BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

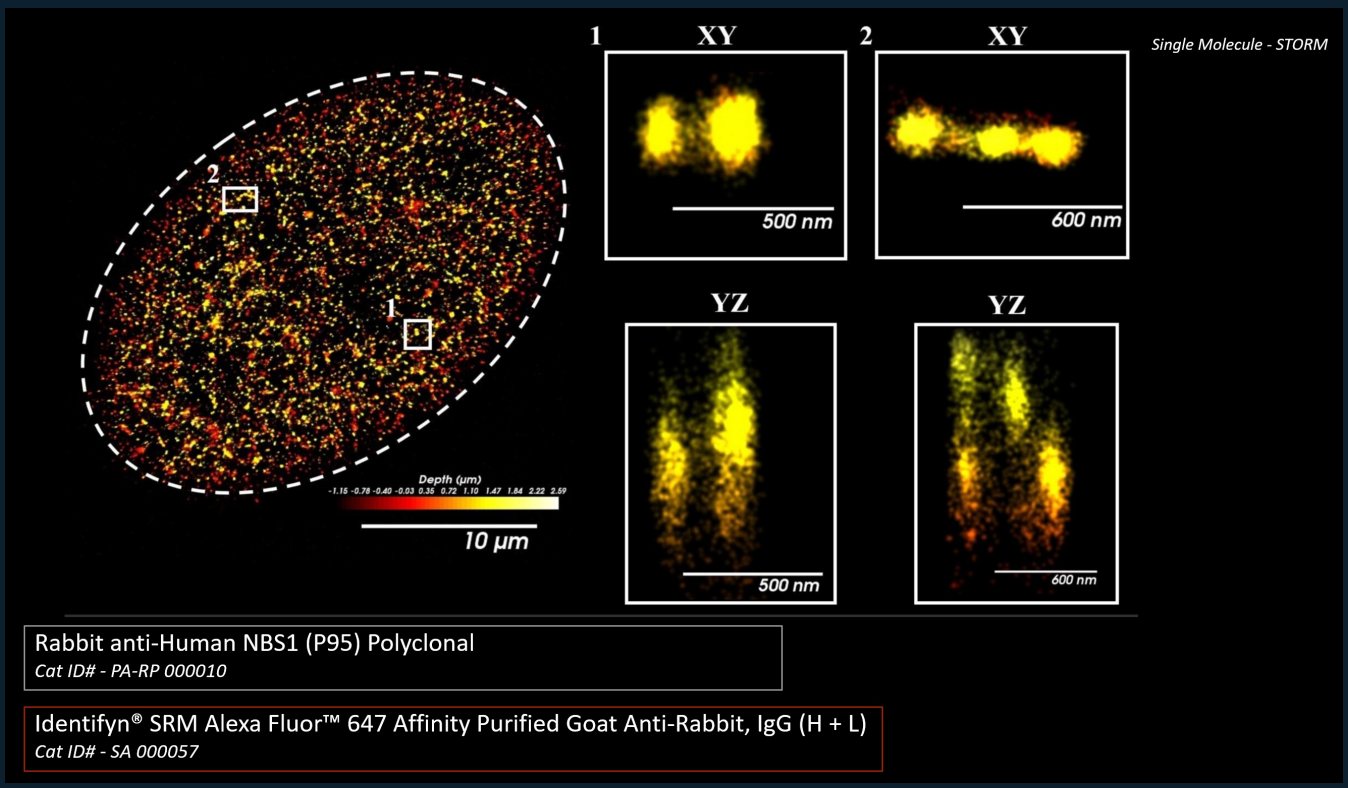
NBS1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human NBS1 (P95) Polyclonal

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RP-000010 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for NBS1, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Single-molecule STORM -> Control

BENNETT ASSESSMENT

The preserved PA-RP-000010 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RP-000010 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 647	3; 50 Cy 5; AF532-49014; 49 DAPI; 625 - 655; 515 - 545; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 555; 647	2; 50 Cy 5; AF532-49014; 625 - 655; 515 - 545; 665 - 715; 555 - 595; 653
Confocal	405/DAPI; 488; 555; 647	2; None; 639 nm @ 0.2%; 561 nm @ 0.2%; 653; 553; 668; 568
Airyscan	405/DAPI; 488; 555; 647; 750	2; None; 639 nm @ 0.3%; 561 nm @ 1.10%; 405 nm @ 0.2%; 653; 553; 353
SIM	405/DAPI; 488; 555; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820 #2-SR; 640; 561
SIM²	405/DAPI; 488; 555; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820 #2-SR; 640; 561
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	1; None; 639 nm @ 0.2%; 653; 668

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.. Structured source metadata values: 11.

VISIBLE OBSERVATION

A preserved presentation image is available for Western blot. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Western blot evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Not stated in extracted source metadata. Objective: not explicitly stated. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 25.00%; X-Cite Xylis Lamp Intensity @ 15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as not stated, and records detected dye/channel descriptors as 405/DAPI; 555; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63x/1.25 Oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Z-Stack: 81 Slices (8.0 µm); Channels: 2; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 975 X 889; 310 X 342; Image Size (Pixels): 975 X 889; 310 X 342; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 20.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 46.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield SIM — Apotome. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 555; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield SIM — Apotome evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 161 Slices (4.8 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 468 X 514; Image Size (Pixels): 468 X 514; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29 µs; Frame Time: 4.97 s. Illumination: Laser Wavelength: 639 nm @ 0.2%; 561 nm @ 0.2%. Optical/scan: Pinhole: 1.00 AU / 64 µm; 1.00 AU / 56 µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 7.9 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

A preserved presentation image is available for Confocal. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Confocal evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 5 OF 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 121 Slices (3.6 µm); Channels: 2; Scaling (Per Pixel): 0.035 nm X 0.035 nm X 0.030 nm; Image size (Pixels): 830 X 908; 159 X 137; Image Size (Pixels): 830 X 908; 159 X 137; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.04 µs; Frame Time: 19.77 s. Illumination: Laser Wavelength: 639 nm @ 0.3%; 561 nm @ 1.10%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 272 µm; Scan Zoom: 1.9; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution 10.0 (3D, Auto); SuperResolution 9.9 (3D, Auto). Structured source metadata values: 65.

VISIBLE OBSERVATION

A preserved presentation image is available for Airyscan. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 647; 750.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Airyscan evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens. Platform: 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:. Acquisition: 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): 721 X 827; 199 X 175; Image Size (Pixels): 721 X 827; 199 X 175; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @ 3.0%; 561 nm @ 2.0%; Illumination Wavelength: 640; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens. Platform: 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:. Acquisition: 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; 147 X 138; Image Size (Pixels): 746 X 782; 147 X 138; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @ 3.0%; 561 nm @ 2.0%; Illumination Wavelength: 640; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 54.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60x/1.30 Silicon Oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 79.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Single-molecule STORM evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 161 Slices (4.8 µm); Channels: 1; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; 0.059 µm X 0.059 µm; Image size (Pixels): 468 X 514; 1503 X 1503; Image Size (Pixels): 468 X 514; 1503 X 1503; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29 µs; 0.26 µs; Frame Time: 4.97 s; 5.63 s. Illumination: Laser Wavelength: 639 nm @ 0.2%. Optical/scan: Pinhole: 1.00 AU / 64 µm; Scan Zoom: 1.7; 1.5; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 8.3 (3D, Auto). Structured source metadata values: 53.

VISIBLE OBSERVATION

A preserved presentation image is available for Control. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Control evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved PA-RP-000010 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 0.035 nm X 0.035 nm X 0.030 nm -> 0.03529 µm X 0.03529 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=830 X 908; Image Size (Scaled)=29.29 µm X 32.04 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 0.042 nm X 0.042 nm X 0.030 nm -> 0.04222 µm X 0.04222 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=721 X 827; Image Size (Scaled)=30.44 µm X 34.92 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 0.042 nm X 0.042 nm X 0.030 nm -> 0.04223 µm X 0.04223 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=746 X 782; Image Size (Scaled)=31.50 µm X 33.02 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["24 hours"], "Control": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

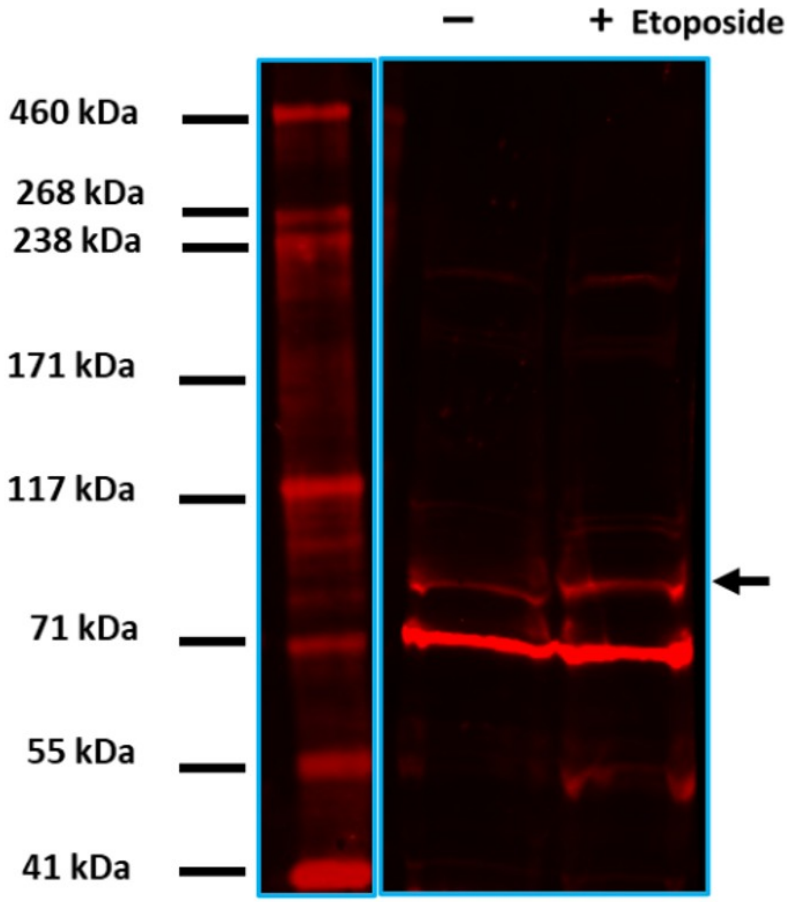
This dossier preserves the complete available evidence progression for PA-RP-000010. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	Not stated	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	S. Khanal

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human NBS1 (P95) Polyclonal, IgG
Cat ID# - PA-RP 000010

Expected MW: 85 kDa

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis on NuPAGE™ Tris-Acetate Mini Protein Gels (3-8%, 1.0 mm) in a Mini Gel Tank. Thermo Fisher™ HiMark™ Pre-stained Protein Standard was used as a molecular weight marker.

Post-electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with 1X PBS. The blocked membrane was incubated with Rabbit anti-Human NBS1 (P95) Polyclonal, IgG for 20 hours, followed by five washes in 1X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour and washed 5 times with 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

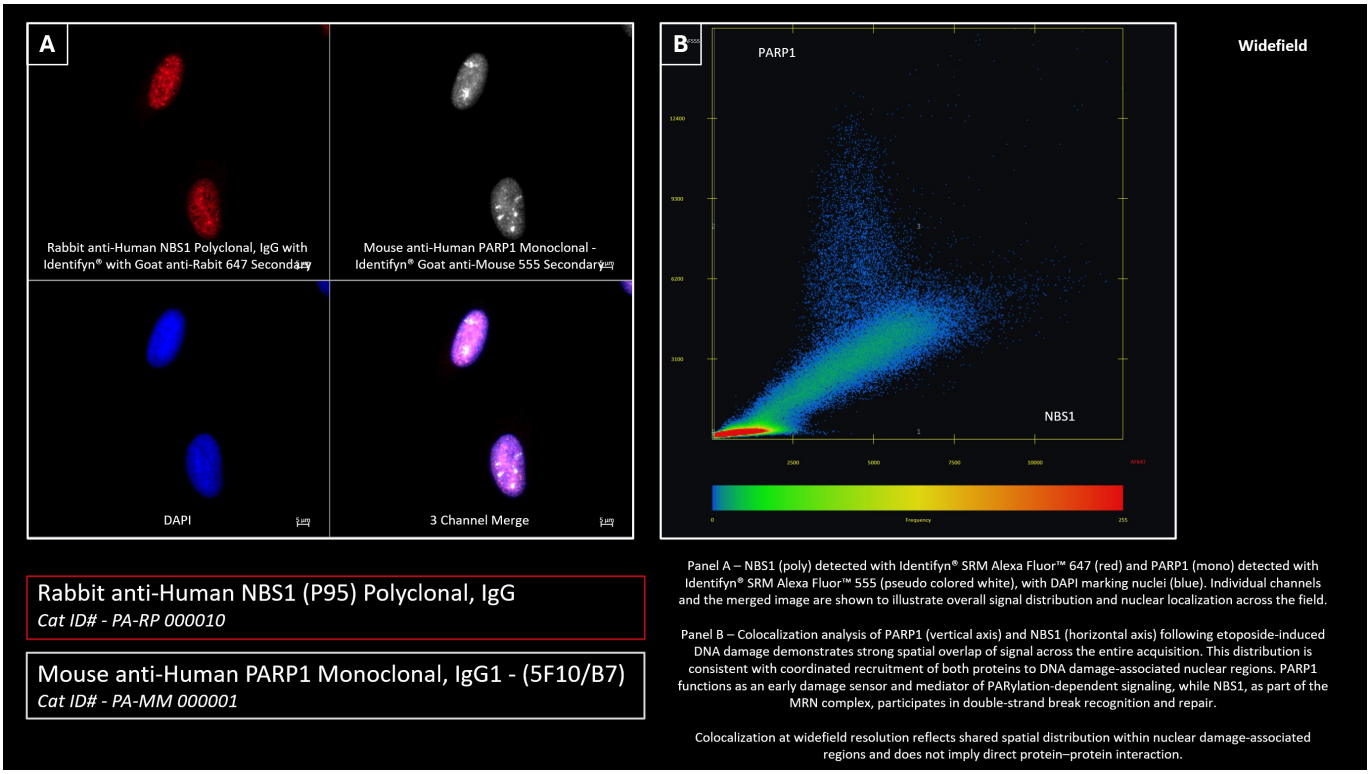
NONE

Image: S. Khanal

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D31E03D008E49AFA90FE8FF8C4E4DF8CE768CBDBA5282CAE42721D670BF6CE5F
Method PDF SHA-256	37D5C375DDD2F756002C87C725FE785E057B7EB102C3232F24FDF03098ECFA2D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	Not stated
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 647
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Not stated
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	50 Cy 5 | AF532-49014 | 49 DAPI
Beam Splitter	660 | 550 | 395
Filter Excitation Wavelength	625 - 655 | 515 - 545 | 335 - 383
Filter Emission Wavelength	665 - 715 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 25.00% | X-Cite Xylis Lamp Intensity @ 15.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	250 ms | 150 ms | 20 ms
Excitation Wavelength	653 | 553 | 353
Emission Wavelength	668 | 568 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1x
Depth of Focus	1.03 µm | 0.88 µm | 0.72 µm
Binning Mode	2,2
Image Measurement	Entire Field

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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).

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18 µm X 112.59 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 1.03 µm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.88 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human NBS1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panels B and C

Horizontal Axis - AF647, Threshold 89, Range Auto
Vertical Axis - AF555, Threshold 0.00, Range Auto

Image Measurement = Entire Field

Summary

Panel A - NBS1 (poly) detected with Identifyn® SRM Alexa Fluor™ 647 (red) and PARP1 (mono) detected with Identifyn® SRM Alexa Fluor™ 555 (pseudocolored white), with DAPI marking nuclei (blue). Individual channels and the merged image are shown to illustrate overall signal distribution and nuclear localization across the field.

Panel B - Colocalization analysis of PARP1 (vertical axis) and NBS1 (horizontal axis) following etoposide-induced DNA damage demonstrates strong spatial overlap of signal across the entire acquisition. This distribution is consistent with the coordinated recruitment of both proteins to DNA-damage-associated nuclear regions. PARP1 functions as an early damage sensor and mediator of PARylation-dependent signaling, while NBS1, as part of the MRN complex, participates in double-strand break recognition and repair.

Colocalization at widefield resolution reflects shared spatial distribution within nuclear damage-associated regions and does not imply direct protein-protein interaction.

Image Correction

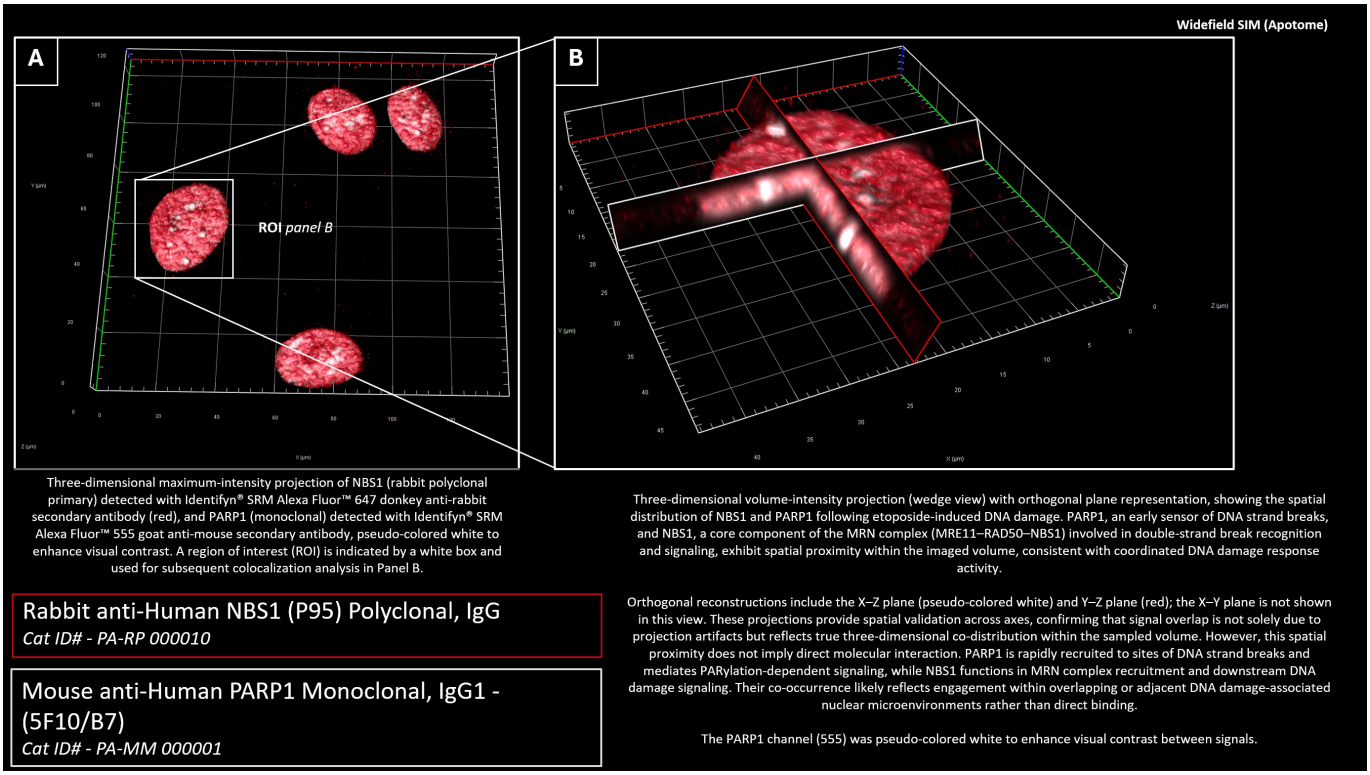
Identifyn® SRM Alexa Fluor™ 555 was pseudocolored white instead of the default orange for visual contrast.

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Widefield
Instrument	Not stated in extracted metadata
Microscope configuration	Not stated in extracted metadata
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5D8196E784215B8ADB485D3A6A0920A721A3229A78F06D800D5A645A0FE630ED
Method PDF SHA-256	66287F13C0EB6643410010B9402997FB08856D9D33DA3DB78745EAAFD44006A8

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Three-dimensional maximum-intensity projection of NBS1 (rabbit polyclonal primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudo-colored white to enhance visual contrast. A region of interest (ROI) is indicated by a white box and used for subsequent colocalization analysis in Panel B.

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation, showing the spatial distribution of NBS1 and PARP1 following etoposide-induced DNA damage. PARP1, an early sensor of DNA strand breaks, and NBS1, a core component of the MRN complex (MRE11–RAD50–NBS1) involved in double-strand break recognition and signaling, exhibit spatial proximity within the imaged volume, consistent with coordinated DNA damage response activity.

Orthogonal reconstructions include the X–Z plane (pseudo-colored white) and Y–Z plane (red); the X–Y plane is not shown in this view. These projections provide spatial validation across axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this spatial proximity does not imply direct molecular interaction. PARP1 is rapidly recruited to sites of DNA strand breaks and mediates PARylation-dependent signaling, while NBS1 functions in MRN complex recruitment and downstream DNA damage signaling. Their co-occurrence likely reflects engagement within overlapping or adjacent DNA damage-associated nuclear microenvironments rather than direct binding.

The PARP1 channel (555) was pseudo-colored white to enhance visual contrast between signals.

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

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 647
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63x/1.25 Oil M27
Z-Stack	81 Slices (8.0 µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	975 X 889 | 310 X 342
Image Size (Scaled)	139.29 µm X 127.00 µm | 44.29 µm X 48.86 µm
Bit Depth	14 Bit
Image Center Position	X: 45.29 mm, Y: 49.81 mm
Stage Position	X: 45.29 mm, Y: 49.81 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | AF532-49014
Beam Splitter	660 | 550
Filter Excitation Wavelength	625 - 655 | 515 - 545
Filter Emission Wavelength	665 - 715 | 555 - 595
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time	200 ms
Excitation Wavelength	653 | 553
Emission Wavelength	668 | 568
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1x
Depth of Focus	1.30 µm | 1.00 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% all channels, Ramp 50% 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)
X-Y	Not Used in Data Analysis

FIELD	SOURCE-DOCUMENTED VALUE
X-Z	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-14% | 0%
Clip Y	30° | -30°
Clip Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 975 X 889
Image Size (Scaled) = 139.29 µm X 127.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.29 mm, Y: 49.81 mm
Stage Position = X: 45.29 mm, Y: 49.81 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI

Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 310 X 342
Image Size (Scaled) = 44.29 µm X 48.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.29 mm, Y: 49.81 mm
Stage Position = X: 45.29 mm, Y: 49.81 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.30 µm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1x
Depth of Focus = 1.00 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing - Panel A

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 50% 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)

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 50% 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 - Panel B

Clipping planes may be introduced in Panel B to highlight the NBS1 Polyclonal and the PARP1 Mouse Monoclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, or, in this case, removing parts of the image in the X-Z and Y-Z planes.

X-Y = Not Used in Data Analysis

X-Z = Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -14%
Clip Y = 30°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%
Clip Y = -30°
Clip Z = 0°

Summary

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation, showing the spatial distribution of NBS1 and PARP1 following etoposide-induced DNA damage. PARP1, an early sensor of DNA strand breaks, and NBS1, a core component of the MRN complex (MRE11-RAD50-NBS1) involved in double-strand break recognition and signaling, exhibit spatial proximity within the imaged volume, consistent with coordinated DNA damage response activity.

Orthogonal reconstructions include the X-Z plane (pseudocolored white) and Y-Z plane (red); the X-Y plane is not shown in this view. These projections provide spatial validation across axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this spatial proximity does not imply direct molecular interaction. PARP1 is rapidly recruited to sites of DNA strand breaks and mediates PARylation-dependent signaling, while NBS1 functions in MRN complex recruitment and downstream DNA damage signaling. Their co-occurrence likely reflects engagement within overlapping or adjacent nuclear microenvironments associated with DNA damage rather than direct binding.

Image Correction

The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

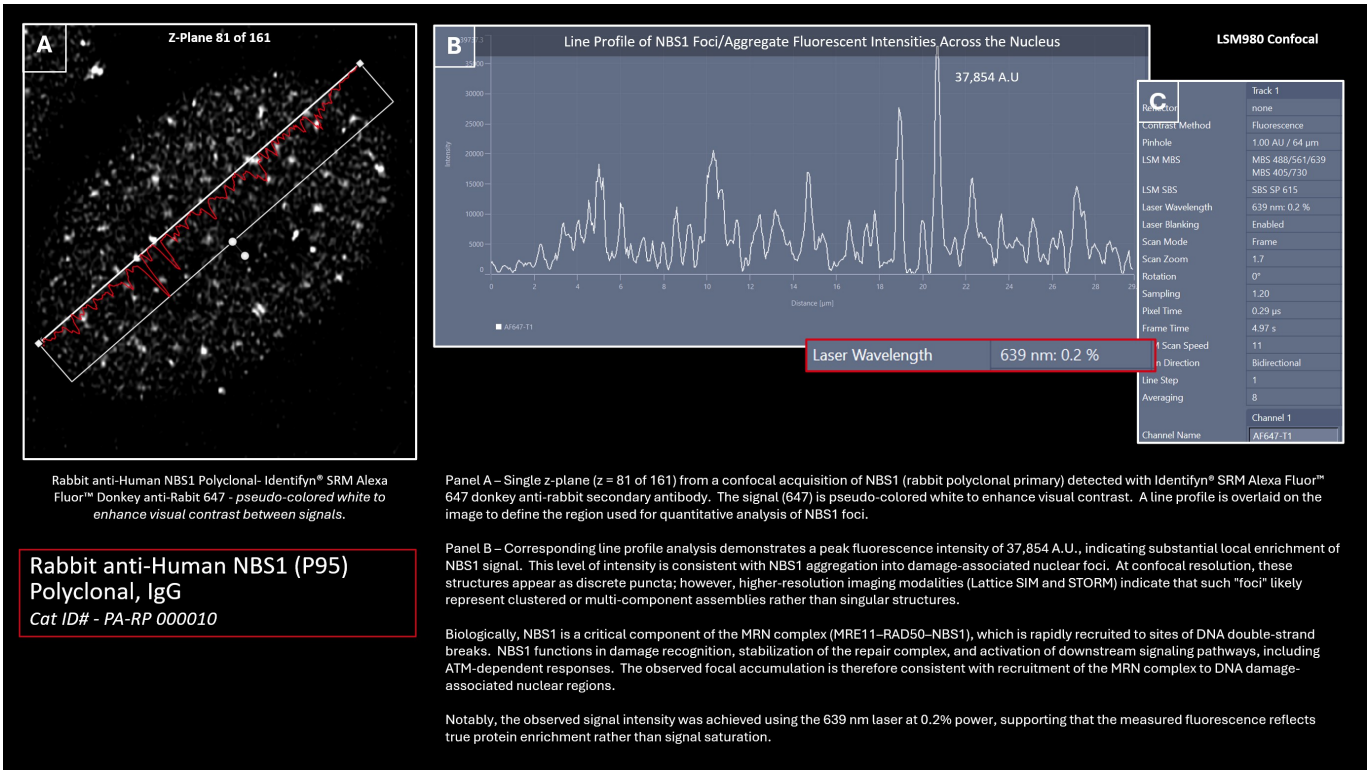
SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6D73E5AD1B8FEE1BA461C64818F5BBA478FEB7A1744572DD249B976A013E80AA

SOURCE PROVENANCE

Method PDF SHA-256	B10DCCA3D9B376673F18F2F7B0AC181D88FDD64C20521CA69F4848137840760C

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human NBS1 Polyclonal- Identifyn® SRM Alexa Fluor™ Donkey anti-Rabit 647 - *pseudo-colored white to enhance visual contrast between signals.*

Rabbit anti-Human NBS1 (P95) Polyclonal, IgG
Cat ID# - PA-RP 000010

Panel A – Single z-plane (z = 81 of 161) from a confocal acquisition of NBS1 (rabbit polyclonal primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody. The signal (647) is pseudo-colored white to enhance visual contrast. A line profile is overlaid on the image to define the region used for quantitative analysis of NBS1 foci.

Panel B – Corresponding line profile analysis demonstrates a peak fluorescence intensity of 37,854 A.U., indicating substantial local enrichment of NBS1 signal. This level of intensity is consistent with NBS1 aggregation into damage-associated nuclear foci. At confocal resolution, these structures appear as discrete puncta; however, higher-resolution imaging modalities (Lattice SIM and STORM) indicate that such "foci" likely represent clustered or multi-component assemblies rather than singular structures.

Biologically, NBS1 is a critical component of the MRN complex (MRE11–RAD50–NBS1), which is rapidly recruited to sites of DNA double-strand breaks. NBS1 functions in damage recognition, stabilization of the repair complex, and activation of downstream signaling pathways, including ATM-dependent responses. The observed focal accumulation is therefore consistent with recruitment of the MRN complex to DNA damage-associated nuclear regions.

Notably, the observed signal intensity was achieved using the 639 nm laser at 0.2% power, supporting that the measured fluorescence reflects true protein enrichment rather than signal saturation.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / CONFOCAL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	161 Slices (4.8 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	468 X 514
Image Size (Scaled)	27.53 µm X 30.23 µm
Bit Depth	16 Bit
Image Center Position	X: 74.44 mm, Y: 45.97 mm
Stage Position	X: 74.44 mm, Y: 45.97 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 64 µm | 1.00 AU / 56 µm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS SP 615 | Mirror
Laser Wavelength	639 nm @ 0.2% | 561 nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.20
Pixel Time	0.29 µs
Frame Time	4.97 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 553
Emission Wavelength	668 | 568
Effective NA	1.4
Detection Wavelength	645 - 700 | 560 - 620
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.5 (3D, Auto) | Filter: 7.9 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 29.8), Y (Min 0.0 and Max 39373)
Panel A - Single z-plane (z	81 of 161) from a confocal acquisition of NBS1 (rabbit polyclonal primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody. The signal (647) is pseudocolored white to enhance visual contrast. A line profile is overlaid on the image to define the region used for quantitative analysis of NBS1 foci.

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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).

PARP1 Monoclonal counterstained with Identifyn® SRM Alexa Fluor™ 555 was acquired but not shown in the data analysis.

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Showing Z-Plane 81 of 161

Z-Stack = 161 Slices (4.8 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 468 X 514
Image Size (Scaled) = 27.53 µm X 30.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.44 mm, Y: 45.97 mm
Stage Position = X: 74.44 mm, Y: 45.97 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 64 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

555 - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 561 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 560 - 620
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.9 (3D, Auto)

Profile View Display - Panels A & B

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 29.8), Y (Min 0.0 and Max 39373)

Measured at Z-plane 81 of 161

Zen Acquisition Info Tab - Panel C

Shown are the acquisition parameters for the 647 channel (NBS1) collected using the 639 nm laser at 0.2% power. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Summary

Panel A - Single z-plane (z = 81 of 161) from a confocal acquisition of NBS1 (rabbit polyclonal primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody. The signal (647) is pseudocolored white to enhance visual contrast. A line profile is overlaid on the image to define the region used for quantitative analysis of NBS1 foci.

Panel B - Corresponding line profile analysis demonstrates a peak fluorescence intensity of 37,854 A.U., indicating substantial local enrichment of NBS1 signal. This level of intensity is consistent with NBS1 aggregation into

damage-associated nuclear foci. At confocal resolution, these structures appear as discrete puncta; however, higher-resolution imaging modalities (Lattice SIM and STORM) indicate that such "foci" likely represent clustered or multi-component assemblies rather than singular structures.

Biologically, NBS1 is a critical component of the MRN complex (MRE11-RAD50-NBS1), which is rapidly recruited to sites of DNA double-strand breaks. NBS1 functions in damage recognition, stabilization of the repair complex, and activation of downstream signaling pathways, including ATM-dependent responses. The observed focal accumulation is therefore consistent with the recruitment of the MRN complex to DNA-damage-associated nuclear regions.

Notably, the observed signal intensity was achieved with the 639 nm laser at 0.2% power, supporting the conclusion that the measured fluorescence reflects true protein enrichment rather than signal saturation.

Image Correction

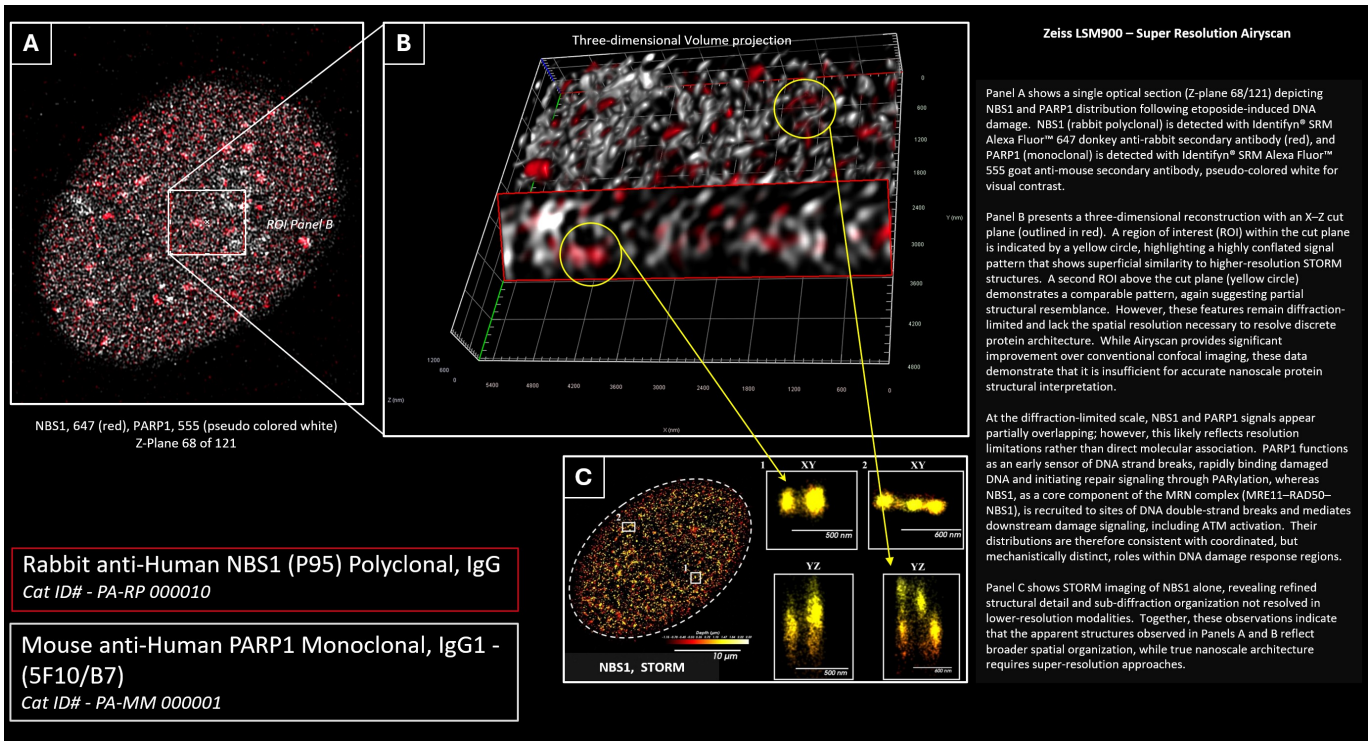
The NBS1 channel (647) was pseudocolored white to enhance visual contrast between signals.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9FE565C2561E40609BB4EF861DF3852DE91A33BBC6774567CD100B806E567B81
Method PDF SHA-256	3994FDB7493A24B1E68630AEA7493569028418D3ECC7FE12E023D6EE57FAFF12

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647; 750
METADATA VALUES	65

STRUCTURED ACQUISITION METADATA / AIRYSCAN

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	121 Slices (3.6 µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.03000 µm
Image size (Pixels)	830 X 908 | 159 X 137
Image Size (Scaled)	29.29 µm X 32.04 µm | 5.61 µm X 4.83 µm
Bit Depth	16 Bit
Image Center Position	X: 66.25 mm, Y: 51.54 mm
Stage Position	X: 66.25 mm, Y: 51.54mm | X: 66.25 mm, Y: 51.54 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 272 µm | 5.00 AU / 235 µm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 525 | SBS SP 505
Laser Wavelength	639 nm @ 0.3% | 561 nm @ 1.10% | 405 nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.9
Rotation	0°
Sampling	2.00
Pixel Time	1.04 µs
Frame Time	19.77 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 553 | 353
Emission Wavelength	668 | 568 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 530 - 582 | 420 - 480, 495 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 10.0 (3D, Auto) | SuperResolution 9.9 (3D, Auto) | SuperResolution 8.1 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, 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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Not Used in Data Analysis
X-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	24%
Clip Y	30°
Clip Z	0°
Y-Z	Not Used in Data Analysis

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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 LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 68 of 121, Panel A

Z-Stack = 121 Slices (3.6 µm)

Channels = 2
Scaling (Per Pixel) = 0.035 nm X 0.035 nm X 0.030 nm
Image size (Pixels) = 830 X 908
Image Size (Scaled) = 29.29 µm X 32.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.25 mm, Y: 51.54 mm
Stage Position = X: 66.25 mm, Y: 51.54mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume Projection, Panel B

Z-Stack = 121 Slices (3.6 µm)

Channels = 2
Scaling (Per Pixel) = 0.035 nm X 0.035 nm X 0.030 nm
Image size (Pixels) = 159 X 137
Image Size (Scaled) = 5.61 µm X 4.83 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.25 mm, Y: 51.54 mm
Stage Position = X: 66.25 mm, Y: 51.54 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 272 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 525
Laser Wavelength = 561 nm @ 1.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 530 - 582
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.9 (3D, Auto)

DAPI - Not Shown in Data Analysis, but Collected in Image Acquisition

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 235 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420 - 480, 495 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.1 (3D, 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 and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, 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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel B

Clipping planes may be introduced to highlight the NBS1 Polyclonal and the PARP1 Mouse Monoclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, or, in this case, removing parts of the image in the X-Z plane.

X-Y = Not Used in Data Analysis

X-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 24%
Clip Y = 30°
Clip Z = 0°

Y-Z = Not Used in Data Analysis

STORM - Panel C

Shown from the same product data (NBS1), set to demonstrate the resolution limitations of Airyscan, however, better than confocal, in determining localization and structural details.

Summary

Panel A shows a single optical section (Z-plane 68/121) depicting NBS1 and PARP1 distribution following etoposide-induced DNA damage. NBS1 (rabbit polyclonal) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) is detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudocolored white for visual contrast.

Panel B presents a three-dimensional reconstruction with an X-Z cut plane (outlined in red). A region of interest (ROI) within the cut plane is indicated by a yellow circle, highlighting a highly conflated signal pattern that superficially resembles higher-resolution STORM structures. A second ROI above the cut plane (yellow circle) demonstrates a comparable pattern, again suggesting partial structural resemblance. However, these features remain diffraction-limited and lack the spatial resolution necessary to resolve discrete protein architecture. While Airyscan provides significant improvements over conventional confocal imaging, these data demonstrate that it is insufficient for accurate nanoscale interpretation of protein structure.

At the diffraction-limited scale, NBS1 and PARP1 signals appear partially overlapping; however, this likely reflects resolution limitations rather than direct molecular association. PARP1 functions as an early sensor of DNA strand breaks, rapidly binding damaged DNA and initiating repair signaling through PARylation, whereas NBS1, as a core component of the MRN complex (MRE11-RAD50-NBS1), is recruited to sites of DNA double-strand breaks and mediates downstream damage signaling, including ATM activation. Their distributions are therefore consistent with coordinated, but mechanistically distinct, roles within DNA damage response regions.

Panel C shows STORM imaging of NBS1 alone, revealing refined structural detail and sub-diffraction organization not resolved in lower-resolution modalities. Together, these observations indicate that the apparent structures observed in Panels A and B reflect broader spatial organization, while true nanoscale architecture requires super-resolution approaches.

Image Correction

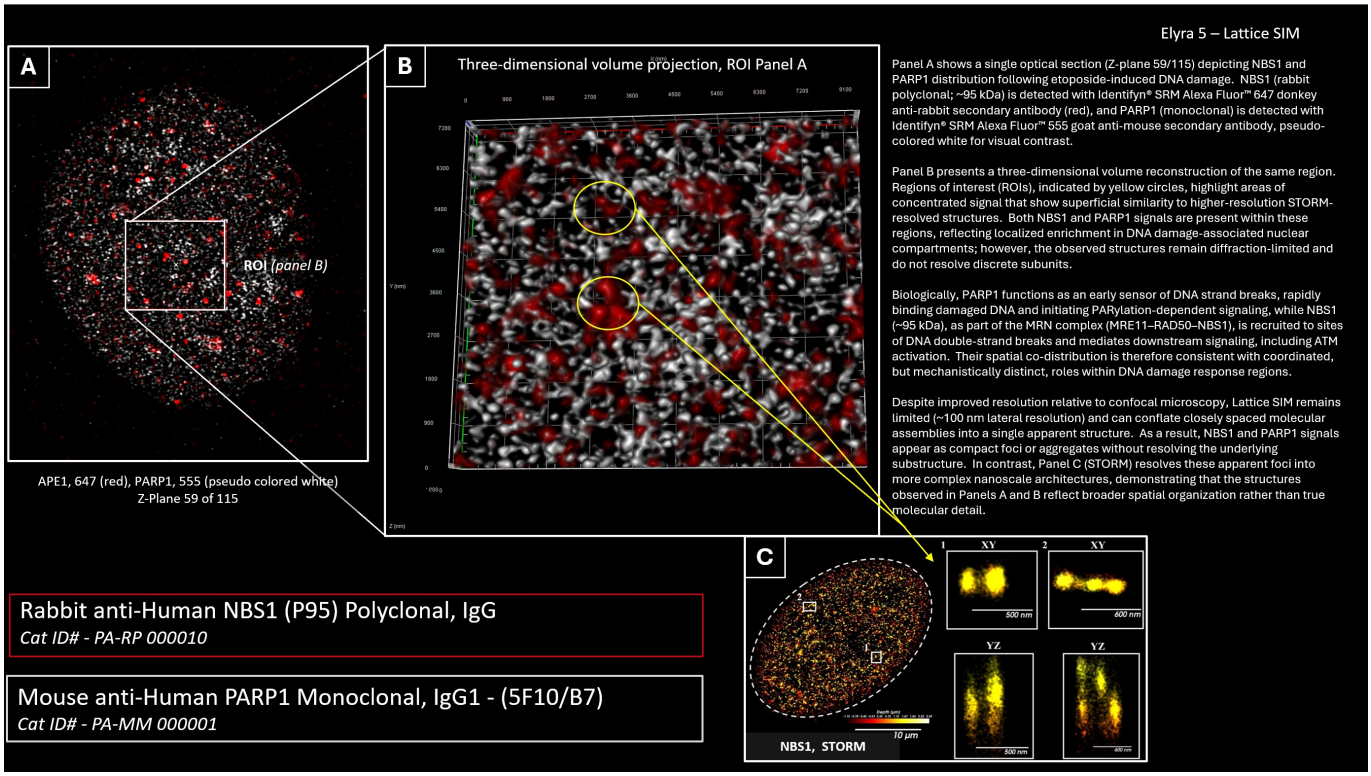
The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	13A257E5C1CF2647101D2CAF9C42AD7883EDF5CE392C21D7915A8A9F2C2D2913
Method PDF SHA-256	ACB35330AA062921DCC9D50EFD1A7367E4A0A2ABC6B661E4D1BB6F4A0213F182

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	53

STRUCTURED ACQUISITION METADATA / SIM

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	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:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens
Z-Stack	115 Slices (3.42 µm)
Channels	2
Scaling (Per Pixel)	0.04222 µm X 0.04222 µm X 0.03000 µm
Image size (Pixels)	721 X 827 | 199 X 175
Image Size (Scaled)	30.44 µm X 34.92 µm | 8.40 µm X 7.39 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
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 | 561
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820 #2-SR
Excitation Wavelength	640 | 561
Emission Wavelength	729 | 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms
Depth of Focus	1.13 µm | 0.92 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 3.0% | 561 nm @ 2.0%
Frame Time	1.59 s | 1.33 s
Scan Direction	Unidirectional
Grating	27.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.5745 (647), 10.8128 (555)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, 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 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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:

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) = 721 X 827
Image Size (Scaled) = 30.44 µm X 34.92 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 Volume 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) = 199 X 175
Image Size (Scaled) = 8.40 µm X 7.39 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 -

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 = 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 = 27.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.5745 (647), 10.8128 (555)
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View - Panel A

Shows Rabbit anti-Human NBS1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, 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 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product data set to demonstrate the resolution limitations of Lattice SIM, however, better than Airyscan, in determining localization and structural details. Moreover, the signal pattern is consistent with the Airyscan data and is more closely resolution-limited than that of a single-molecule image from STORM.

Summary

Panel A shows a single optical section (Z-plane 59/115) depicting NBS1 and PARP1 distribution following etoposide-induced DNA damage. NBS1 (rabbit polyclonal; ~95 kDa) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) is detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudocolored white for visual contrast.

Panel B presents a three-dimensional volume reconstruction of the same region. Regions of interest (ROIs), indicated by yellow circles, highlight areas of concentrated signal that show superficial similarity to higher-resolution STORM-resolved structures. Both NBS1 and PARP1 signals are present within these regions, reflecting localized enrichment in DNA damage-associated nuclear compartments; however, the observed structures remain diffraction-limited and do not resolve discrete subunits.

Biologically, PARP1 functions as an early sensor of DNA strand breaks, rapidly binding damaged DNA and initiating PARylation-dependent signaling, while NBS1 (~95 kDa), as part of the MRN complex (MRE11-RAD50-NBS1), is recruited to sites of DNA double-strand breaks and mediates downstream signaling, including ATM activation. Their spatial co-distribution is therefore consistent with coordinated yet mechanistically distinct roles within DNA damage response regions.

Despite improved resolution relative to confocal microscopy, Lattice SIM remains limited (~100 nm lateral resolution) and can conflate closely spaced molecular assemblies into a single apparent structure. As a result, NBS1 and PARP1 signals appear as compact foci or aggregates without resolving the underlying substructure. In contrast, Panel C (STORM) resolves these apparent foci into more complex nanoscale architectures, demonstrating that the structures observed in Panels A and B reflect broader spatial organization rather than true molecular detail.

Image Correction

The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

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

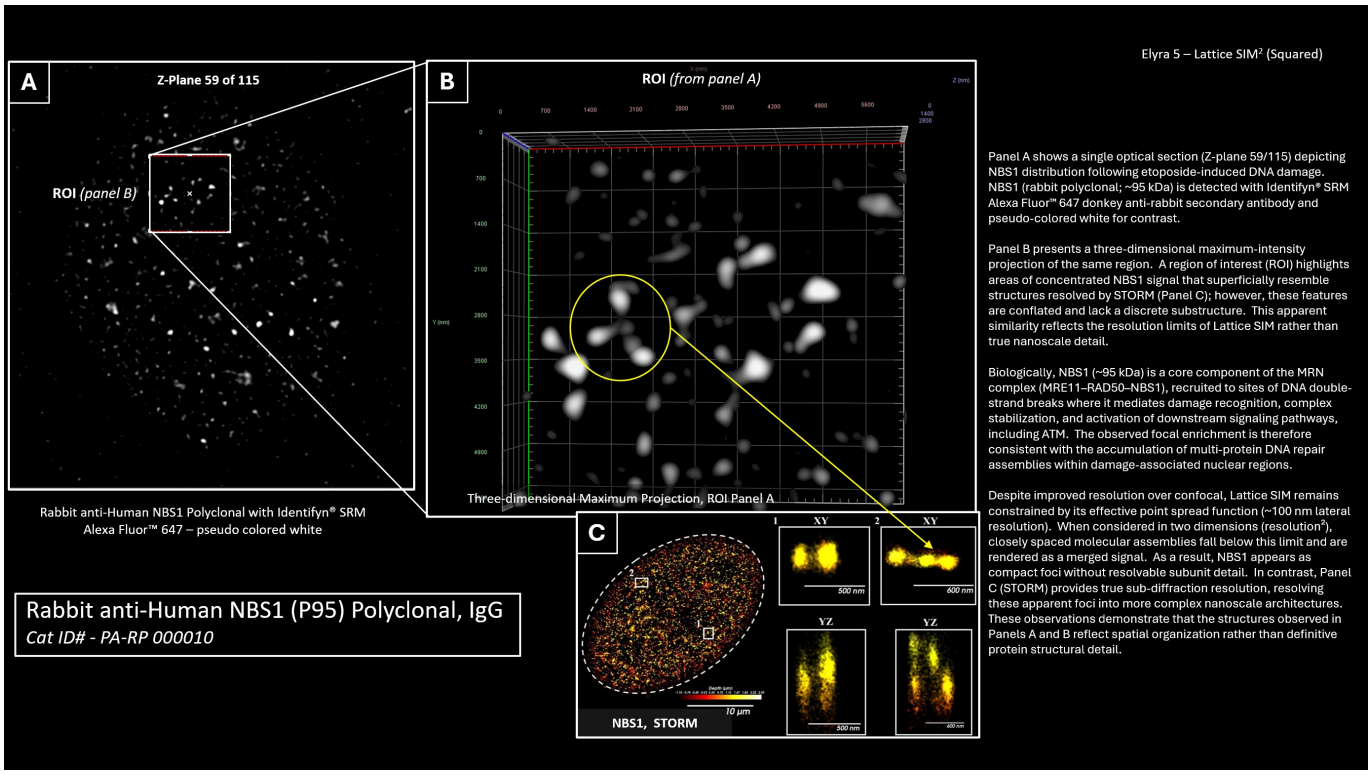
SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	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:
Structured source metadata values	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	674B3FBB3A3B5AE443D5BAEFF47CE50CC019708DD219554F7DF9D2306984EDF2
Method PDF SHA-256	BD09D4811501C2B6BDD186773F855DE6A22CB4D89BCBE0D4650CD7B2A28E8034

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	54

STRUCTURED ACQUISITION METADATA / SIM²

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	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:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens
Z-Stack	115 Slices (3.42 µm)
Channels	2
Scaling (Per Pixel)	0.04223 µm X 0.04223 µm X 0.03000 µm
Image size (Pixels)	746 X 782 | 147 X 138
Image Size (Scaled)	31.50 µm X 33.02 µm | 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
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 | 561
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820 #2-SR
Excitation Wavelength	640 | 561
Emission Wavelength	729 | 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms
Depth of Focus	1.13 µm | 0.92 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 3.0% | 561 nm @ 2.0%
Frame Time	1.59 s | 1.33 s
Scan Direction	Unidirectional
Grating	27.5 µm grating | 36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
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)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

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:

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
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 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 (resolution²), 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

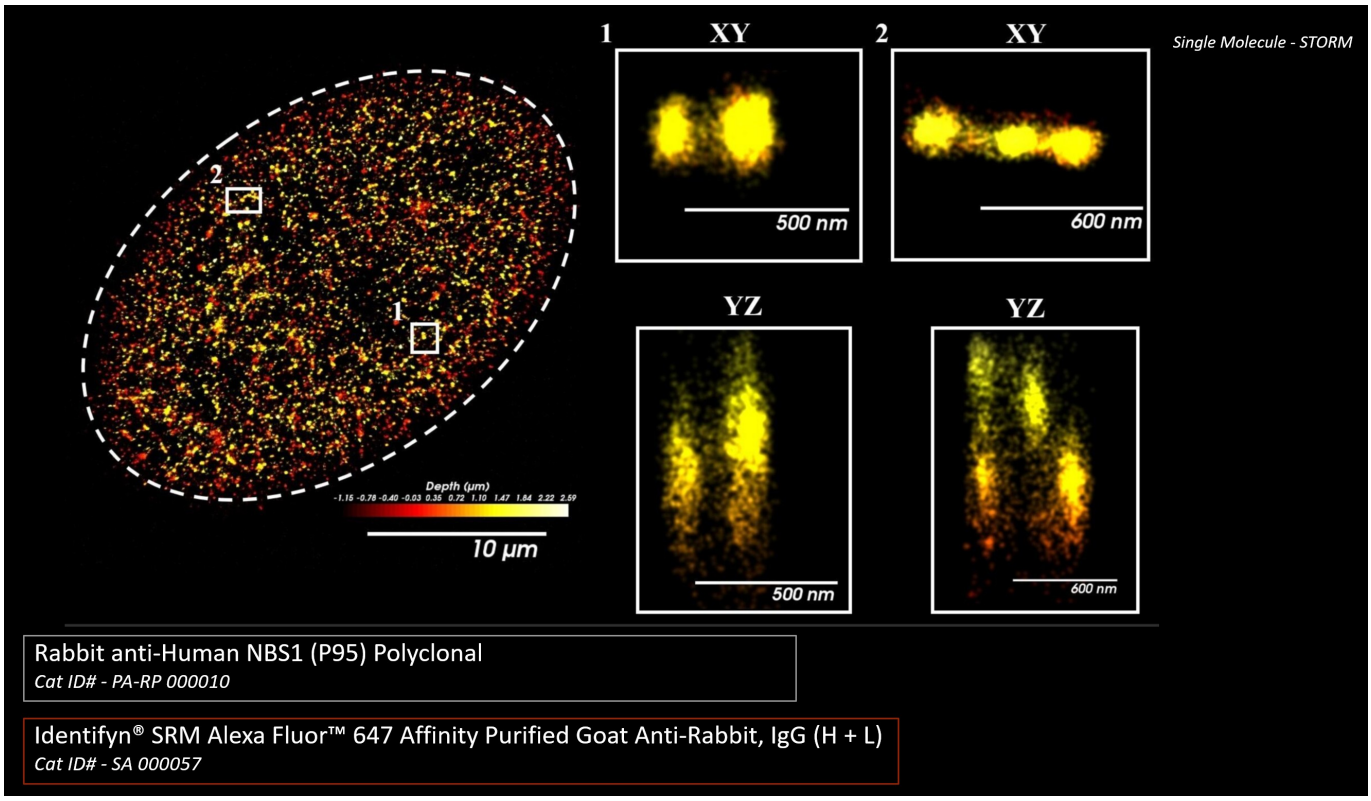
SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	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:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	192C6141326717A4FF8949D2183631BEA5BA65EF3800E3CE7EABDB7BF577C63D
Method PDF SHA-256	56F3E22F855129CA1F058116ACC3902258E6CBB5469245086194C53BEF4E54F4

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	79

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Objective	Olympus 60x/1.30 Silicon Oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
AutoFocus Drift Correction	On
Biplane Module	635 nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Coarse Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100 ms | 20 ms
Super Resolution Camera Exposure Time	20 ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20 ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200 µm
Capture Mode	Live
Piezo Current	182.2 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under AutoFocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
AutoFocus Mode	Pre-Capture
SuperRes	50 µm is selected
Piezo Record	Begin: 182.2; End: 184
SuperRes 640 nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	9
Cycles	17
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	50
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 25
Sizing Mode	Constant
Particle size	25 nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.35
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	30
Axial precision	65
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Compute Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human NBS1 (P95) Polyclonal, IgG
Cat ID# - PA-RP 000010

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

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.

Method

HeLa cells were grown in a µ-Slide 8 Well high-Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM 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 NBS1 (P95) Polyclonal, IgG was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3 times in Identifyn®'s proprietary Wash Buffer, and 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 1:200. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn®'s Calibration Standard Operating Procedure, which can be found here.

https://identifyn.com/storm-calibration

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, 'Record' is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1
Number of Simultaneous Probes: 1
Number of Reference Probes: 1
Camera: Both Cameras
Color Channel: Both Channels
Probe Capture Mode: Interleaved
Z -Stack Capture Mode: Interleaved
Multiple Location Capture: Not selected
Post Record Reference: Not Selected
Fluidics: Not Selected
AutoFocus Drift Correction: On

Microscope Configuration

Biplane Module: 635 nm Dichroic
Objective: Silicon Selected
Stage Insert: Rectangular Selected
Coarse Focus Position: 8-Well Chamber Selected
Dichroic: SuperRes
PSF: (Red, Orange)
Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%
Widefield Camera Exposure Time: 100 ms
Super Resolution Camera Exposure Time: 20 ms
Widefield Camera Binning: 1
Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20 ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200 µm
Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization - The red-bordered box identifies the area of the SuperRes view and defines a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 182.2 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100 ms
Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under AutoFocus was selected.

AutoFocus - Calibration Values

Current Position: -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
AutoFocus Mode: Pre-Capture

View Mode

SuperRes: 50 µm is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - Positioning of the target Cell or Subsection of the Target Cell is optimized and optimally focused.

Piezo Record: Begin: 182.2; End: 184

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640 nm: 0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input

Reference Probe 1 Laser control 640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20 ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 9
Cycles: 17
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 50

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to default values.

Localization

Cutout Width: 16 px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1 px
Fiducial at Max Accumulation: Not selected
Max Particles Per Frame: No Max
Background SLPE Removal: Not Selected
Cutout/Background Removal: Not Selected
Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 25

Region of Interest -

Not selected

Display -

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 25 nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.35
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 8 Intervals
Pixel Size: 50 nm
Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

Radial precision: 30
Axial precision: 65

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1 µm
Maximum Particle Count to Form Cluster: 10
Compute Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

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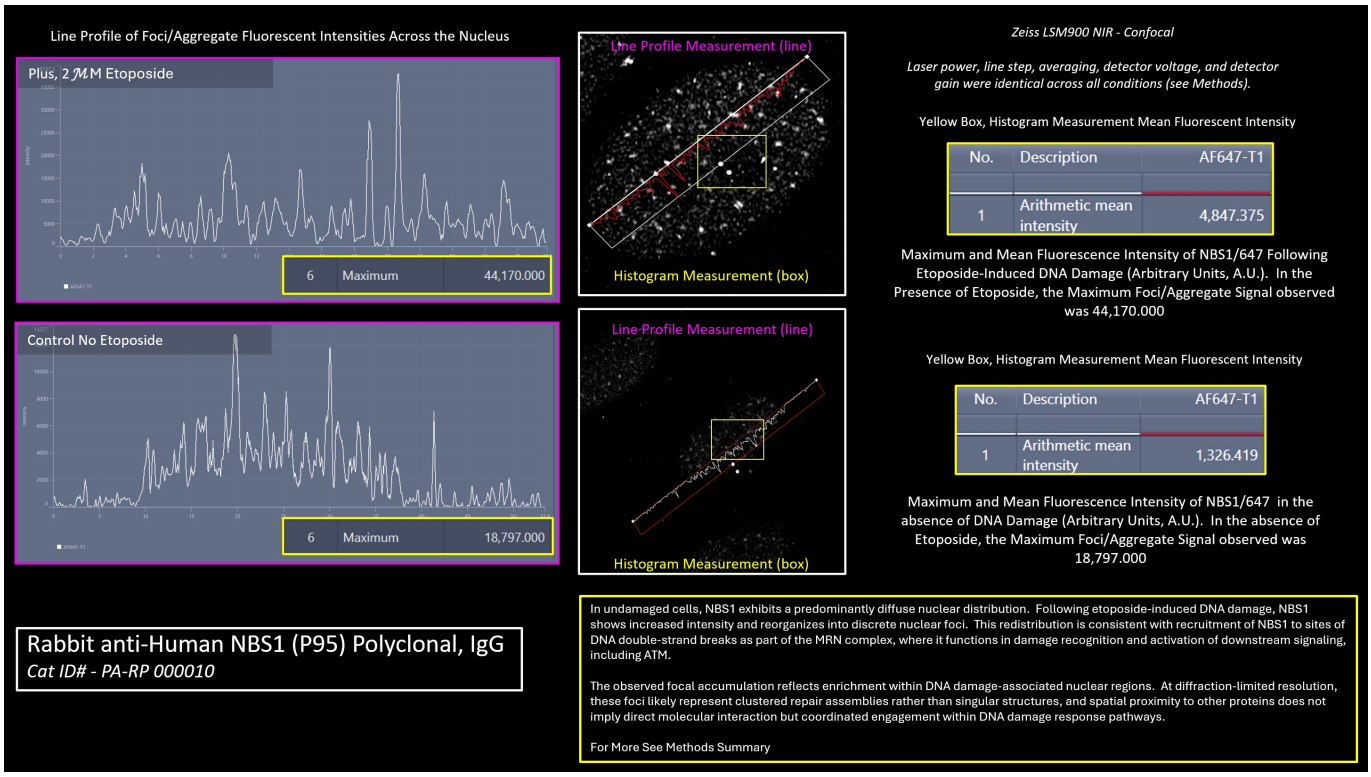
Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Structured source metadata values	79
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DADCDA1BE8E2E928E1591C66CE5CB777F27395FCBEECB9F1B024B0A821382B93
Method PDF SHA-256	238F991ACB180D5B2DCE08C8AF55F3DA8D5C540D624FC0701767D0A5139AB89A

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	53

STRUCTURED ACQUISITION METADATA / CONTROL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	161 Slices (4.8 µm)
Channels	1
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm | 0.059 µm X 0.059 µm
Image size (Pixels)	468 X 514 | 1503 X 1503
Image Size (Scaled)	27.53 µm X 30.23 µm | 88.38 µm X 88.38 µm
Bit Depth	16 Bit
Image Center Position	X: 74.44 mm, Y: 45.97 mm | X: 80.77 mm, Y: 54.03 mm
Stage Position	X: 74.44 mm, Y: 45.97 mm | X: 80.77 mm, Y: 54.03 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 64 µm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS SP 615
Laser Wavelength	639 nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7 | 1.5
Rotation	0°
Sampling	1.20
Pixel Time	0.29 µs | 0.26 µs
Frame Time	4.97 s | 5.63 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	645 - 700
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.5 (3D, Auto) | Filter: 8.3 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 | Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 29.8), Y (Min 0.0 and Max 39373) | X and Y axes were set to Auto - X (Min 0.0 and Max 53.40), Y (Min 0.0 and Max 13377)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000)
Arithmetic Mean intensity	4847.375 | 795.313

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human NBS1 (P95) Polyclonal, IgG
Cat ID# - PA-RP 000010

Identifyn® Secondary Antibody

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

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.

Method

Plus Damage -

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 -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human NBS1 (P95) Polyclonal, 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 and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

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 NBS1 (P95) Polyclonal, 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 and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Showing Z-Plane 81 of 161, Plus Etoposide

Z-Stack = 161 Slices (4.8 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 468 X 514
Image Size (Scaled) = 27.53 µm X 30.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.44 mm, Y: 45.97 mm
Stage Position = X: 74.44 mm, Y: 45.97 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - No Etoposide.

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image size (Pixels) = 1503 X 1503
Image Size (Scaled) = 88.38 µm X 88.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 80.77 mm, Y: 54.03 mm
Stage Position = X: 80.77 mm, Y: 54.03 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Plus Etoposide-Induced DNA Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 64 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

647 - Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 64 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.3 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 29.8), Y (Min 0.0 and Max 39373)

This panel measures NBS1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~40K A.U. at its maximum.

Measured at Z-plane 81 of 161

Profile View Display - Minus DNA Damage, Bottom

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 53.40), Y (Min 0.0 and Max 13377)

This panel measures NBS1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~12K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000)

Arithmetic Mean intensity = 4847.375

The histogram was generated from the yellow-boxed region shown to quantify the overall NBS1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - Minus DNA Damage, Bottom

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000)

Arithmetic Mean intensity = 795.313

The histogram was generated from the yellow-boxed region shown to quantify the overall NBS1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, NBS1 exhibits a low-to-moderate, predominantly diffuse nuclear distribution, consistent with its role in basal genome surveillance. Following etoposide-induced DNA damage, NBS1 shows a marked increase in mean intensity and reorganizes into discrete nuclear foci, with aggregate signal substantially elevated relative to baseline.

This redistribution is consistent with activation of the DNA damage response and rapid recruitment of NBS1 to sites of DNA double-strand breaks as part of the MRN complex (MRE11-RAD50-NBS1). In this context, NBS1 functions early in the damage response, facilitating break recognition, stabilizing the repair complex, and activating downstream signaling pathways, including ATM-dependent checkpoint activation.

The observed focal accumulation reflects the concentration of NBS1 within damage-associated nuclear microenvironments, where multi-protein repair assemblies form. While these foci represent active DNA damage response domains, their appearance at diffraction-limited resolution likely reflects clustered or overlapping repair complexes rather than singular structures.

The increase in signal intensity and organization supports NBS1's role as a critical upstream mediator of double-strand break repair, coordinating both structural and signaling components of the response. Spatial proximity to other repair factors does not imply direct molecular interaction but rather coordinated engagement within shared DNA damage response pathways.

Collectively, these data support a model in which NBS1 redistribution following genotoxic stress reflects rapid recruitment to DNA damage sites and integration into higher-order repair assemblies, reinforcing its role as an essential regulator of double-strand break recognition and signaling.

Image Corrections

The NBS1 channel (647) was pseudocolored white to enhance visual contrast in displaying measurements.

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RP-000010
Stage	Control
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B4875C8D1DEB6BAE54C7BADDEB4AF2D238D7B65A40B551063FA04045656AD77C
Method PDF SHA-256	EE3C14A0C4F5F9DE14BE7E756B6358F7D9FB1469B93703D05B4231E0D2D5C909