

Identify Standards for Optical Microscopy Methods™**Identify® 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, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identify® 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 LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)***Z-Stack = 60 Slices (2.95µm)***

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm X 50.00nm
Image Size (Pixels) = 2588 X 2588
Image Size (Scaled) = 91.35µm X 91.35µm
Bit Depth = 16 Bit
Image Center Position = X: 78.66mm, Y: 50.05mm
Stage Position = X: 78.66mm, Y: 50.05mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.31 AU / 236µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @.02%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.81µs
Frame Time = 12.92s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4

Detection Wavelength = 495-569
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.1 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 182µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.81µs
Frame Time = 12.92s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-464
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.0 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 4% (488), Threshold 4% (DAPI), Ramp 96% (488), Ramp 96% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness 135%, Azimuth 80° , Elongation -130° , Enabled Directional Light,
Enabled Tone Mapping

Projection = View Angle 37°, Scale Z 155%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

None

Image by P. Raut

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