

**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™ 594 Affinity Purified Goat Anti-Rabbit, F(ab')<sub>2</sub> (H + L)***Cat ID# - SA 000052***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')<sub>2</sub> (H + L)***Cat ID# - SA 000019*

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<sup>+</sup>; 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® 594 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 LSM 900 Confocal, 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) - Panel A****Z-Stack = 160 Slices (4.70µm)**

Channels = 2

Scaling (Per Pixel) = 30.18nm X 30.18nm X 30.00nm

Image Size (Pixels) = 1096 X 1096

Image Size (Scaled) = 33.08 $\mu$ m X 33.08 $\mu$ m  
Bit Depth = 16 Bit  
Image Center Position = X: 78.66mm, Y: 51.92mm  
Stage Position = X: 78.66mm, Y: 51.92mm  
ROI Center Offset = X: 0.00 $\mu$ m, Y: 0.00 $\mu$ m

**Z Stack - (3D) - Region of Interest Panel C****Z-Stack = 160 Slices (4.70 $\mu$ m)**

Channels = 2  
Scaling (Per Pixel) = 30.18nm X 30.18nm X 30.00nm  
Image Size (Pixels) = 405 X 423  
Image Size (Scaled) = 12.22 $\mu$ m X 12.77 $\mu$ m  
Bit Depth = 16 Bit  
Image Center Position = X: 78.66mm, Y: 51.92mm  
Stage Position = X: 78.66mm, Y: 51.92mm  
ROI Center Offset = X: 0.00 $\mu$ m, Y: 0.00 $\mu$ m

**594 -**

Reflector = None  
Contrast Method = Fluorescence  
Pinhole = 5.00 AU / 257 $\mu$ m  
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)  
Laser Wavelength = 561nm @0.4%  
Laser Blanking = Enabled  
Scan Mode = Frame  
Scan Zoom = 3.0  
Rotation = 0°  
Sampling = 2.34  
Pixel Time = 0.69 $\mu$ s  
Frame Time = 4.07s  
LSM Scan Speed = 8  
Scan Direction = Bidirectional  
Line Step = 1  
Averaging = 4  
Excitation Wavelength = 590  
Emission Wavelength = 618  
Effective NA = 1.4  
Detection Wavelength = 580-650  
Imaging Device = Airyscan  
Detector Type = GaAsP-PMT  
Detector Gain = 850 V  
Detector Offset = 0  
Detector Digital Gain = 1.0  
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

**488 -**

Reflector = None  
Contrast Method = Fluorescence

Pinhole = 5.00AU / 217µm  
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)  
Laser Wavelength = 488nm @0.1%  
Laser Blanking = Enabled  
Scan Mode = Frame  
Scan Zoom = 3.0  
Rotation = 0°  
Sampling = 2.34  
Pixel Time = 0.69µs  
Frame Time = 4.07s  
LSM Scan Speed = 8  
Scan Direction = Bidirectional  
Line Step = 1  
Averaging = 4  
Excitation Wavelength = 493  
Emission Wavelength = 517  
Effective NA = 1.4  
Detection Wavelength = 480 - 550  
Imaging Device = Airyscan  
Detector Type = GaAsP-PMT  
Detector Gain = 800 V  
Detector Offset = 0  
Detector Digital Gain = 1.0  
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

### **Post Processing - AiryScan 3D Processing**

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

### **3D Image Processing - Panel A**

3D Maximum Projection = Precise  
Appearance = Threshold 1% (594), Threshold 1% (488), 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% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 55% of all channels  
Background = Black  
Light = Azimuth 42°, Elongation -86°, Enabled Tone Mapping  
Projection = View Angle 50°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Summary -

**Panel A** – Here, we present a 3-dimensional Maximum Projection of the complete image acquisition, comprising 160 individual Z-planes, with a scan zoom of 3.5, to examine the PARP1 nuclear signal.

**Panel B** – Here, we provide a 2-dimensional image using z-plane 79 of the 160 planes collected. Additionally, the Region of Interest (ROI) we have chosen to examine is shown, further providing structural detail in Airyscan below the diffraction limit.

**Panel C** – Here, we present a 3-dimensional Surface Projection of the ROI shown in panel B. We employ Surface Projection to provide further detail of the signal structure, which, in sub-diffraction applications such as our widefield or widefield SIM, appears to be a uniform, large single circular foci. We note that Surface Modeling is exaggerated to a point. However, there is a structural detail not visible in diffraction-limited applications that may be teased out using Airyscan and the Zen Modeling tools; again, we note that while we are making no biological claims, it is interesting that structural detail continues to be elucidated as we “step down” in resolution.

**Panel D** - Here, we provide transparency by showing the settings used in the acquisition of this image, noting the laser line used for the PARP1/594 staining (561 nm, @0.1%). We also provide all other settings, including the fact that the image was only averaged four times.

## Image Corrections

None

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Image by J.K. Bennett

Analysis and Presentation by B.T. Bennett

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