

Identifyn Standards for Optical Microscopy Methods™Identifyn® Primary Antibody**Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M)***Cat ID# - PA-RR 000003*Identifyn® Secondary Antibodies**Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)***Cat ID# - SA 000019***Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)***Cat ID# - SA 000063*

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. 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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - *Panel A, Total Image Acquisition***Z-Stack = 132 Slices (3.93µm)**

Channels = 3

Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 66.72 μ m X 66.72 μ m
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel C, Region of Interest

Z-Stack = 47 Slices (2.3 μ m) - The Gallery tool was used to reduce the z-stack to provide clarity

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 1162 X 1078
Image Size (Scaled) = 15.33 μ m X 14.22 μ m
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13 μ m
Binning Mode = 2,2
Laser Wavelength = 640nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5 μ m grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR

Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @7.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 2
Channels = 1
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.4461
Input SNR = N/A
Regularization Weight = N/A
Iterations = N/A
Filter = N/A
Fitting = N/A

Normalization = Automatic
Experimental PSF = N/A

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% DAPI, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 20% all channels, Ambient Light 0% all channels, Specular Light 100% all channels, Shininess 57% of all channels
Background = Black
Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary -

Panel A - Presents a 3-dimensional Maximum Projection of the totality of the image acquisition, 132 z-planes, 3.93 microns.

Panel B - Presents a 2-dimensional image at z-plane 71 of 132. Additionally, a Region of Interest (ROI) is indicated by the white box to be shown in panel C.

Panel C - Presents a 3-dimensional surface projection of the ROI chosen from panel B. The ROI is intended to demonstrate the power of super-resolution SIM in elucidating further the structural details that we began to see in the LSM Plus confocal image and super resolution Airyscan.

Panel D - Transparency in image acquisition, displaying the settings. Note that the PARP1/647 staining utilizes 1.0% of the 640 nm laser with a 120 ms exposure time and a 1.59 s frame time. The grating period was 36.5.

Image Corrections

None

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