

**Identifyn Standards for Optical Microscopy Methods™****Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L)***Cat ID# - SA 000015*

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, [Mitochondria Antibody \(MA5-12017\)](#), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using [ProLong™ Diamond Antifade Mountant with DAPI cat#P36962](#).

**Microscope**

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

**Single Plane (2D)**

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) = 835 X 787

Image Size (Scaled) = 119.29µm X 112.43µm

Bit Depth = 14 Bit

Image Center Position = X: 42.33mm, Y: 51.12mm

Stage Position = X: 42.33mm, Y: 51.12mm

ROI Center Offset = X: 1.14µm, Y: 0.00µm

**488 -**

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 -550

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @25%

Exposure Time = 200ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.25

Imaging Device = Axiocam 807

Camera Adapter = 1X

Depth of Focus = 1.00µm

Binning Mode = 2,2

**DAPI -**

Reflector = 49 DAPI

Beam Splitter = 395

Filter Excitation Wavelength = 335-383

Filter Emission Wavelength = 420-470  
Contrast Method = Fluorescence  
Light source = X-Cite Xylis Lamp Intensity @5%  
Exposure Time = 30ms  
Excitation Wavelength = 353  
Emission Wavelength = 465  
Effective NA = 1.25  
Imaging Device = AxioCam 807  
Camera Adapter = 1X  
Depth of Focus = 0.90µm  
Binning Mode = 2,2

### **Post Processing** - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.  
Normalization, Phase Corrector, Fourier Filter, and Deconvolution **were NOT used.**

### **Image Corrections**

None

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Image by J.K. Bennett

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