

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 followed this, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), which was 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) = 66.72µm X 66.72µm
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: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels D/E, Region of Interest**Z-Stack = 132 Slices (3.93µm)**

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 744 X 828
Image Size (Scaled) = 9.82µm X 10.93µm
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: 0.00µm, Y: 0.00µm

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 = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm 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 - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
Sharpness Setting = Standard
Auto Sharpness = On
Sharpness = 8.8687 (647), 9.4576 (555), 8.0281 (DAPI)
Order Combination = Weiner Filter
Regularization = None
Regularization Weight = 0.000
Iterations = 3
Filter - No Filter
Fitting = Fast Fit

Histogram = Scale to Min/Max

3D Image Processing - Panels D/E

3D Volume Projection = Precise

Appearance = Threshold 2% (647), Threshold 2% (488), Threshold 2% (405), Ramp 98% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, 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)

Clipping Image Process - Panels D/E

The Clipping plane is introduced to demonstrate signal specificity and localization.

X-Y = Active was selected, and invisible was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment and both the DAPI and PARP1 signals. A purple outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.

Summary -

Panel A - 3-dimensional maximum projection of the image acquisition

Panel B - Shows optimal focal plane within the z-stack for DAPI (35/126)

Panel C - Shows the optimal focal plane within the z-stack for PARP1 (56/126)

Panel D - Shows PARP1 structural signal in a 3-dimensional volume surface view from the top, showing where the cut plane is placed.

Panel E - Shows the Z-axis view of the cut plane and the localization of both DAPI and PARP1 (albeit z-aberration is evident)

Evidentiary Conclusion -

21 Z-Planes exist between optimal focal plane for DAPI vs. PARP1, using a 30nm step size = 630nm between PARP1 and nucleolar center – suggest that super resolution may with some degree of confidence that the PARP1 is not actually with nuclear space as all diffraction limited images show, but somewhere either partially in or out. There are 21 Z-Planes available between the optimal focal plane for DAPI and PARP1, with a step size of 30 nm, resulting in a distance of 630 nm from PARP1 to the nucleolar center. This suggests that, with a certain degree of confidence, PARP1 may not be located within the nuclear space as indicated by all diffraction-limited images. Instead, it may be situated either partially inside or outside of it. While this does not constitute a definitive conclusion, further studies utilizing super-resolution techniques could provide more clarity on this finding. This certainly does not represent fact, but additional studies of localization using super resolution could “shine more light” on this finding.

Image Corrections

None

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