BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

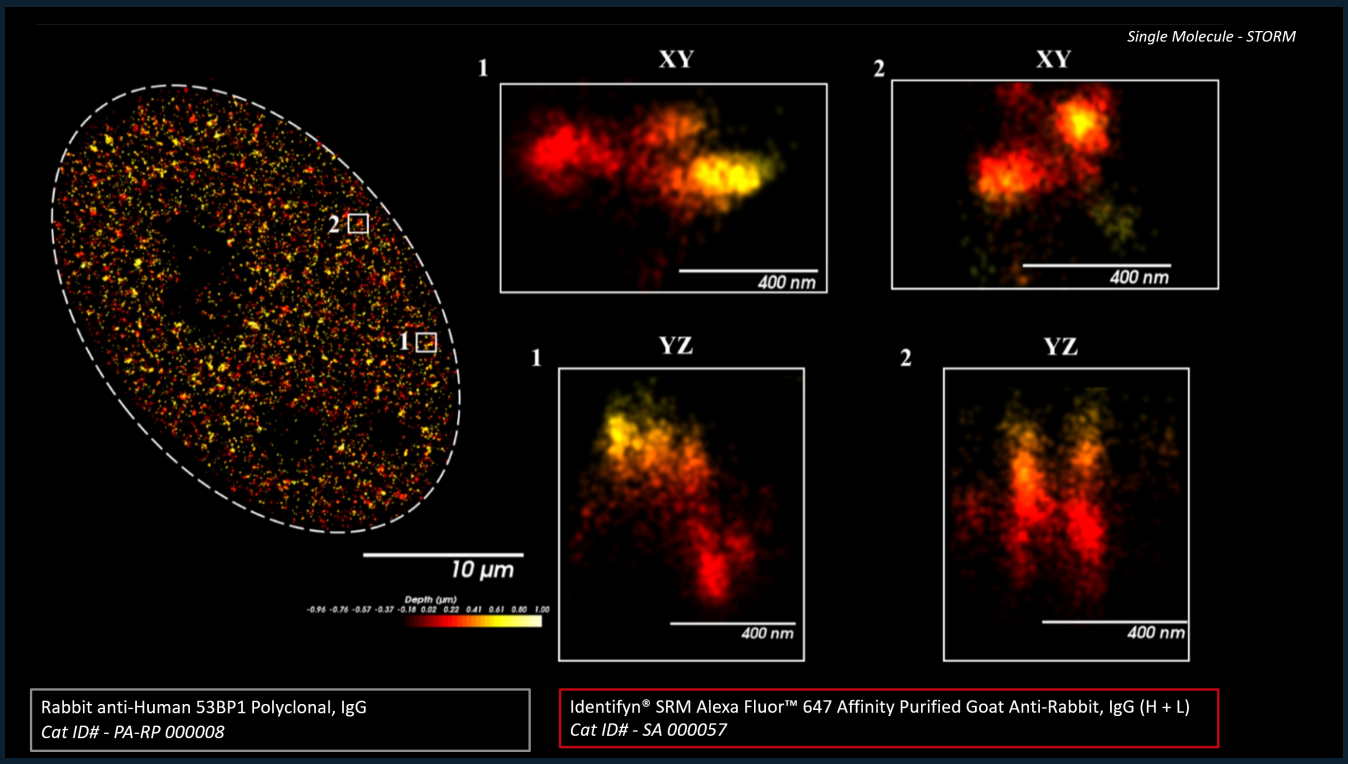
53BP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human 53BP1 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-000008 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

UNPUBLISHED / PENDING SCIENTIST REVIEW / NO PUBLICATION AUTHORIZED

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 53BP1, 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-000008 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-000008 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; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	3; None; 639nm @0.1%; 488nm @0.04%; 405nm @0.3%; 653; 493; 353
Airyscan	405/DAPI; 488; 647	3; None; 639nm @0.08%; 488nm @0.04%; 405nm @0.3%; 653; 493; 353
SIM	405/DAPI; 488; 647	3; 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-SR; T3-Axiocam 820 #2-SR
SIM²	405/DAPI; 488; 647	3; 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-SR; T3-Axiocam 820 #2-SR
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	1 (647); None; 639nm @0.10%; 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(s). 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: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1000 X 1010; Image Size (Pixels): 1000 X 1010; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

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 ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 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: 57 Slices (5.6 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 1307 X 1045; 682 X 695; Image Size (Pixels): 1307 X 1045; 682 X 695; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 150 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25; 1.4. 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: 58.

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; 488; 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: 64 Slices (1.89 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1017 X 1017; Image Size (Pixels): 1017 X 1017; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.33µs; Frame Time: 3.36s. Illumination: Laser Wavelength: 639nm @0.1%; 488nm @0.04%. Optical/scan: Pinhole: 1.00AU / 65µm; 1.00AU / 51µm; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Effective NA: 1.4. 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; LSM Plus Mode: Filter: 7.9 (3D, Auto); Filter: 7.0 (3D, Auto). Structured source metadata values: 60.

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; 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: 141 Slices (4.2 µm); Channels: 3; Scaling (Per Pixel): 35.90 nm X 35.90 nm X 30.00 nm; Image size (Pixels): 1793 X 1793; 863 X 640; Image Size (Pixels): 1793 X 1793; 863 X 640; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.15µs; Frame Time: 17.86s. Illumination: Laser Wavelength: 639nm @0.08%; 488nm @0.04%. Optical/scan: Pinhole: 5.00AU / 328µm; 5.00AU / 250µm; Scan Zoom: 2.1; LSM Scan Speed: 6; Scan Direction: Bidirectional; Effective NA: 1.4. 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; AiryScan Mode: Filter: 10.0 (3D, Auto); Filter: 9.8 (3D, Auto). Structured source metadata values: 68.

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; 647.

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. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 165 Slices (4.89 µm); Channels: 3; 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 5056 X x 5056; 1737 X x 1475; Image Size (Pixels): 5056 X x 5056; 1737 X x 1475; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.00%; Illumination Wavelength: 640; 488. 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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 15°, 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: 70.

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; 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. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 165 Slices (4.89 µm); Channels: 3; 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1349 X x 1626; 265 X x 238; Image Size (Pixels): 1349 X x 1626; 265 X x 238; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 25°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); 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: 68.

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; 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: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 80.

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: Channels: 1 (647); Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; 0.059 µm X 0.059 µm; Image size (Pixels): 1017 X 1017; 1737 X 1737; Image Size (Pixels): 1017 X 1017; 1737 X 1737; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.33µs; 0.22µs; Frame Time: 3.36s; 6.49s. Illumination: Laser Wavelength: 639nm @0.10%. Optical/scan: Pinhole: 1.00AU / 65µm; 1.00AU / 66µm; Scan Zoom: 2.2; 1.3; LSM Scan Speed: 12; 11; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.9 (3D, Auto); Filter: 6.6 (3D, Auto). Structured source metadata values: 54.

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-000008 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): 35.90 nm X 35.90 nm X 30.00 nm -> 0.03590 µm X 0.03590 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)=1793 X 1793; Image Size (Scaled)=63.29 µm X 63.29 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 1.70%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 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)=5056 X x 5056; Image Size (Scaled)=106.75 µm X 106.75 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 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)=1349 X x 1626; Image Size (Scaled)=28.48 µm X 34.33 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 200µM -> Etoposide = 2 µM. The governing scientist identified every 200 µM occurrence as a technician transcription error. The canonical historical concentration is 2 µM. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Single-molecule STORM": ["12 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

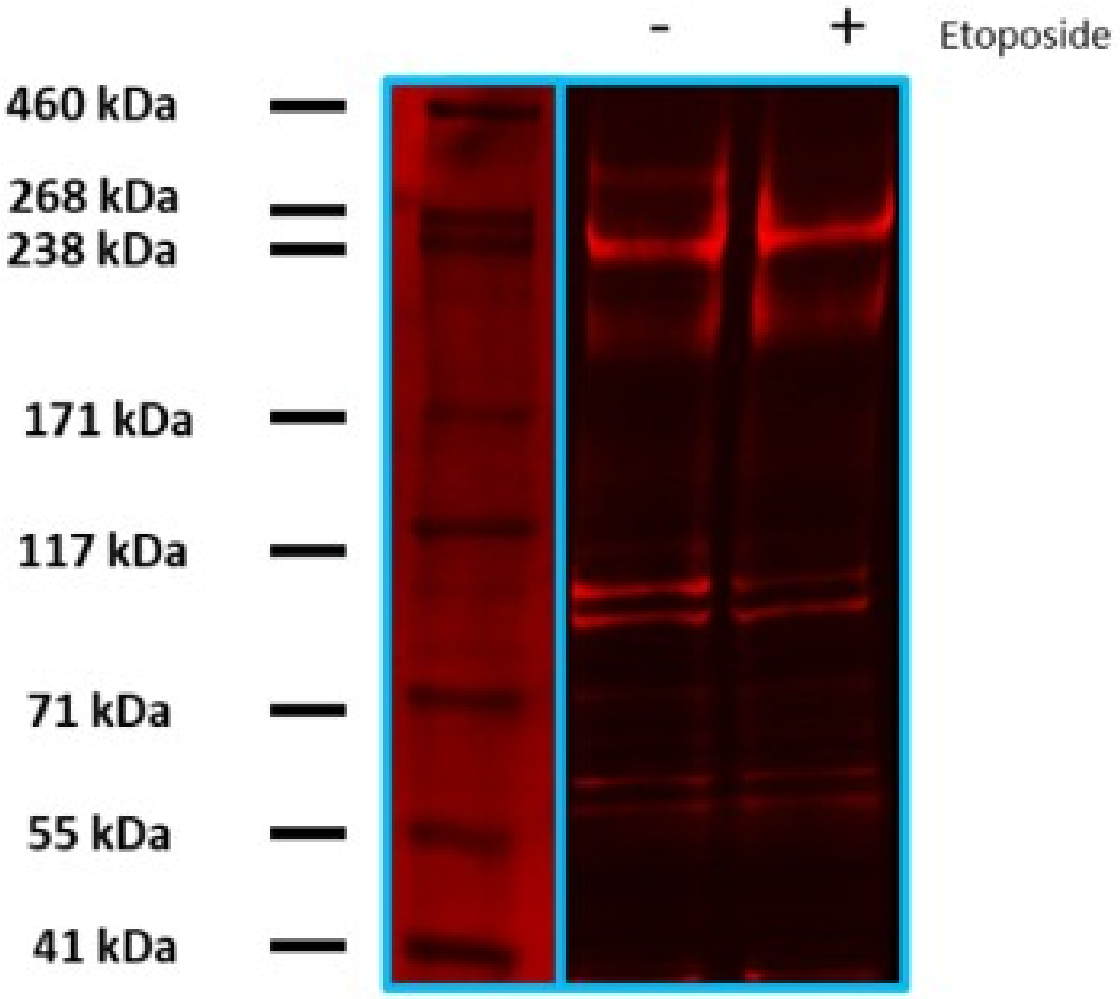
This dossier preserves the complete available evidence progression for PA-RP-000008. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	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(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	632nm
Emission Filter	676±37nm
Detector	PMT
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Expected MW: 214 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and 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 using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. The Thermo Fisher's 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 PBS. The blocked membrane was incubated with Rabbit anti-Human 53BP1 Polyclonal, IgG for 20 hours, followed by 5X PBS washes. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with 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(s)

Scan Settings

Detection mode = Fluorescence
Laser = 632nm
Emission Filter = 676±37nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small

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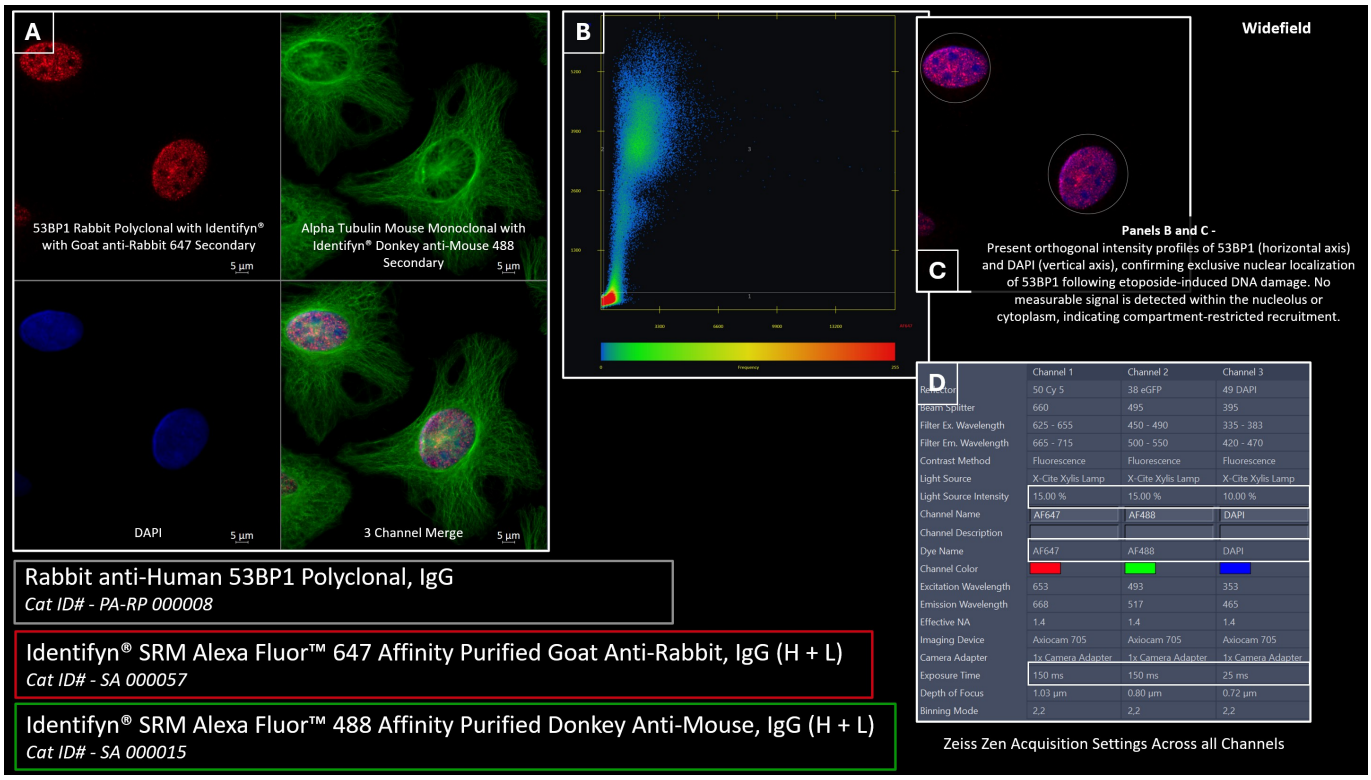
SOURCE PROVENANCE

Catalog	PA-RP-000008
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C258CC31AC27C8476D671CB56B67284D706741FC496C174708A38B4A67FB50F2
Method PDF SHA-256	BC2348830EC6EB6FC9CEF8F9A925D2873012A89CA06C1859EE48F333FD2ACC9D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	37

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	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	1000 X 1010
Image Size (Scaled)	109.52 µm X 110.62 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 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 25 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1000 X 1010
Image Size (Scaled) = 109.52 µm X 110.62 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 @15.00%
Exposure Time = 150 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

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 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 = 25 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 features Rabbit anti-Human 53BP1 Polyclonal, IgG, visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Colocalization View - Panel B, and Image Panel C, note the circular white mask used to take measurements.

Horizontal Axis - AF647, Threshold 190, Range Auto
Vertical Axis - DAPI, Threshold 386, Range Auto

Zeiss Zen Acquisition Settings - Panel D

Shown are the acquisition settings across all three channels, including LED intensity and exposure times, along with all other relevant parameters, provided for methodological transparency.

Summary

Panels B and C present orthogonal intensity profiles of 53BP1 (horizontal axis) and DAPI (vertical axis), demonstrating nuclear localization of 53BP1 following etoposide-induced DNA damage. No measurable signal is detected within the nucleolus or cytoplasm, consistent with compartment-restricted recruitment. Panel D: Imaging acquisition parameters are reported for methodological transparency: 53BP1 counterstained with an Identifyn® 647 secondary antibody was acquired with 15% LED input and a 150 ms exposure time in Zeiss ZEN.

Image Correction

None

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

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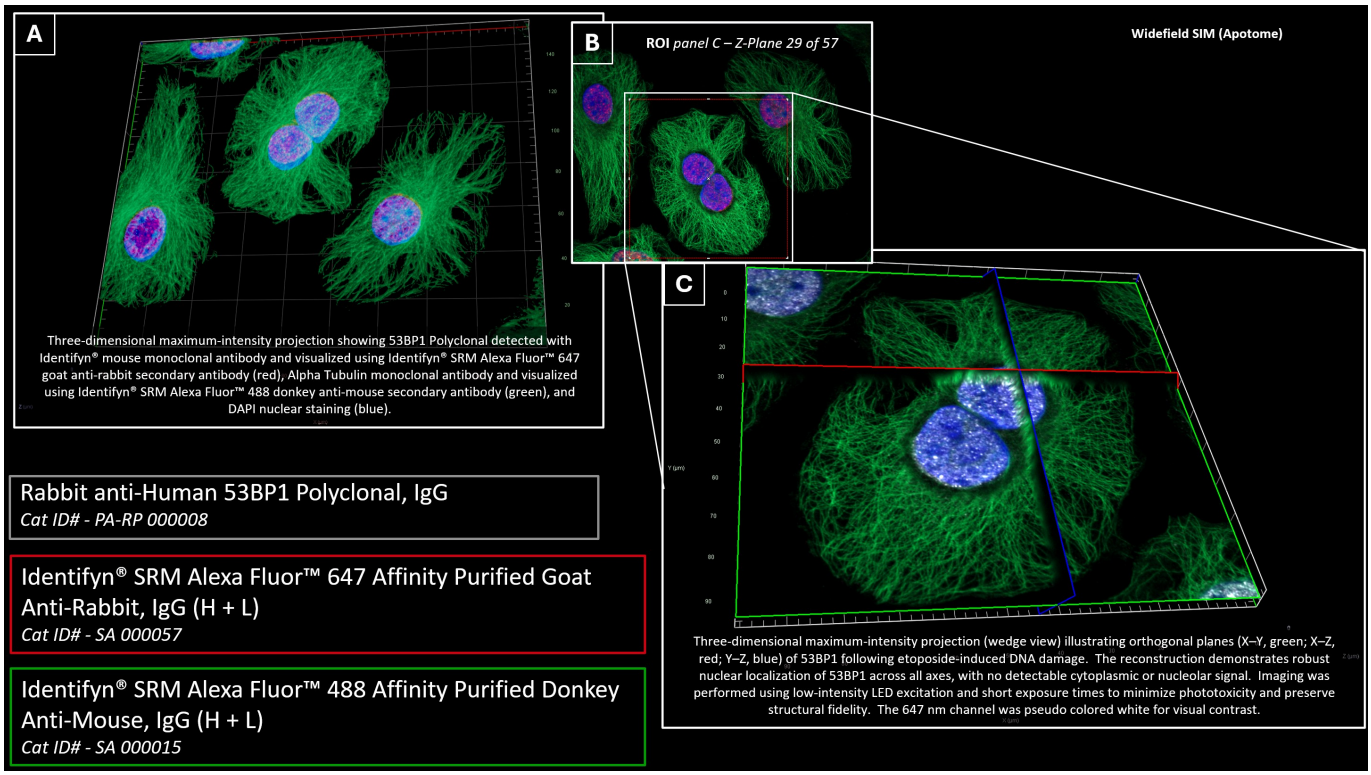
SOURCE PROVENANCE

Catalog	PA-RP-000008
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A2E6AB50B0740A89C24C293743F059373CB7C798F80D46E21780A3CE55E0FEE6
Method PDF SHA-256	E6FFA06908D266B787A3351FFA30F957C78C636C4889E26C0FC03752E5FCCC01

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	58

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	57 Slices (5.6 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	1307 X 1045 | 682 X 695
Image Size (Scaled)	186.71 µm X 148.29 µm | 98.86 µm X 99.29 µm
Bit Depth	14 Bit
Image Center Position	X: 63.02 µm, Y: 50.49 µm
Stage Position	X: 63.02 µm, Y: 50.49 µm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 25 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.25 | 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.00 µm | 0.72 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

FIELD	SOURCE-DOCUMENTED VALUE
Position of Clip	0% | -17% | -16%
Clip X	0°
Clip Z	0°
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.
Clip Y	45° | -45°
Y-Z	Activate was selected, textured opaque was chosen, and a blue 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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss 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 = 57 Slices (5.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 1307 X 1045
Image Size (Scaled) = 186.71 µm X 148.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 63.02 µm, Y: 50.49 µm
Stage Position = X: 63.02 µm, Y: 50.49 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Single Plane (2D) - Panel B, Z-Plane 29 of 57, (Dimension Shown in Panel A)

Z Stack - (3D) - Panel C

Z-Stack = 57 Slices (5.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 682 X 695
Image Size (Scaled) = 98.86 µm X 99.29 µm
Image Center Position = X: 63.02 µm, Y: 50.49 µm
Stage Position = X: 63.02 µm, Y: 50.49 µm
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 @15.00%
Exposure Time = 150 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

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 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 = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.72 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 Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

Clipping planes are introduced in Panel B to highlight the 53BP1 Rabbit Polyclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = 0%
Clip X = 0°
Clip Z = 0°

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 = -17%
Clip Y = 45°
Clip Z = 0°

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

Position of Clip = -16%
Clip Y = -45°
Clip Z = 0°

Summary

Widefield structured illumination microscopy (Apotome) imaging of 53BP1 following etoposide-induced DNA damage reveals robust and highly specific nuclear localization. Panel A presents the full three-dimensional acquisition, rendered as a maximum-intensity projection, that captures the global nuclear distribution of 53BP1 across the entire z-stack. Panel B highlights a single optical section (Z-plane 29 of 57), with a defined region of interest selected for higher-order three-dimensional analysis. This region is further examined using a three-dimensional maximum-intensity projection (wedge view), which illustrates orthogonal planes (X-Y, green; X-Z, red; Y-Z, blue) and enables visualization of 53BP1 localization across all spatial axes. The signal is confined to the nuclear compartment, with no detectable cytoplasmic or nucleolar staining, consistent with 53BP1's established role as a chromatin-associated DNA damage response factor. The clarity, confinement, and reproducibility of the nuclear signal underscore the high specificity and low background of the polyclonal 53BP1 antibody, supporting its suitability for three-dimensional imaging of the DNA damage response under low-phototoxicity conditions.

Image Correction

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

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

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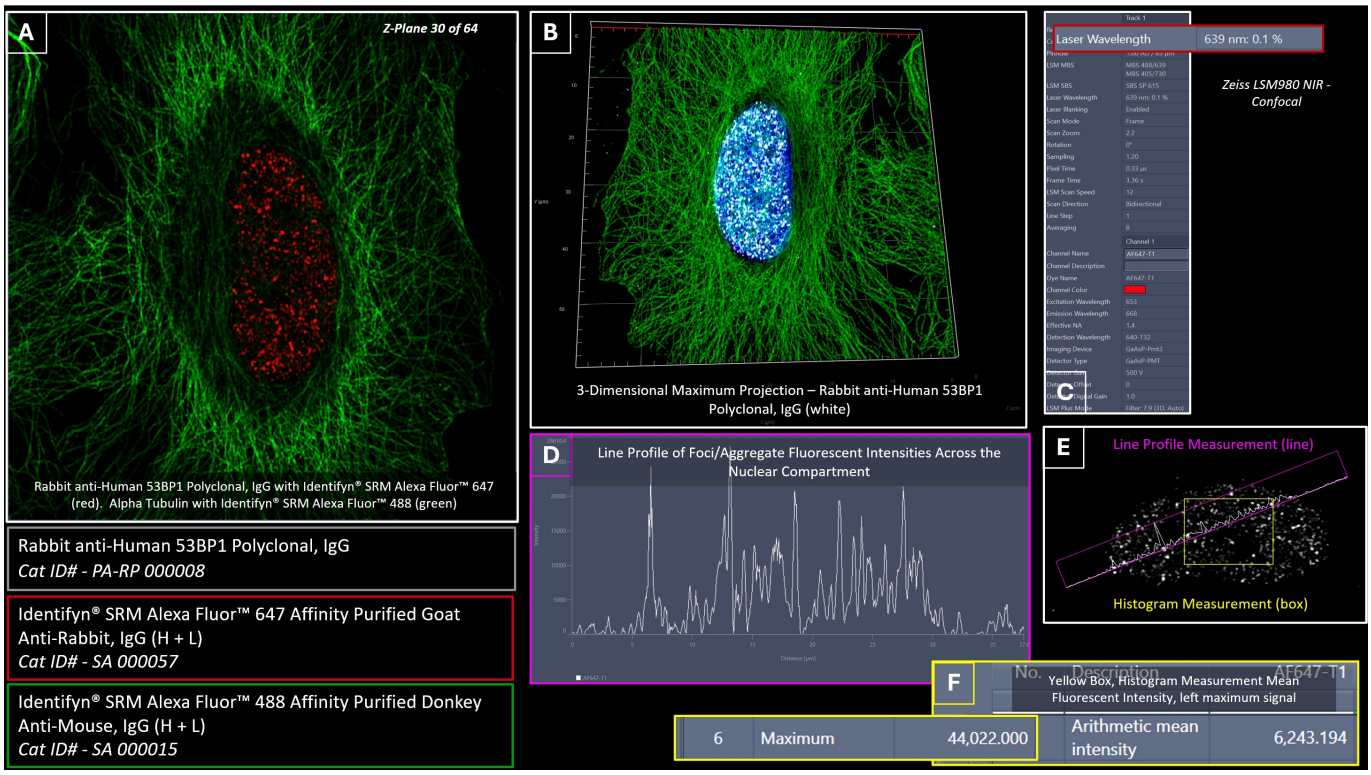
SOURCE PROVENANCE

Catalog	PA-RP-000008
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	58
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CA2C1204E5ABA67FC1604A470C1C56807415A137F509278C49501CD3BE955632
Method PDF SHA-256	2ABF32F21E46A782BEBDA9B747BEAE98B197EBA43946E7CD76AFE11433A600DA

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	60

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	64 Slices (1.89 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1017 X 1017
Image Size (Scaled)	59.81 µm X 59.81 µm
Bit Depth	16 Bit
Image Center Position	X: 86.79 mm, Y: 50.23 mm
Stage Position	X: 86.79 mm, Y: 50.23 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 65µm | 1.00AU / 51µm | 1.00AU / 43µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615 | SBS SP 505 | Mirror
Laser Wavelength	639nm @0.1% | 488nm @0.04% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.20
Pixel Time	0.33µs
Frame Time	3.36s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	640 - 732 | 511 - 550 | 430 - 480
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3
Detector Type	GaAsP-PMT | GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.9 (3D, Auto) | Filter: 7.0 (3D, Auto) | Filter: 6.5 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
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 37.6), Y (Min 0 and Max 28610)
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 and Max 20000)

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss 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:

Single Plane (2D) - Z-Plane 30 of 64 - Panels A & E

Z Stack - (3D) - Panel B

Z-Stack = 64 Slices (1.89 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1017 X 1017
Image Size (Scaled) = 59.81 µm X 59.81 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.79 mm, Y: 50.23 mm
Stage Position = X: 86.79 mm, Y: 50.23 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
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)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 480
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.5 (3D, Auto)

2-Dimensional View - Panel A

Panel features Rabbit anti-Human 53BP1 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L).

Image was taken from Z-stack Acquisition at plane 30 of 64

3D Image Processing - Panel B

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

Note that the 53BP1, counterstained with 647, was pseudo-colored white from red in Zeiss Zen, to enhance visual contrast.

LSM 980 Zen Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel (53BP1 Rabbit Polyclonal with Identifyn 647 secondary) using the 639nm laser at 0.1%.

Not shown, the 488 channel (Alpha Tubulin) using the 488nm laser at 0.04%, and DAPI using the 405nm laser at 0.3%

Profile View Display - Panels D & E

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 37.6), Y (Min 0 and Max 28610)

This panel measures 53BP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 28K A.U. at its maximum.

Measured at Z-plane 30 of 64

Histogram Display - Panels E & F

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 and Max 20000)

The histogram was generated from the yellow-boxed region shown in Panel E to quantify the overall 53BP1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition (see control), complementing the line-profile analysis presented alongside it.

Summary -

Confocal imaging was used to characterize the spatial distribution and intensity of 53BP1 following DNA damage, combining two-dimensional visualization with three-dimensional reconstruction and quantitative signal analysis.

Panel A presents a representative two-dimensional optical section (Z-plane 30 of 64) acquired from a Z-stack, showing Rabbit anti-Human 53BP1 Polyclonal IgG detected with an Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibody, together with α-tubulin labeled using a mouse monoclonal primary and an Identifyn® SRM Alexa Fluor™ 488 secondary antibody. This view demonstrates strong nuclear enrichment of 53BP1 relative to the cytoskeletal counterstain, consistent with a DNA damage-associated chromatin response.

Panel B displays three-dimensional image processing of the same acquisition rendered as a precise maximum-intensity projection, using standardized thresholding and tone-mapping parameters to preserve structural fidelity and quantitative integrity. The projection highlights discrete intranuclear 53BP1 foci and aggregates distributed throughout the nuclear volume, with minimal background and no detectable cytoplasmic signal. For visual clarity, the 647 nm channel corresponding to 53BP1 was pseudocolored white instead of its default red, without altering the underlying intensity values.

Panel C documents the acquisition conditions for the 53BP1 channel, demonstrating that a high-contrast nuclear signal was achieved using extremely low laser power (639 nm at 0.1%), with similarly low excitation applied for the 488 nm α-tubulin and 405 nm DAPI channels (not shown). These conditions underscore the sensitivity of the detection and minimize photobleaching and phototoxicity.

Quantitative analysis is shown in Panels D and E, where line-profile measurements across an arbitrary intranuclear axis reveal prominent 53BP1 DNA damage-induced foci, with peak signal intensities reaching approximately 28,000 arbitrary units at Z-plane 30 of 64. Complementary histogram analysis (Panels E and F), generated from a defined

nuclear region of interest, quantifies the overall 53BP1 response following damage induction and demonstrates a marked shift in signal distribution relative to control conditions. Together, the spatial confinement, high signal-to-background ratio, and reproducibility across orthogonal analyses support the specificity and performance of the polyclonal 53BP1 antibody for quantitative three-dimensional DNA damage response imaging under low-excitation conditions.

Image Corrections

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

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

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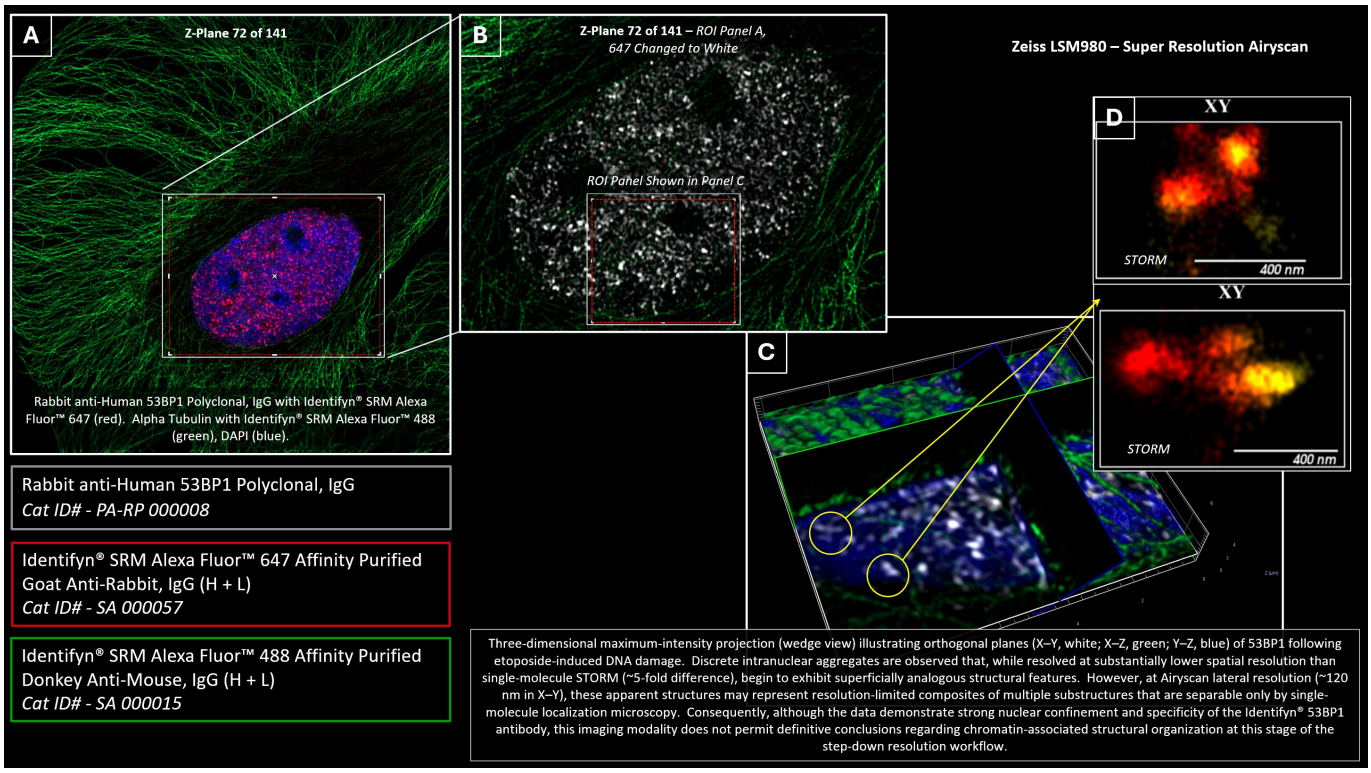
SOURCE PROVENANCE

Catalog	PA-RP-000008
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	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BD471BE23C7B98B8355236BBD27FB9962626DDF88F40BC9492F36628BD299DFE
Method PDF SHA-256	C95D91F62A0872F50DFC1DAFC51BE137971795CB59010B7A6F97AF7EC2C2F23C

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	68

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
Channels	3
Scaling (Per Pixel)	0.03590 µm X 0.03590 µm X 0.03000 µm
Image Size (Pixels)	1793 X 1793 | 863 X 640 | 289 X 249
Image Size (Scaled)	63.29 µm X 63.29 µm | 30.46 µm X 22.59 µm | 10.20 µm X 8.79 µm
Bit Depth	16 Bit
Image Center Position	X: 85.47 mm, Y: 48.09 mm
Stage Position	X: 85.47 mm, Y: 48.09 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	141 Slices (4.2 µm)
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 328µm | 5.00AU / 250µm | 5.00AU / 214µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 640 | SBS SP 550 | SBS SP 505
Laser Wavelength	639nm @0.08% | 488nm @0.04% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	2.00
Pixel Time	1.15µs
Frame Time	17.86s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 499 - 548 | 422 - 477
Imaging Device	Airyscan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	Filter: 10.0 (3D, Auto) | Filter: 9.8 (3D, Auto) | Filter: 8.4 (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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	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	-49% | -14% | -20%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	30° | -30°
Y-Z	Activate was selected, textured opaque was chosen, and a blue 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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss 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:

Single Plane (2D) - Z-Plane 72 of 141 - Panel A

Channels = 3
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image Size (Pixels) = 1793 X 1793
Image Size (Scaled) = 63.29 µm X 63.29 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.47 mm, Y: 48.09 mm
Stage Position = X: 85.47 mm, Y: 48.09 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-Plane 72 of 141- Panel B, ROI Panel A

Channels = 3
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image Size (Pixels) = 863 X 640
Image Size (Scaled) = 30.46 µm X 22.59 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.47 mm, Y: 48.09 mm
Stage Position = X: 85.47 mm, Y: 48.09 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm
Image Size (Pixels) = 289 X 249
Image Size (Scaled) = 10.20 µm X 8.79 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.47 mm, Y: 48.09 mm
Stage Position = X: 85.47 mm, Y: 48.09 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 640
Laser Wavelength = 639nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15µs
Frame Time = 17.86s
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 = Filter: 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15µs
Frame Time = 17.86s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Filter: 9.8 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.15µs
Frame Time = 17.86s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Filter: 8.4 (3D, Auto)

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human 53BP1 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI,

Panel B, the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast.

Image was taken from Z-stack Acquisition at plane 72 of 141.

3D Image Processing

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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

Clipping planes are introduced in Panel B to highlight the 53BP1 Rabbit Polyclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = 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 = -49%
Clip X = 0°
Clip Z = 0°

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

Position of Clip = -20%
Clip Y = -30°
Clip Z = 0°

Panel D - STORM Image from the same data set, DATA

Summary -

Using the Identifyn® Standards for Optical Microscopy Methods™, we continue a step-down, multi-modal imaging analysis to evaluate the localization, organization, and detectability of 53BP1 following etoposide-induced DNA damage. HeLa cells were labeled with a Rabbit anti-human 53BP1 Polyclonal IgG and detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, with α-tubulin and DAPI serving as cytoskeletal and nuclear references, respectively. Across all imaging modalities and analytical views, the 53BP1 signal was robustly confined to the nuclear compartment, with no detectable cytoplasmic or extranuclear labeling, demonstrating high specificity and low background of the Identifyn® antibody reagents.

Two-dimensional optical sections (Panels A and B) acquired from a 141-slice Z-stack illustrate a discrete intranuclear 53BP1 signal relative to α-tubulin, with enhanced contrast achieved by pseudocoloring the 647-nm channel from its default red to white without altering underlying intensity values. Three-dimensional volume reconstructions (Panel C) generated from the same region of interest reveal punctate and aggregated distributions of 53BP1 throughout the nuclear volume. Orthogonal clipping and wedge-based volume projections further confirm nuclear confinement across X-Y, X-Z, and Y-Z planes, enabling direct visualization of spatial continuity and depth.

All data were acquired on a Zeiss LSM 980 equipped with Airyscan detection under highly sensitive, low-excitation conditions (639 nm at 0.08%, 488 nm at 0.04%, 405 nm at 0.3%), underscoring the robustness of the observed signal while minimizing photobleaching and phototoxicity. Quantitative line-profile and histogram analyses demonstrate strong signal-to-background ratios and reproducible intensity distributions across nuclear regions, supporting consistent detection across the dataset. (confocal)

While three-dimensional Airyscan imaging is beginning to reveal structural features superficially analogous to those observed by single-molecule localization microscopy, the lateral resolution limit of Airyscan (~120 nm in X-Y)

constrains structural interpretation. Apparent aggregates at this resolution may represent resolution-limited composites of multiple substructures that are separable only by STORM, as shown in Panel D using the same dataset. Consequently, although these data robustly establish nuclear localization and antibody specificity, they do not permit definitive conclusions regarding chromatin-associated structural organization at this stage of the step-down resolution workflow.

Given the central role of 53BP1 in DNA double-strand break signaling, pathway choice, and genome stability, accurate identification of its nuclear localization and distribution following etoposide-induced damage is essential for interpreting repair pathway engagement. The Super Resolution Airyscan data continues to establish a rigorous, low-power imaging foundation upon which higher-resolution modalities can be applied to resolve damage-induced chromatin architecture with greater structural fidelity.

Image Corrections

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

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

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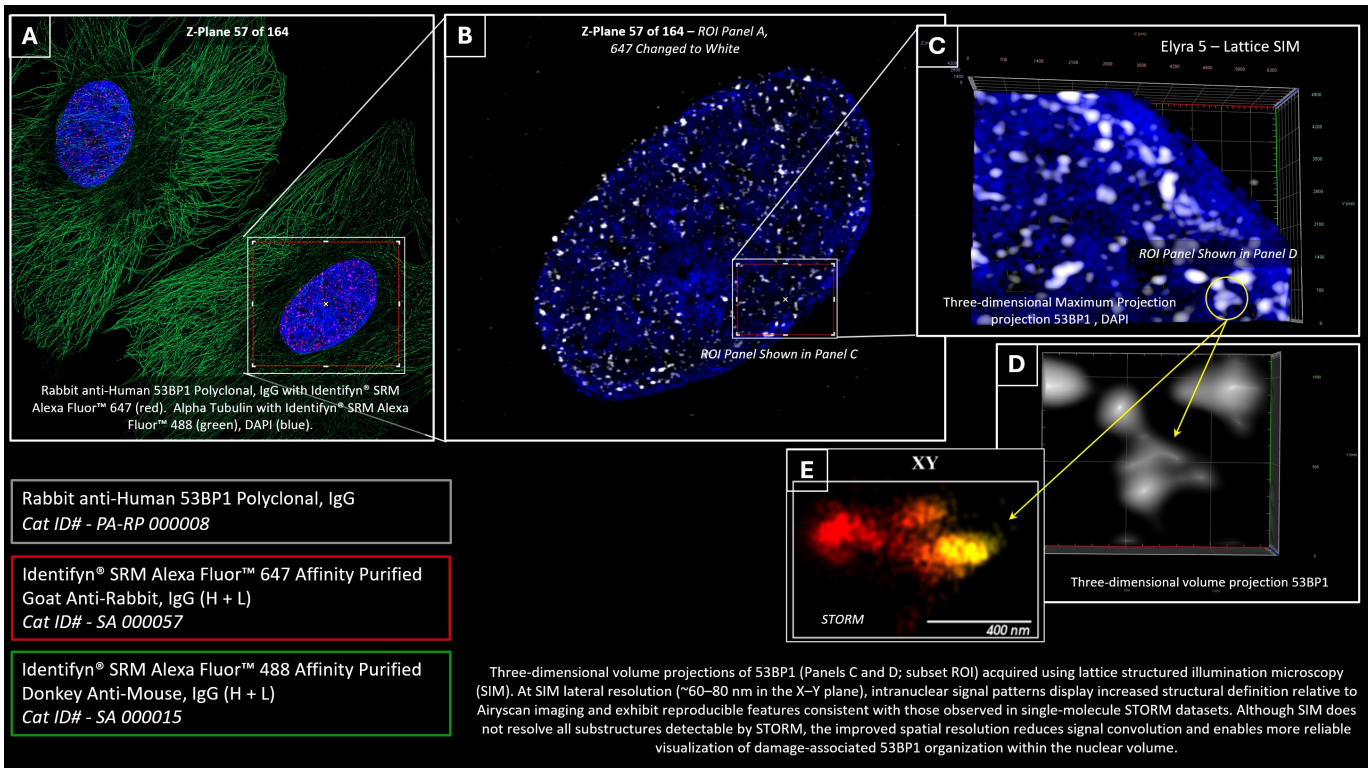
SOURCE PROVENANCE

Catalog	PA-RP-000008
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	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A21C95E86EE82DD808345F571FA51AB57C30EEBBCCC90ED5778AEA1959DEE830
Method PDF SHA-256	6951FDB90F9605C84B9170EA518857342BD25220806CF81820D80AF398242FD6

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	70

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 Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	3 | 2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	5056 X x 5056 | 1737 X x 1475 | 328 X x 389 | 64 X x 54
Image Size (Scaled)	106.75 µm X 106.75 µm | 36.67 µm X 31.14 µm | 6.29 µm X 5.05 µm | 1.35 µm X 1.14 µm
Bit Depth	16 Bit
Image Center Position	X: 61.24 mm, Y: 40.78 mm
Stage Position	X: 61.24 mm, Y: 40.78 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	165 Slices (4.89 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 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
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	100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.00% | 405 nm @2.00%
Frame Time	1.33 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 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	10.5799 (647), 11.1375 (488), 10.1489 (DAPI)
Fitting	Fast Fit
Normalization	Auto
Panel B	the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast, and tubulin (488) is not shown.
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 1% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 120%, 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) | View Angle 15°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

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

Single Plane (2D) - Z-plane 57 of 164 total - Panel A

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 5056 X x 5056
Image Size (Scaled) = 106.75 µm X 106.75 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.78 mm
Stage Position = X: 61.24 mm, Y: 40.78 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 57 of 164 total - Panel B

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1737 X x 1475
Image Size (Scaled) = 36.67 µm X 31.14 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.78 mm
Stage Position = X: 61.24 mm, Y: 40.78 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 165 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 328 X x 389
Image Size (Scaled) = 6.29 µm X 5.05 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.78 mm
Stage Position = X: 61.24 mm, Y: 40.78 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 165 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 64 X x 54
Image Size (Scaled) = 1.35 µm X 1.14 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.24 mm, Y: 40.78 mm
Stage Position = X: 61.24 mm, Y: 40.78 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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 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 = 10.5799 (647), 11.1375 (488), 10.1489 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human 53BP1 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI.

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

Image was taken from Z-stack Acquisition at plane 57 of 164.

3D Image Processing - Panel C

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

3D Image Processing - Panel D

3D Volume Projection = Precise
Appearance = Threshold 1% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 15°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - STORM Image from the same data set, DATA

Summary -

Super-resolution lattice structured illumination microscopy (SIM) was used to further characterize the intranuclear distribution of 53BP1 following etoposide-induced DNA damage within the Identifyn Standards for Optical Microscopy Methods™ step-down framework. HeLa cells were labeled using a Rabbit anti-Human 53BP1 Polyclonal IgG detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, with α-tubulin and DAPI serving as cytoskeletal and nuclear references, respectively. Across all lattice SIM views, the 53BP1 signal was strictly confined to the nuclear compartment, consistent with its established role as a chromatin-associated mediator of DNA double-strand break (DSB) repair.

Two-dimensional lattice SIM optical sections (Panels A and B) acquired from a 164-slice Z-stack reveal punctate and aggregated intranuclear 53BP1 distributions with substantially increased spatial definition relative to Airyscan imaging. Three-dimensional volume reconstructions (Panel C) and higher-magnification region-of-interest analyses (Panel D) further resolve damage-associated 53BP1 assemblies throughout the nuclear volume. At lattice SIM lateral resolution (~60-80 nm in X-Y), these signal patterns display reproducible organizational features that are consistent with the known accumulation of 53BP1 at DSB-flanking chromatin domains involved in non-homologous end joining (NHEJ).

Functionally, 53BP1 recruitment to damaged chromatin contributes to NHEJ pathway engagement by restricting DNA end resection and promoting repair factor assembly in contexts where homologous recombination is disfavored. While lattice SIM does not resolve all substructures detectable by single-molecule localization microscopy, the improved resolution reduces signal convolution and enables more reliable visualization of damage-associated 53BP1 organization within the nuclear volume. Direct comparison with STORM imaging from the same dataset (Panel E) indicates that apparent aggregates at SIM resolution may represent composites of multiple sub-assemblies that are separable only at the single-molecule level.

Collectively, these data position lattice SIM as a critical intermediate modality for assessing 53BP1 nuclear organization in the context of DSB repair and NHEJ pathway engagement. Although definitive chromatin-level structural conclusions require higher-resolution localization approaches, accurate mapping of 53BP1 distribution at this resolution provides essential context for understanding repair pathway choice and the spatial regulation of the DNA damage response following genotoxic stress.

Image Corrections

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

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

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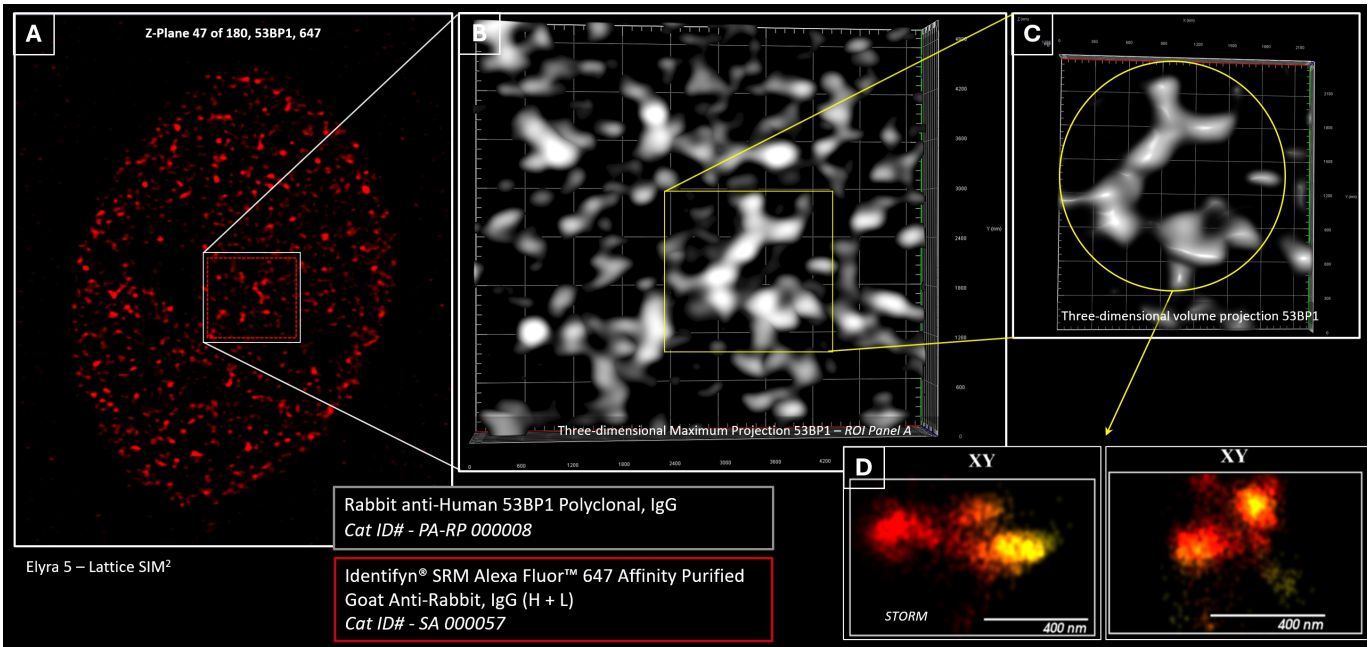
SOURCE PROVENANCE

Catalog	PA-RP-000008
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F255021FFCA117DC9D5AE4ACCEB6AF8C678B1739D1847F51F016F6A40F2F33A0
Method PDF SHA-256	36BBBF9ADD140941F642A7022179E9CE0B0B8D70A0DE05F77630D6B342C2BFA3

ORIGINAL IMAGE EVIDENCE / SIM²



Three-dimensional maximum- and volume-projection views of 53BP1 (Panels C and D, respectively; subset ROI) acquired using lattice structured illumination microscopy squared (SIM²). At SIM² lateral resolution (~40–60 nm in the X–Y plane), intranuclear signal patterns display markedly increased structural definition relative to Airyscan and Latrice SIM imaging and exhibit reproducible features that are consistent with those observed in single-molecule STORM datasets. Although SIM² does not resolve all substructures detectable by STORM, the improved spatial resolution substantially reduces signal convolution and enables more reliable visualization of damage-associated 53BP1 organization within the nuclear volume. In this context, SIM² represents an intermediate resolution modality that bridges SIM and STORM, capturing structural features that closely approximate STORM-level organization while remaining limited in its ability to resolve the full underlying substructure. As such, SIM² provides a critical and informative step within the Identifyn step-down microscopy methodology for evaluating DNA damage–associated protein organization prior to single-molecule localization analysis.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	68

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 Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	3 | 2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1349 X x 1626 | 265 X x 238 | 106 X x 113
Image Size (Scaled)	28.48 µm X 34.33 µm | 5.59 µm X 5.02 µm | 2.24 µm X 2.39 µm
Bit Depth	16 Bit
Image Center Position	X: 61.20 mm, Y: 36.61 mm
Stage Position	X: 61.20 mm, Y: 36.61 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	165 Slices (4.89 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 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
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	100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.00% | 405 nm @2.00%
Frame Time	1.33 s | 676.00 ms
Scan Direction	Unidirectional
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
Panels B and C	the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast, and tubulin (488) is not shown.
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 0% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 90%, Enabled Tone Mapping | Brightness 125%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 25°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

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

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

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

Z Stack - (3D) - Panel B

Z-Stack = 165 Slices (4.89 µm)

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

Z Stack - (3D) - Panel C

Z-Stack = 165 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 106 X x 113
Image Size (Scaled) = 2.24 µm X 2.39 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.20 mm, Y: 36.61 mm
Stage Position = X: 61.20 mm, Y: 36.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 - (Not Shown)

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI - (Not Shown)

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.00%
Frame Time = 676.00 ms
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 - Panels A

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

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

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

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 90%, Enabled Tone Mapping
Projection = View Angle 25°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 0% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 125%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - STORM Image from the same data set, DATA

Summary -

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

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

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

Image Corrections

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

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

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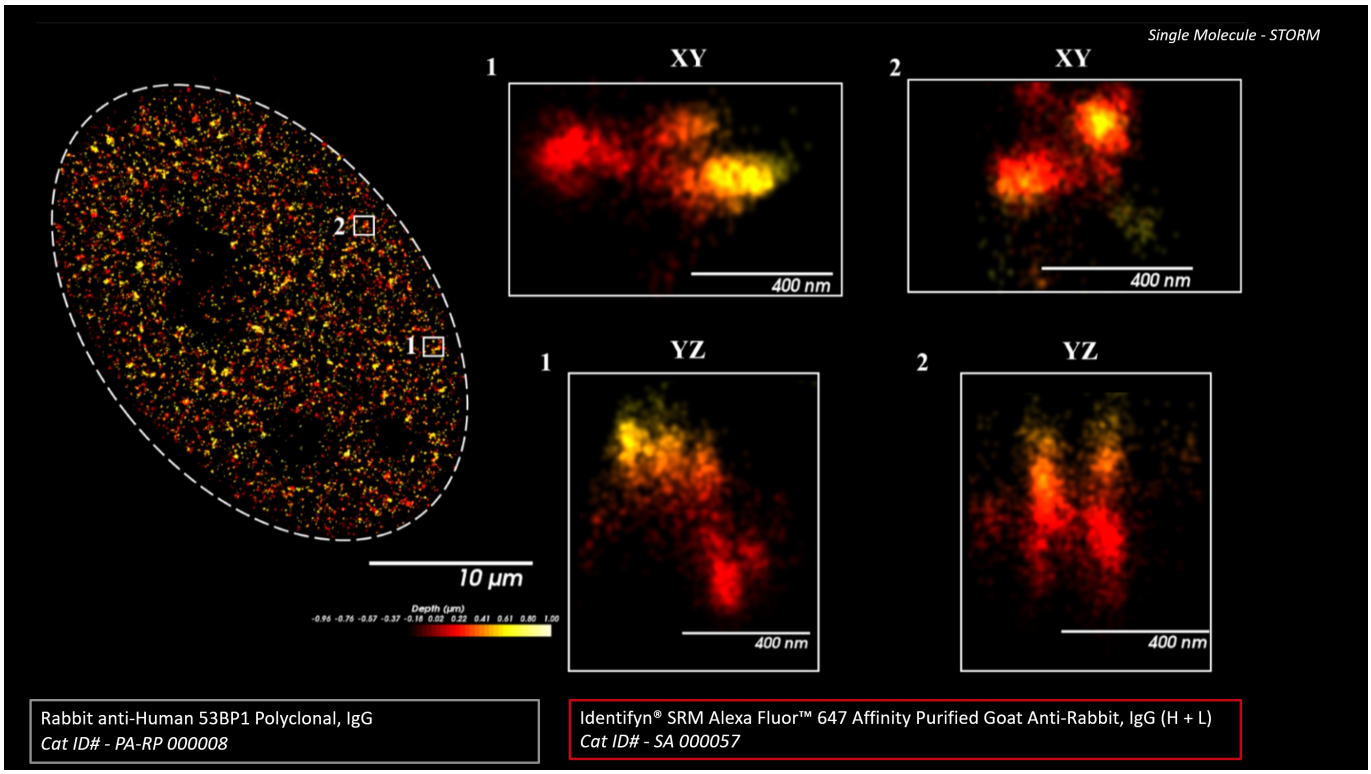
SOURCE PROVENANCE

Catalog	PA-RP-000008
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	98250C32D26308BBC7A5BE2A77FAD60ACC22C9E6864DE4D7DC73085C7A5E1549
Method PDF SHA-256	6CCA31DF234147BB01889B3C36A7BB9BA17A0BE6CBFDA8FA6C55B26342AC95C8

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	80

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	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
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	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	182.5 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	100ms
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	50µm is selected
Piezo Record	Begin: 182.5; End: 182.5
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 30000
Z Positions	1
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	90%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
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: 15
Sizing Mode	Radial Precision
Particle size	5nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.12
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	10 Intervals
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-999.71 to +999.89
Radial precision	30
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1um
Maximum Particle Count to Form Cluster	10
Computer 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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

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

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

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

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

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

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

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

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

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

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 200µM Etoposide-spiked complete growth media for 12 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human 53BP1 Polyclonal was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn®'s proprietary Wash Buffer, and Identifyn®'s 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X 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: 635nm Dichroic
Objective: Silicon Selected
Stage Insert: Rectangular Selected
Course 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: 100ms
Super Resolution Camera Exposure Time: 20ms
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: 20ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200µm
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: 182.5 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: 100ms
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: 50µm 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: 182.5; End: 182.5

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640nm: 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: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 30000
Z Positions: 1
Cycles: 1
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635nm Dichroic)

Laser Power: 90%

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1px
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: 15

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: Radial Precision
Particle size: 5nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.12
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 10 Intervals
Pixel Size: 50nm
Smoothing: 8.00

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

PSF Z offset: -999.71 to +999.89
Radial precision: 30
Axial precision: 70

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: 0.1um
Maximum Particle Count to Form Cluster: 10
Computer 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

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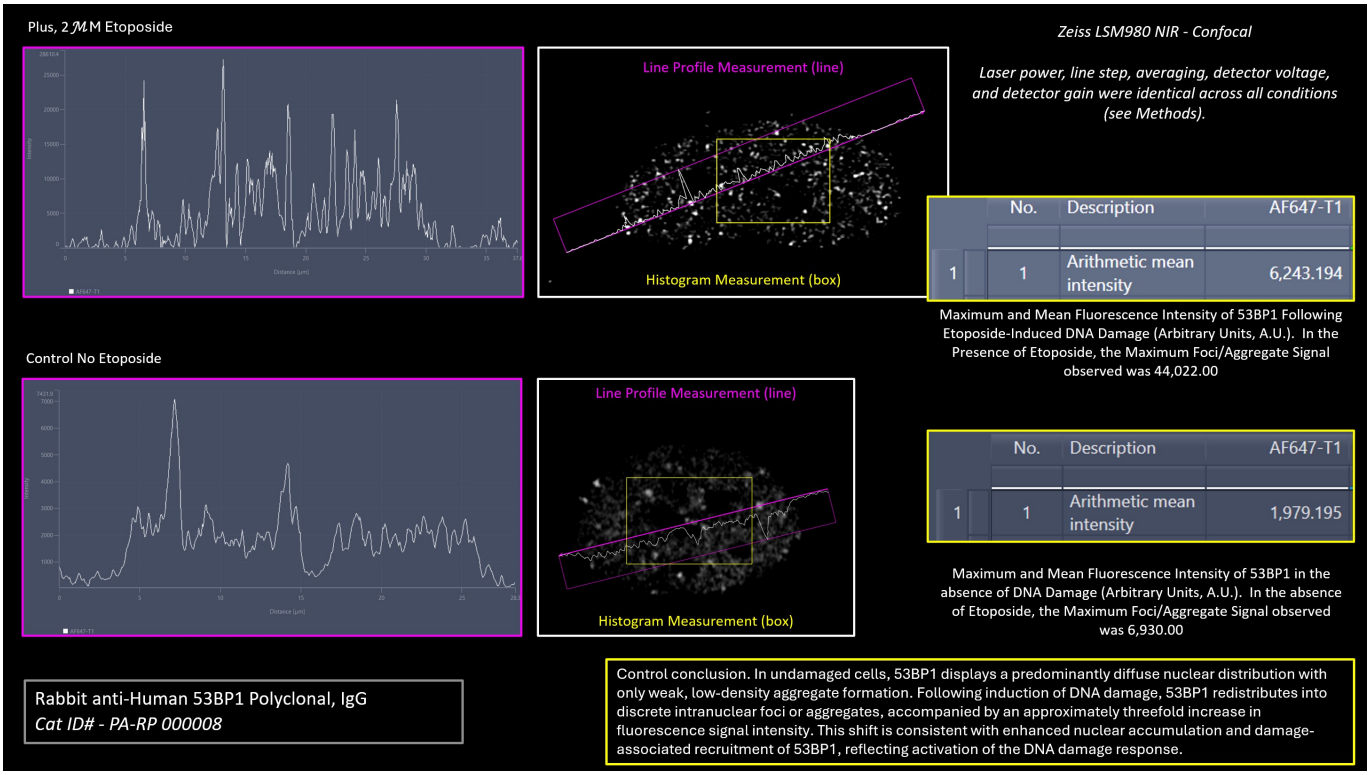
SOURCE PROVENANCE

Catalog	PA-RP-000008
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	80
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	36AA236F8E18680F836DB7872CE82FC4A93883312BF66F652266AC34C35AC8FC
Method PDF SHA-256	18D6E203553E097AEEFE6F6EFB50DABD90AE3CB7A16CF2E336B9A457B28EC9AF

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	54

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
Channels	1 (647)
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm | 0.059 µm X 0.059 µm
Image Size (Pixels)	1017 X 1017 | 1737 X 1737
Image Size (Scaled)	59.81 µm X 59.81 µm | 102.19 µm X 102.19 µm
Bit Depth	16 Bit
Image Center Position	X: 86.79 mm, Y: 50.23 mm | X: 82.25 mm, Y: 48.06 mm
Stage Position	X: 86.79 mm, Y: 50.23 mm | X: 82.25 mm, Y: 48.06 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 65µm | 1.00AU / 66µm
LMS MBS	MBS 488/639 & 405/730 | MBS 488/561 & 405/730
LMS SBS	SBS SP 615 | Mirror
Laser Wavelength	639nm @0.10%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2 | 1.3
Rotation	0°
Sampling	1.20
Pixel Time	0.33µs | 0.22µs
Frame Time	3.36s | 6.49s
LSM Scan Speed	12 | 11
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	640 - 732 | 640 - 668
Imaging Device	GaAsP-Pmt3
Detector Type	GaAsP-PMT
Detector Gain	500 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.9 (3D, Auto) | Filter: 6.6 (3D, Auto)
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 37.6), Y (Min 0 and Max 28610) | X and Y axes were set to Auto - X (Min 0.0 and 28.3 Max), Y (Min 36 and Max 7432)
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 and Max 20000) | X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000)

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 53BP1 Polyclonal, IgG
Cat ID# - PA-RP 000008

Identifyn® Secondary Antibodies

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

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

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

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

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

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

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

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

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

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

Method

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

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962. (Not Shown, but in sample)

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:

Single Plane (2D) - Z-Plane 30 of 64 - Plus DNA Damage, Top

Channels = 1 (647)
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1017 X 1017
Image Size (Scaled) = 59.81 µm X 59.81 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.79 mm, Y: 50.23 mm
Stage Position = X: 86.79 mm, Y: 50.23 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Single Plane - Minus DNA Damage, Bottom

Channels = 1 (647)
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image Size (Pixels) = 1737 X 1737
Image Size (Scaled) = 102.19 µm X 102.19 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.25 mm, Y: 48.06 mm
Stage Position = X: 82.25 mm, Y: 48.06 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Etoposide-Induced DNA Damage, Top

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
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)

647 - NO Induced DNA Damage, Bottom

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 66µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror
Laser Wavelength = 639nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.22µs
Frame Time = 6.49s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 668
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.6 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

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 37.6), Y (Min 0 and Max 28610)

This panel measures 53BP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~44K A.U. at its maximum.

Measured at Z-plane 71 of 141.

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 28.3 Max), Y (Min 36 and Max 7432)

This panel measures 53BP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~6K 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 and Max 20000)

The histogram was generated from the yellow-boxed region shown to quantify the overall 53BP1 protein response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed signal increase 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 and Max 5000)

The histogram was generated from the yellow-boxed region shown to quantify the overall 53BP1 protein within a defined nuclear area in the absence of DNA-damage induction. This measurement provides additional support for the observed signal increase relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, 53BP1 displays a predominantly diffuse nuclear distribution with only weak, low-density aggregate formation. Following induction of DNA damage, 53BP1 redistributes into discrete intranuclear foci or aggregates, accompanied by an approximately threefold increase in fluorescence signal intensity. This shift is consistent with enhanced nuclear accumulation and damage-associated recruitment of 53BP1, reflecting activation of the DNA damage response.

Image Corrections

None

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

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SOURCE PROVENANCE

Catalog	PA-RP-000008
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	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AAABC028612A075D726CA9CD98A69492C911D138AD8A0C759067437115D14921
Method PDF SHA-256	8D9B2EB04F2FD0BB116D5010733C50F261ED7283F2EEB9F41E807886E19AE177