Identifyn Standards for Optical Microscopy Methods™
Identifyn® Primary Antibody
Rabbit anti-Human NBS1 (P95) Polyclonal, IgG
Cat ID# - PA-RP 000010

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody
Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

NBS1 (Nijmegen breakage syndrome 1) plays a crucial part in the cellular response to DNA double-strand breaks in both the Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) pathways and is a component of the MRE11/RAD50/NBS1 complex (MRN). NBS1 acts as a sensor of DNA double-strand breaks and, via its interaction with γH2AX, recruits MRE11 and RAD50 to the site of damage, processes the DNA ends, prepares them for repair, and facilitates the recruitment of other repair factors. NBS1 also plays a role in coordinating the cellular response to DNA damage with the cell cycle. It interacts with various cell cycle checkpoint proteins, such as ATM (ataxia-telangiectasia mutated), to halt the cell cycle progression, allowing time for DNA repair. NBS1 also contributes to telomere maintenance and participates in detecting and repairing telomere damage, helping prevent genomic instability associated with telomere dysfunction. Dysfunction or mutations in NBS1 can lead to genomic instability and predispose individuals to cancer and other genetic disorders.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human NBS1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:

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Z Stack - (3D) - *2D at Z-Plane 59 of 115, Panel A*
Z-Stack = 115 Slices (3.42 µm)

Channels = 2
Scaling (Per Pixel) = 0.042 nm X 0.042 nm X 0.030 nm
Image size (Pixels) = 746 X 782
Image Size (Scaled) = 31.50 µm X 33.02 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.15 mm, Y: 36.14 mm
Stage Position = X: 63.15 mm, Y: 36.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - *3D Maximum Projection, Panel B*
Z-Stack = 115 Slices (3.42 µm)

Channels = 2
Scaling (Per Pixel) = 0.042 nm X 0.042 nm X 0.030 nm
Image size (Pixels) = 147 X 138
Image Size (Scaled) = 6.21 µm X 5.83 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.15 mm, Y: 36.14 mm
Stage Position = X: 63.15 mm, Y: 36.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 3.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

555 - *Not Shown in Data Analysis*

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

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Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T2-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 2.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm 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 - *Panel A*

Shows Rabbit anti-Human NBS1 (P95) Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody, pseudocolored white. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Maximum Projection in Panel B.

3D Image Processing - *Panel B*

3D Maximum Projection = Precise
Appearance = Threshold 2%, Ramp 0%, Maximum 100%
Background = Black
Light = Brightness 100%, Enabled Directional Lighting, Enabled Tone Mapping.
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - *Panel C*

Shown from the same dataset to illustrate the resolution characteristics of Lattice SIM². While SIM² provides improved lateral resolution relative to both Airyscan and Lattice SIM —enhancing localization and partially refining structural features—the observed signal

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remains resolution-limited. The overall pattern is consistent with that seen in Airyscan and SIM datasets, but with modestly improved feature separation.

Summary

Panel A shows a single optical section (Z-plane 59/115) depicting NBS1 distribution following etoposide-induced DNA damage. NBS1 (rabbit polyclonal; ~95 kDa) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody and pseudocolored white for contrast.

Panel B presents a three-dimensional maximum-intensity projection of the same region. A region of interest (ROI) highlights areas of concentrated NBS1 signal that superficially resemble structures resolved by STORM (Panel C); however, these features are conflated and lack a discrete substructure. This apparent similarity reflects the resolution limits of Lattice SIM2 rather than true nanoscale detail.

Biologically, NBS1 (~95 kDa) is a core component of the MRN complex (MRE11–RAD50–NBS1), recruited to sites of DNA double-strand breaks where it mediates damage recognition, complex stabilization, and activation of downstream signaling pathways, including ATM. The observed focal enrichment is therefore consistent with the accumulation of multi-protein DNA repair assemblies within damage-associated nuclear regions.

Despite improved resolution over confocal, Lattice SIM remains constrained by its effective point spread function (~100 nm lateral resolution). When considered in two dimensions (resolution^2), closely spaced molecular assemblies fall below this limit and are rendered as a merged signal. As a result, NBS1 appears as compact foci without resolvable subunit detail. In contrast, Panel C (STORM) provides true sub-diffraction resolution, resolving these apparent foci into more complex nanoscale architectures. These observations demonstrate that the structures observed in Panels A and B reflect spatial organization rather than definitive protein structural detail.

Image Correction

The NBS1 channel (647) was pseudocolored white to enhance visual contrast between signals.

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

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