

Identifyn Standards for Optical Microscopy Methods™Identifyn Primary Antibody**Rabbit anti-Human 53BP1 Polyclonal, IgG***Cat ID# - PA-RP 000008*Identifyn® Secondary Antibodies**Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

53BP1 (P53-binding protein 1) is a crucial player in the DNA damage response (DDR), particularly in the repair of double-strand breaks (DSBs). It has a range of functions that contribute to genomic stability and an appropriate response to DNA damage. The following are the prominent roles and functions of 53BP1 in DNA repair:

DNA Damage Sensing: 53BP1 is among the early proteins recruited to DNA damage sites. It recognizes and binds to the phosphorylated form of H2AX (γ-H2AX), a histone variant phosphorylated at double-strand break sites. This ability to localize to DNA damage sites helps to recruit other proteins involved in DNA repair and signaling.

Non-Homologous End Joining (NHEJ): 53BP1 significantly promotes NHEJ, a pathway to repair double-strand breaks, especially in the G1 phase of the cell cycle when a homologous template is unavailable. 53BP1 promotes this pathway by:

Facilitating Synapsis: 53BP1 helps align the broken DNA ends, preparing them for ligation by the NHEJ machinery.

Restricting End Resection: By inhibiting the end resection process (a step in homologous recombination), 53BP1 ensures that NHEJ remains the primary repair mechanism for DSBs in cells not in the S or G2 phase.

Interaction with Other DNA Repair Proteins: 53BP1 interacts with other proteins to promote NHEJ, such as RIF1 (Rap1 Interacting Factor 1) and the Shieldin complex. These interactions help determine the repair pathway choice, favoring NHEJ over homologous recombination when appropriate.

DNA Damage Signaling: 53BP1 is involved in amplifying DNA damage signaling, contributing to the recruitment of additional repair proteins and activating cell cycle checkpoints. This signaling function helps maintain genomic stability by ensuring that cells do not progress through the cell cycle with unrepaired DNA damage.

Tumor Suppression and Genome Stability: 53BP1 has tumor suppressive properties, given its role in promoting accurate repair and maintaining genomic stability. Its ability to ensure proper repair of DSBs and its interaction with other DDR proteins make it an essential player in preventing mutations and chromosomal aberrations that can lead to cancer.

In summary, 53BP1 is a multifaceted protein in the DNA damage response, promoting non-homologous end joining, facilitating DNA damage signaling, and contributing to genomic stability. Its interactions with other DNA repair proteins and its role in regulating the choice of repair pathways are crucial for maintaining cellular integrity and preventing tumorigenesis.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with [Thermo Fisher Fish Serum Blocker \(cat#37527X3\)](#). The primary antibody, Rabbit anti-Human 53BP1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature 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, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 47 of 180 total - Panel A

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1349 X x 1626
Image Size (Scaled) = 28.48 µm X 34.33 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.20 mm, Y: 36.61 mm
Stage Position = X: 61.20 mm, Y: 36.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 165 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 265 X x 238
Image Size (Scaled) = 5.59 µm X 5.02 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.20 mm, Y: 36.61 mm
Stage Position = X: 61.20 mm, Y: 36.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 165 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 106 X x 113
Image Size (Scaled) = 2.24 μ m X 2.39 μ m
Bit Depth = 16 Bit
Image Center Position = X: 61.20 mm, Y: 36.61 mm
Stage Position = X: 61.20 mm, Y: 36.61 mm
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 = 100 ms
Depth of Focus = 1.13 μ m
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

488 - (Not Shown)

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 = 100 ms
Depth of Focus = 0.81 μ m
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 μ m grating

DAPI - (Not Shown)

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 = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @2.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 2
 Algorithm = SIM2
 Input SNR = Low
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Normalization = Auto

2-Dimensional View - Panels A

Panel features Rabbit anti-Human 53BP1 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L). ***Not shown, but collected,*** are Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L), and DAPI.

Panels B and C: the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast, and tubulin (488) is not shown.

Image was taken from Z-stack Acquisition at plane 47 of 180.

3D Image Processing - Panel B

3D Maximum Projection = Precise

Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 90%, Enabled Tone Mapping

Projection = View Angle 25°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Volume Projection = Precise

Appearance = Threshold 0% all channels, Ramp 98% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 125%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - STORM Image from the same data set, [DATA](#)

Summary -

Three-dimensional maximum- and volume-projection views of 53BP1 (Panels C and D, respectively; subset ROI) acquired using lattice structured illumination microscopy squared (SIM²) under the Identifyn Standards for Optical Microscopy Methods™. HeLa cells were labeled with a Rabbit anti-Human 53BP1 Polyclonal IgG (Identifyn; PA-RP 000008) and detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies (SA 000057). At SIM² lateral resolution (~40–60 nm in the X–Y plane), intranuclear 53BP1 signal patterns display markedly increased structural definition relative to Airyscan (~120 nm) and Lattice SIM (~60–80 nm) imaging and exhibit reproducible organizational features consistent with those observed in single-molecule STORM datasets from the same sample.

Although SIM² does not resolve all substructures detectable by STORM, the improved spatial resolution substantially reduces signal convolution, enabling more reliable visualization of damage-associated 53BP1 organization throughout the nuclear volume. Apparent aggregates observed at lower resolution increasingly separate into defined intranuclear assemblies at SIM², suggesting that SIM- and Airyscan-resolved structures represent resolution-limited composites of finer subunits. In this context, SIM² provides an intermediate visualization regime that closely approximates STORM-level organization while remaining constrained by ensemble resolution limits.

Biologically, the accurate spatial mapping of 53BP1 at this resolution is directly relevant to its established role in DNA double-strand break signaling and non-homologous end joining (NHEJ), where recruitment to γ-H2AX-marked chromatin domains and interactions with downstream factors such as RIF1 and the Shieldin complex influence repair pathway choice. While definitive chromatin-level structural conclusions require single-molecule localization microscopy, SIM² constitutes a critical and informative step within the Identifyn step-down microscopy workflow, enabling biologically meaningful assessment of DNA damage-associated protein organization prior to STORM analysis.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B, C, and D.

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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