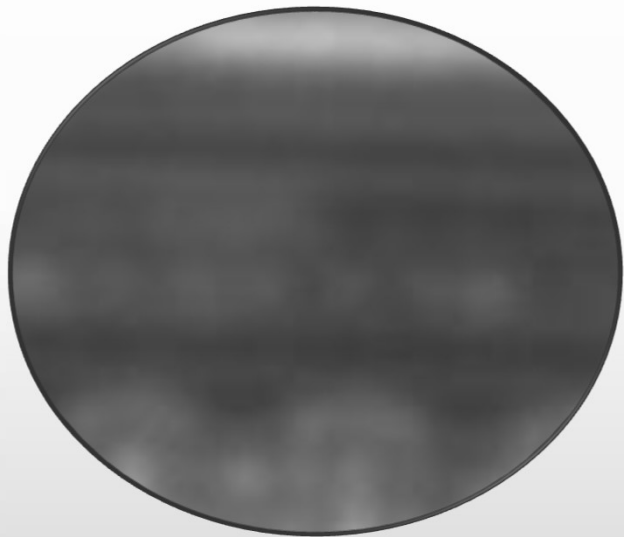
State-of-the-art best-of-class Image  
(From world leading microscopy company)



OptoNano Microsphere-assisted Image

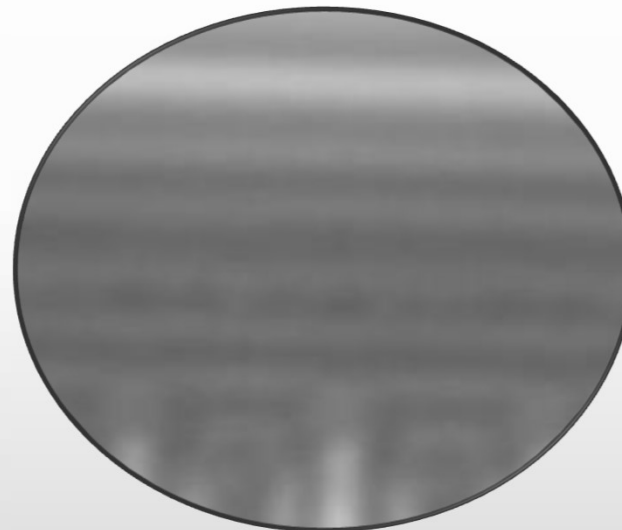
# Zoom in on selected regions

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!