BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

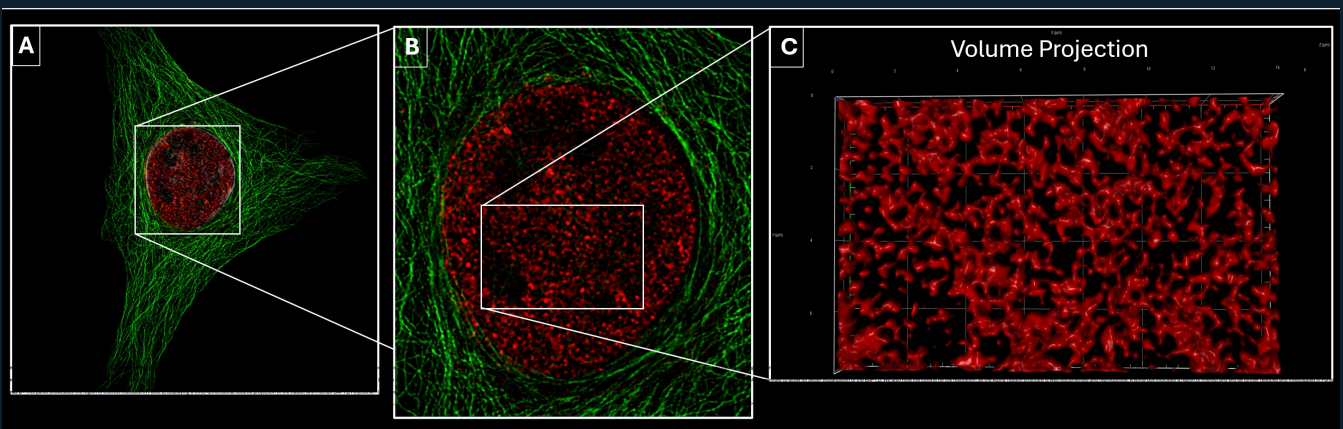
BAP1.P

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BAP1.p (Serine 592) Polyclonal - Identifyn® SRM Alexa Fluor™ 594

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN SIM -> SIM²

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A - This panel displays a 2-dimensional view at z-plane 57 of 113, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red). Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green) and DAPI nuclear staining (blue, pseudo colored to dark grey for visual contrast) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 57 of 113 (same z-plane as previous panel), from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red) and the Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as Alpha Tubulin structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. And using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage. Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000050 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for BAP1.p, 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

BENNETT ASSESSMENT

The preserved DC-000050 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 / NIR-BOUNDED PARTIAL STEPDOWN

The preserved DC-000050 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

The record preserves 7 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

No separate control record is preserved; none is inferred from method or template language.

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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 45 Texas Red; 38 eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561 nm @0.2%; 488 nm @0.06%; 405 nm @0.2%; 590; 493; 353
Airyscan	405/DAPI; 488; 594; 750	3; None; 561 nm @3.50%; 488 nm @0.1%; 405 nm @0.2%; 590; 493; 353
SIM	405/DAPI; 488; 594	3; 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 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561
SIM²	405/DAPI; 488; 594	3; 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 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561

MODALITY ASSESSMENT / PART 1 OF 7

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 594.

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 7

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): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 100 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 38.

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; 594.

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 7

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: 78 Slices (7.7 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 985 X 805; Image Size (Pixels): 985 X 805; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 100 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @8.00%; X-Cite Xylis Lamp Intensity @12.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 42.

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; 594.

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 7

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: 221 Slices (6.6 µm); Channels: 3; Scaling (Per Pixel): 0.054 µm X 0.054 µm X 0.030 µm; Image size (Pixels): 1101 X 1101; Image Size (Pixels): 1101 X 1101; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.31 µs; Frame Time: 3.63 s. Illumination: Laser Wavelength: 561 nm @0.2%; 488 nm @0.06%. Optical/scan: Pinhole: 1.00 AU / 59 µm; 1.00 AU / 49 µm; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; 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: 7.9 (3D, Auto); 7.1 (3D, Auto). Structured source metadata values: 69.

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; 594.

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 7

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: 261 Slices (7.8 µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1248 X 1248; 523 X 588; Image Size (Pixels): 1248 X 1248; 523 X 588; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.82 µs; Frame Time: 6.25 s. Illumination: Laser Wavelength: 561 nm @3.50%; 488 nm @0.1%. Optical/scan: Pinhole: 5.00 AU / 286 µm; 5.00 AU / 250 µm; Scan Zoom: 3.0; LSM Scan Speed: 7; 6; Scan Direction: Bidirectional; Averaging: 4; 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: 8.3 (3D, Auto); 7.4 (3D, Auto). Structured source metadata values: 70.

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; 594; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 7

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: 113 Slices (3.36 µm); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 2534 X 2200; Image Size (Pixels): 5056 X 5056; 2534 X 2200; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @5.00%; 488 nm @1.50%; Illumination Wavelength: 561; 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); Background: Black. Structured source metadata values: 68.

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; 594.

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 7

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: 113 Slices (3.36 µm); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1766 X 1855; Image Size (Pixels): 5056 X 5056; 1766 X 1855; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @5.00%; 488 nm @1.50%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 30°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 69.

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; 594.

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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000050 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 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)=1248 X 1248; Image Size (Scaled)=44.05 µm X 44.05 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 µm X 0.01689 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 5056; Image Size (Scaled)=85.40 µm X 85.40 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 µm X 0.01689 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 5056; Image Size (Scaled)=85.40 µm X 85.40 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 2µM; 60 nm -> 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.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 592. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human BAP1.p (Serine 592) Polyclonal - Identifyn® SRM Alexa Fluor™ 594

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. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

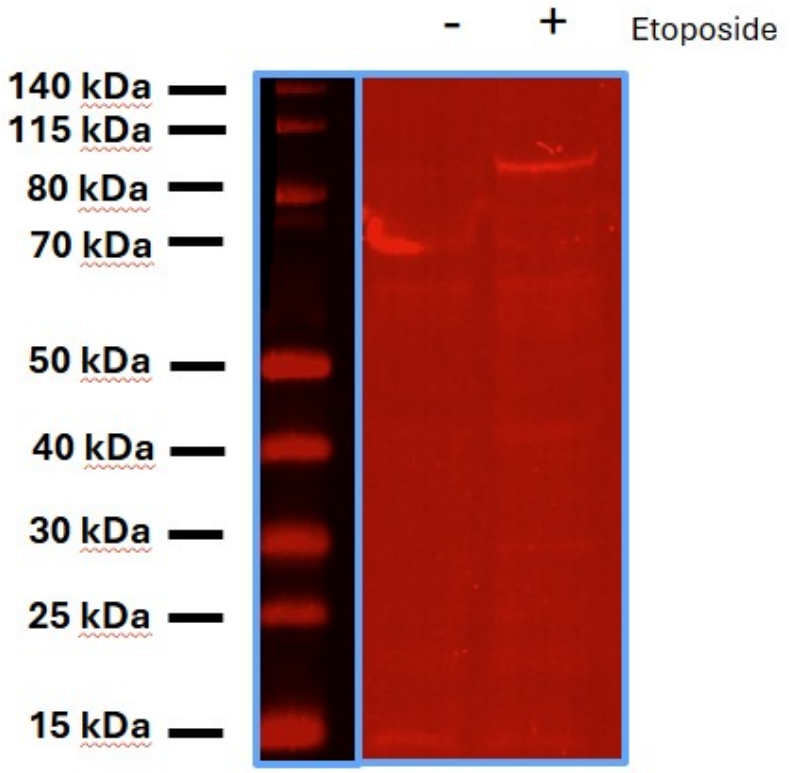
This dossier preserves the complete available evidence progression for DC-000050. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

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	594
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	532nm
Emission Filter	624±40nm
Detector	PMT
Intensity	2
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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Expected Molecular Weight: 100 kDa

HeLa cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down 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™ 4 to 12%, Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, 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 and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG for 2 hours, followed by 5X PBS washes. 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 = 532nm
Emission Filter = 624±40nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000050
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
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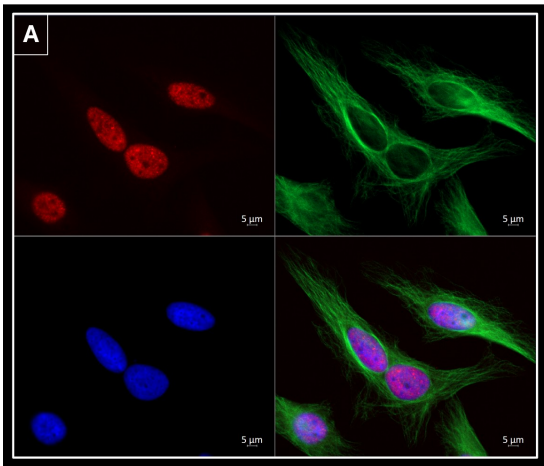
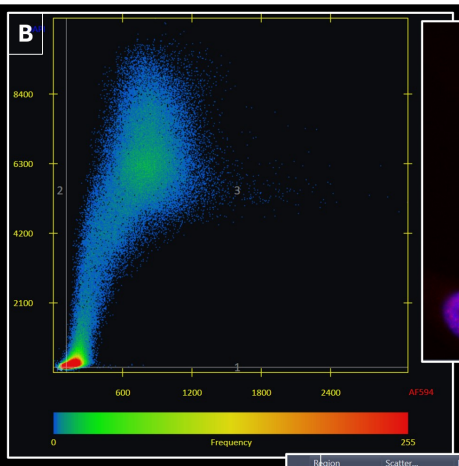
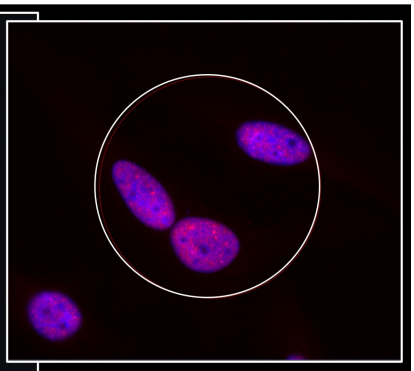
ORIGINAL IMAGE EVIDENCE / WIDEFIELD







C

	Region	Scatter...	Pixel Count	Area(µm²)	Relative...	Pearson	Manders
2	Entire Image	DAPI	214528	2,573.363	17.162	--	--
3	Entire Image	Colocalization	436190	5,232.302	34.894	0.93525	0.92325
4	Entire Image	Background	396814	4,759.968	31.744	--	--
5	Entire Image	Global	1250048	14,994.907	100.000	0.93794	0.88374
6	2 Circle (Diameter)	AF594	28645	343.610	8.092	--	--
7	2 Circle (Diameter)	DAPI	61513	737.877	17.376	--	--
8	2 Circle (Diameter)	Colocalization	231110	2,772.272	65.283	0.92664	0.94039

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate. The upper left section features Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 (red), The upper right displays Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), the lower left section shows DAPI nuclear staining (blue), while the lower right presents a merged image of the three channels.

Panel B – Displays a colocalization view of the DAPI (Y-axis) and Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 (X-axis), showing that BAP1.p is strongly colocalized with DAPI and is thereby, via this 2-dimensional image, within the nuclear compartment as expected for this protein following Etoposide induced DNA damage.

Panel C - Additionally, the Manders' Overlap Coefficient (MOC) metric was employed to analyze the image and quantify the degree of colocalization between the two channels. MOC measures the proportion of signal intensity in one channel that overlaps with the signal in the other channel. The area measured is indicated in the image by a white circular border.

MOC values range from 0 to 1, where a value of 0 indicates no overlap and a value of 1 signifies complete overlap of the signals.
MOC = 0.92664

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	38

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)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	45 Texas Red | 38 eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	100 ms | 25 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2
Manders' Overlap Coefficient	0.94039
MOC	0.92664

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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

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) = 1216 X 1028
Image Size (Scaled) = 133.18 µm X 112.59 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.96 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 @10.00%
Exposure Time = 100 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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG, the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Colocalization View - Panel B & C

Horizontal Axis - AF594, Threshold 118, Range Auto
Vertical Axis - DAPI, Threshold 167, Range Auto
Pixel Count - 28645 (AF594) and 61513 (DAPI)
Manders' Overlap Coefficient = 0.94039

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™SRM Alexa Fluor™ 594 Direct Conjugate. The upper left section features Rabbit anti-Human BAP1.p Polyclonal -Identifyn®

SRM Alexa Fluor™ 594 (red). The upper right displays Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), the lower left section shows DAPI nuclear staining (blue). In contrast, the lower right presents a merged image of the three channels.

Panel B - Displays a colocalization view of the DAPI (Y-axis) and Rabbit anti-Human BAP1.p Polyclonal -Identifyn® SRM Alexa Fluor™ 594 (X-axis), showing that BAP1.p is strongly colocalized with DAPI and is thereby, via this 2-dimensional image, within the nuclear compartment as expected for this protein following Etoposide-induced DNA damage.

Panel C - Additionally, the Manders' Overlap Coefficient (MOC) metric was employed to analyze the image and quantify the degree of colocalization between the two channels. MOC measures the proportion of signal intensity in one channel that overlaps with the signal in the other channel. The area measured is indicated in the image by a white circular border.

MOC values range from 0 to 1, where a value of 0 indicates no overlap and a value of 1 signifies complete overlap of the signals.
MOC = 0.92664

Image Correction

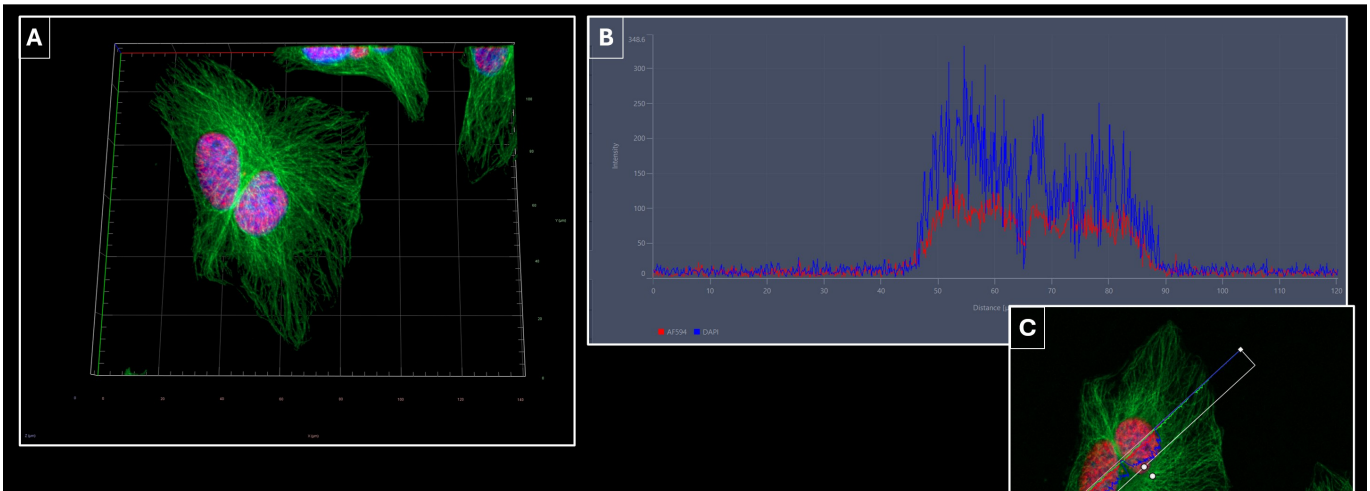
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1323708771B5EAEA530D0EE49E40D760EFA98D39C66F40BD1976377AAEE41D64
Method PDF SHA-256	35C6A74B5746EFBAEBC7D069805AE622847C3374B640C8014FC9F3143953BD2A

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A - This panel presents a 3-dimensional Maximum Projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate 9red), Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (blue) at widefield SIM (Apotome) resolution. The z-stack comprised a total of 78 z-planes, and importantly, tone mapping is disabled as artificial color mapping is not needed.

Panel B - This panel features a line profile display that allows for the measurement of localization and fluorescent intensity. The X-axis represents distance, and the Y-axis represents intensity. Here, we show data regarding Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red), and DAPI (blue), note we omitted the alpha tubulin seen in the image in Panel C, to provide a clear view of the localization of BAP1.p and DAPI in the histogram. The data indicates that the signals for Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 and DAPI nuclear staining occupy the exact location along the X-axis. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 and DAPI are colocalized on Z-plane 41 of 78, meaning they occupy the same area in the image along the X-axis. The Y-axis shows that the Rabbit anti-Human BAP1.p 594 direct conjugate and the DAPI exhibit comparable fluorescent brightness. This indicates a robust signal for the BAP1.p/594 direct conjugate and proper localization following Etoposide-induced DNA damage.

Panel C - This panel displays Z-plane 41 from the z-stack, which contains a total of 78 z-planes for all three channels collected. It importantly shows the position of the line profile measured in Panel B.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	42

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	78 Slices (7.7 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	985 X 805
Image Size (Scaled)	140.71 µm X 115.00 µm
Bit Depth	14 Bit
Image Center Position	X: 47.66 mm, Y: 51.23mm
Stage Position	X: 47.66 mm, Y: 51.23mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	45 Texas Red | 38 HE eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @8.00% | X-Cite Xylis Lamp Intensity @12.00%
Exposure Time	100 ms | 150 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.20 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 120.3), Y (Min 0 and Max 349)

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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

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:

Single Plane (2D) - Z-plane 41 of 78 total - Panel A

Z Stack - (3D)

Z-Stack = 78 Slices (7.7 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 985 X 805
Image Size (Scaled) = 140.71 µm X 115.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 47.66 mm, Y: 51.23mm
Stage Position = X: 47.66 mm, Y: 51.23mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 100 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @12.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 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 @8.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

Profile View Display - Panel B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 120.3), Y (Min 0 and Max 349)

Summary

Panel A - This panel presents a 3-dimensional Maximum Projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRMAlexa Fluor™ 594 Direct Conjugate (red), Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue) at widefield SIM (Apotome) resolution. The z-stack comprised a total of 78 z-planes, and importantly, tone mapping is disabled as artificial color mapping is not needed.

Panel B - This panel features a line profile display that allows for the measurement of localization and fluorescent

intensity. The X-axis represents distance, and the Y-axis represents intensity. Here, we show data regarding Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red), and DAPI (blue), note we omitted the alpha tubulin seen in the image in Panel C, to provide a clear view of the localization of BAP1.p and DAPI in the histogram. The data indicate that the signals for Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 and DAPI nuclear staining occupy the exact location along the X-axis. This finding demonstrates that Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 and DAPI are colocalized on Z-plane 41 of 78, meaning they occupy the same area in the image along the X-axis. The Y-axis shows that the Rabbit anti-Human BAP1.p 594 direct conjugate and the DAPI exhibit comparable fluorescent brightness. This indicates a robust signal for the BAP1.p/594 direct conjugate and proper localization following Etoposide-induced DNA damage.

Panel C - This panel displays Z-plane 41 from the z-stack, which contains a total of 78 z-planes for all three channels collected. This is particularly important as it shows the position of the line profile measured in Panel B.

Image Correction

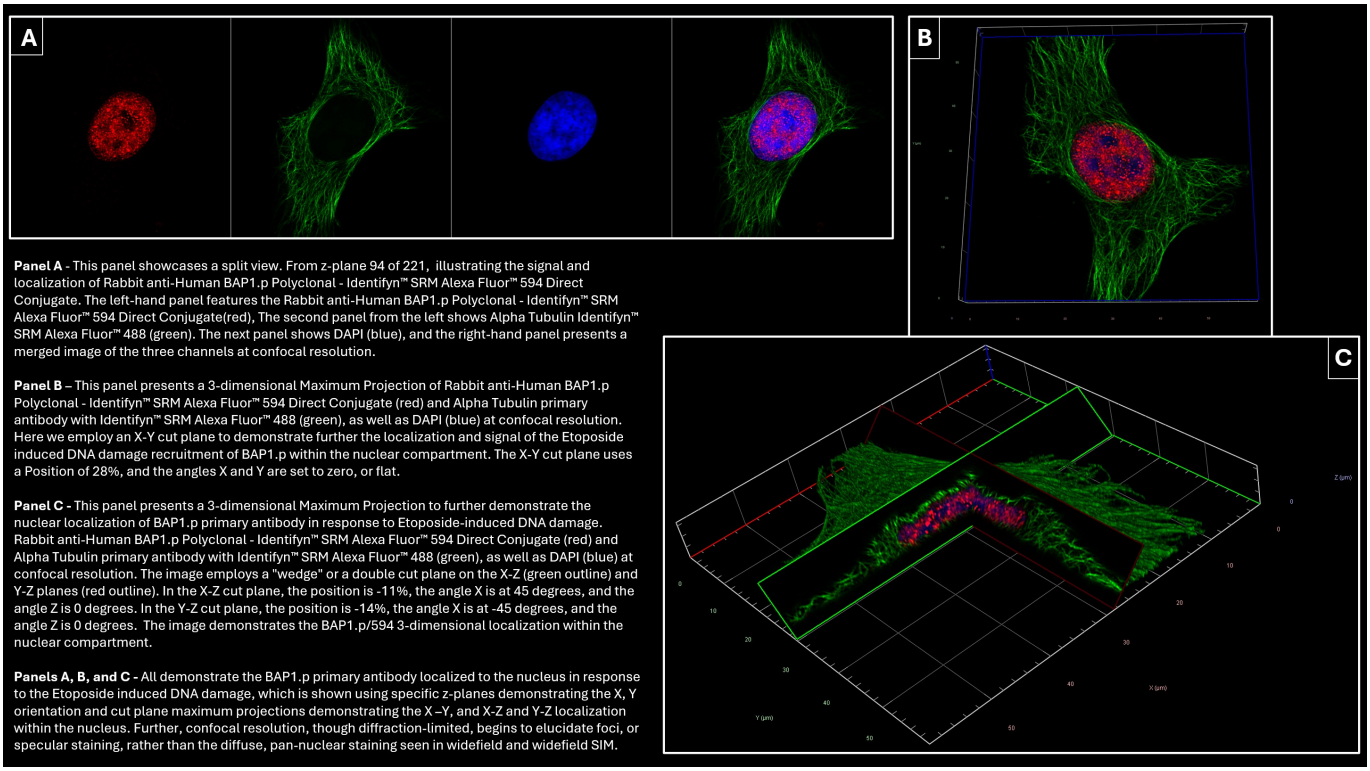
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	42
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	87F18349C5DEC3EA321CC3379FDBA60F06DD77AE882F31EFFE2E70183B62E862
Method PDF SHA-256	E80038A03524BFECD4160801516826B90F61DC8386BB6EA25F10843C29E8909F

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - This panel showcases a split view. From z-plane 94 of 221, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate. The left-hand panel features the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate(red), The second panel from the left shows Alpha Tubulin Identifyn™ SRM Alexa Fluor™ 488 (green). The next panel shows DAPI (blue), and the right-hand panel presents a merged image of the three channels at confocal resolution.

Panel B – This panel presents a 3-dimensional Maximum Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. Here we employ an X-Y cut plane to demonstrate further the localization and signal of the Etoposide induced DNA damage recruitment of BAP1.p within the nuclear compartment. The X-Y cut plane uses a Position of 28%, and the angles X and Y are set to zero, or flat.

Panel C - This panel presents a 3-dimensional Maximum Projection to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a "wedge" or a double cut plane on the X-Z (green outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is -11%, the angle X is at 45 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -14%, the angle X is at -45 degrees, and the angle Z is 0 degrees. The image demonstrates the BAP1.p/594 3-dimensional localization within the nuclear compartment.

Panels A, B, and C - All demonstrate the BAP1.p primary antibody localized to the nucleus in response to the Etoposide induced DNA damage, which is shown using specific z-planes demonstrating the X, Y orientation and cut plane maximum projections demonstrating the X –Y, and X-Z and Y-Z localization within the nucleus. Further, confocal resolution, though diffraction-limited, begins to elucidate foci, or specular staining, rather than the diffuse, pan-nuclear staining seen in widefield and widefield SIM.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	69

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	221 Slices (6.6 µm)
Channels	3
Scaling (Per Pixel)	0.054 µm X 0.054 µm X 0.030 µm
Image Size (Pixels)	1101 X 1101
Image Size (Scaled)	59.92 µm X 59.92 µm
Bit Depth	16 Bit
Image Center Position	X: 77.18 mm, Y: 46.31 mm
Stage Position	X: 77.18 mm, Y: 46.31 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 59 µm | 1.00 AU / 49 µm | 1.00 AU / 44 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @0.2% | 488 nm @0.06% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.30
Pixel Time	0.31 µs
Frame Time	3.63 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	590-650 | 484-549 | 435-480
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.9 (3D, Auto) | 7.1 (3D, Auto) | 6.6 (3D, Auto)
LMS MBS	MBS 488/561, 405/730
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
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	A clipping plane was introduced in Panel B to pronounce regions of special interest. | Clipping planes are introduced in Panel C to pronounce regions of special interest.
X-Y	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	-28% | -11% | -14%
Clip X	0° | 45°
Clip Y	0° | -45°
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 Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a dark red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Bennett Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

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 94 of 221 total - Panel A

Z Stack - (3D) - Panels A, B, & C

Z-Stack = 221 Slices (6.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.054 µm X 0.054 µm X 0.030 µm
Image Size (Pixels) = 1101 X 1101
Image Size (Scaled) = 59.92 µm X 59.92 µm
Bit Depth = 16 Bit
Image Center Position = X: 77.18 mm, Y: 46.31 mm
Stage Position = X: 77.18 mm, Y: 46.31 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590-650
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.9 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 49 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 484-549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.1 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LMS MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435-480
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.6 (3D, Auto)

Split View - Panel A

The first panel on the left features Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG, the second panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the third panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 94 of 221.

3D Image Processing - Panels B & C

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
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 = A clipping plane was introduced in Panel B to pronounce regions of special interest.

X-Y = 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 = -28%
Clip X = 0°
Clip Y = 0°

Clipping Image Process = Clipping planes are introduced in Panel C to pronounce regions of special interest.

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 = -11%
Clip X = 45°
Clip Z = 0°

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

Position of Clip = -14%
Clip Y = -45°
Clip Z = 0°

Summary

Panel A - This panel showcases a split view. From z-plane 94 of 221, illustrating the signal and localization of Rabbit anti-HumanBAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate. The left-hand panel features the Rabbit anti-Human BAP1.pPolyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate(red., The second panel from the left shows Alpha Tubulin Identifyn® SRM Alexa Fluor™ 488 (green). The next panel shows DAPI (blue), and the right-hand panel presents a merged image of the three channels at confocal resolution.

Panel B - This panel presents a 3-dimensional Maximum Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution Here we employ an X-Y cut plane to demonstrate further the localization and signal of the Etoposide induced DNA damage recruitment of BAP1.p within the nuclear compartment. The X-Y cut plane uses a Position of 28%, and the angles X and Y are set to zero, or flat.

Panel C - This panel presents a 3-dimensional Maximum Projection to further demonstrate the nuclear localization of BAP1.p primary antibody in response to Etoposide-induced DNA damage. Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), as well as DAPI (blue) at confocal resolution. The image employs a "wedge" or a double cut plane on the X-Z (green outline) and Y-Z planes (red outline). In the X-Z cut plane, the position is -11%, the angle X is at 45 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the position is -14%, the angle X is at -45 degrees, and the angle Z is 0 degrees. The image demonstrates the BAP1.p/594 3-dimensional localization within the nuclear compartment.

Panels A, B, and C - All demonstrate the BAP1.p primary antibody localized to the nucleus in response to the Etoposide-induced DNA damage, which is shown using specific z-planes demonstrating the X, Y orientation and cut plane maximum projections demonstrating the X-Y, and X-Z and Y-Z localization within the nucleus. Further, confocal resolution, though diffraction-limited, begins to elucidate foci, or specular staining, rather than the diffuse, pan-nuclear staining seen in widefield and widefield SIM.

Image Corrections

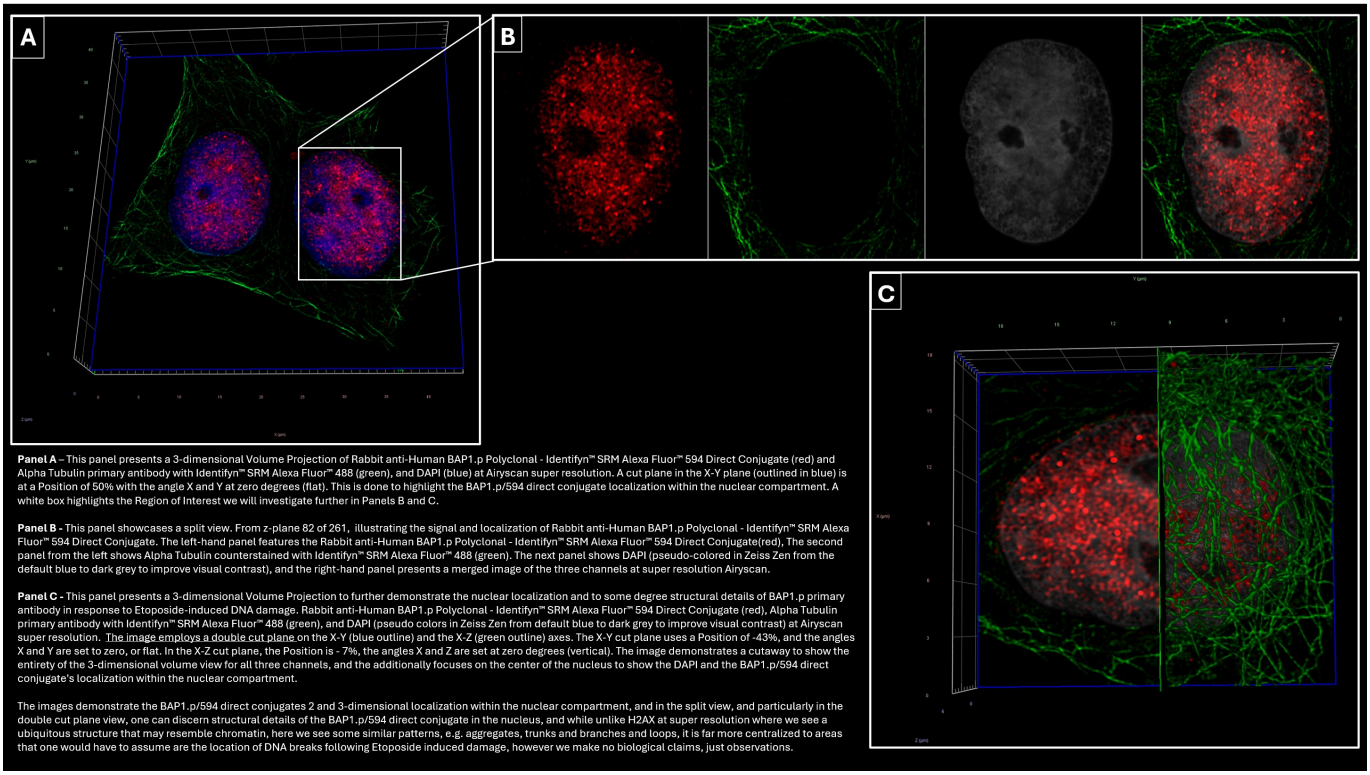
None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1A575AFB557FE7A54F82C83B37149B36D7F8CE2326BBB351E85D1F5EA6340B78
Method PDF SHA-256	DBAF422CFD0A63F04C0A15B0E63B86C7F593FB09058083AD9D49BBF66E638229

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A – This panel presents a 3-dimensional Volume Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (blue) at Airyscan super resolution. A cut plane in the X-Y plane (outlined in blue) is at a Position of 50% with the angle X and Y at zero degrees (flat). This is done to highlight the BAP1.p/594 direct conjugate localization within the nuclear compartment. A white box highlights the Region of Interest we will investigate further in Panels B and C.

Panel B - This panel showcases a split view. From z-plane 82 of 261, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate. The left-hand panel features the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate(red), The second panel from the left shows Alpha Tubulin counterstained with Identifyn™ SRM Alexa Fluor™ 488 (green). The next panel shows DAPI (pseudo-colored in Zeiss Zen from the default blue to dark grey to improve visual contrast), and the right-hand panel presents a merged image of the three channels at super resolution Airyscan.

Panel C - This panel presents a 3-dimensional Volume Projection to further demonstrate the nuclear localization and to some degree structural details of BAP1.p primary antibody in response to Etoposide-induced DNA damage. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red), Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (pseudo colors in Zeiss Zen from default blue to dark grey to improve visual contrast) at Airyscan super resolution. The image employs a double cut plane on the X-Y (blue outline) and the X-Z (green outline) axes. The X-Y cut plane uses a Position of -43%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is - 7%, the angles X and Z are set at zero degrees (vertical). The image demonstrates a cutaway to show the entirety of the 3-dimensional volume view for all three channels, and the additionally focuses on the center of the nucleus to show the DAPI and the BAP1.p/594 direct conjugate's localization within the nuclear compartment.

The images demonstrate the BAP1.p/594 direct conjugates 2 and 3-dimensional localization within the nuclear compartment, and in the split view, and particularly in the double cut plane view, one can discern structural details of the BAP1.p/594 direct conjugate in the nucleus, and while unlike H2AX at super resolution where we see a ubiquitous structure that may resemble chromatin, here we see some similar patterns, e.g. aggregates, trunks and branches and loops, it is far more centralized to areas that one would have to assume are the location of DNA breaks following Etoposide induced damage, however we make no biological claims, just observations.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 750
METADATA VALUES	70

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	261 Slices (7.8 µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1248 X 1248 | 523 X 588
Image Size (Scaled)	44.05 µm X 44.05 µm | 18.46 µm X 20.75 µm
Bit Depth	16 Bit
Image Center Position	X: 74.43 mm, Y: 50.02 mm
Stage Position	X: 74.43 mm, Y: 50.02 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 286 µm | 5.00 AU / 250 µm | 5.00 AU / 219 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 525 | SBS SP 550 | SBS SP 505
Laser Wavelength	561 nm @3.50% | 488 nm @0.1% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.00
Pixel Time	0.82 µs
Frame Time	6.25 s
LSM Scan Speed	7 | 6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	573-627 | 499-548 | 420-480, 495-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	8.3 (3D, Auto) | 7.4 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 3% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | 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	Clipping planes are introduced to pronounce regions of special interest.
X-Y	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	-50% | -43% | -7%
Clip X	0°
Clip Y	0°
3D Volume Projection	Precise
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 Z	0°

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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 261 Slices (7.8 μm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1248 X 1248
Image Size (Scaled) = 44.05 μm X 44.05 μm
Bit Depth = 16 Bit
Image Center Position = X: 74.43 mm, Y: 50.02 mm
Stage Position = X: 74.43 mm, Y: 50.02 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Single Plane (2D) - Z-plane 82 of 261 total - Panel B

Z Stack - (3D) - Panels B & C

Z-Stack = 261 Slices (7.8 μm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 523 X 588
Image Size (Scaled) = 18.46 μm X 20.75 μm
Bit Depth = 16 Bit
Image Center Position = X: 74.43 mm, Y: 50.02 mm
Stage Position = X: 74.43 mm, Y: 50.02 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 286 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @3.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 µs
Frame Time = 6.25 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573-627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.3 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS SP 550
Laser Wavelength = 488 nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 µs
Frame Time = 6.25 s
LSM Scan Speed = 7
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
LSM Plus Mode = 8.3 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 219 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 µs
Frame Time = 6.25 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420-480, 495-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 3% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
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 = Clipping planes are introduced to pronounce regions of special interest.

X-Y = 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 = -50%
Clip X = 0°
Clip Y = 0°

Split View - Panel B

The first panel on the left features Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG, the second panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the third panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 82 of 261.

3D Image Processing - Panel C

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 = Clipping planes are introduced to pronounce regions of special interest.

X-Y = 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 = -43%
Clip X = 0°
Clip Y = 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 = -7%
Clip X = 0°
Clip Z = 0°

Summary

Panel A - This panel presents a 3-dimensional Volume Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue) at Airyscan super resolution. A cut plane in the X-Y plane (outlined in blue) is at a Position of 50% with the angles X and Y at zero degrees (flat). This is done to highlight the BAP1.p/594 direct conjugate localization within the nuclear compartment. A white box highlights the Region of Interest we will investigate further in Panels B and C.

Panel B - This panel showcases a split view from z-plane 82 of 261, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate. The left-hand panel features the Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate(red), The second panel from the left shows Alpha Tubulin counterstained with Identifyn® SRM Alexa Fluor™ 488 (green). The next panel shows DAPI (pseudo-colored in Zeiss Zen from the default blue to dark grey to improve visual contrast), and the right-hand panel presents a merged image of the three channels at super resolution Airyscan.

Panel C - This panel presents a 3-dimensional Volume Projection to further demonstrate the nuclear localization and to some degree structural details of BAP1.p primary antibody in response to Etoposide-induced DNA damage, Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 DirectConjugate (red), Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (pseudo colors in Zeiss Zen from default blue to dark grey to improve visual contrast) at Airyscan super resolution. The image employs a double cut plane on the X-Y (blue outline) and the X-Z (green outline) axes. The X-Y cut plane uses a Position of -43%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is -7%, the angles X and Z are set at zero degrees (vertical). The image demonstrates a cutaway to show the entirety of the 3-dimensional volume view for all three channels, and it additionally focuses on the center of the nucleus to show the DAPI and the BAP1.p/594 direct conjugate's localization within the nuclear compartment.

The images demonstrate the BAP1.p/594 direct conjugates 2 and 3-dimensional localization within the nuclear compartment, and in the split view, and particularly in the double cut plane view, one can discern structural details of the BAP1.p/594 direct conjugate in the nucleus, and while unlike H2AX at super resolution where we see a ubiquitous structure that may resemble chromatin, here we see some similar patterns, e.g. aggregates, trunks and branches and loops, it is far more centralized to areas that one would have to assume are the location of DNA breaks following Etoposide induced damage, however we make no biological claims, just observations.

Image Corrections

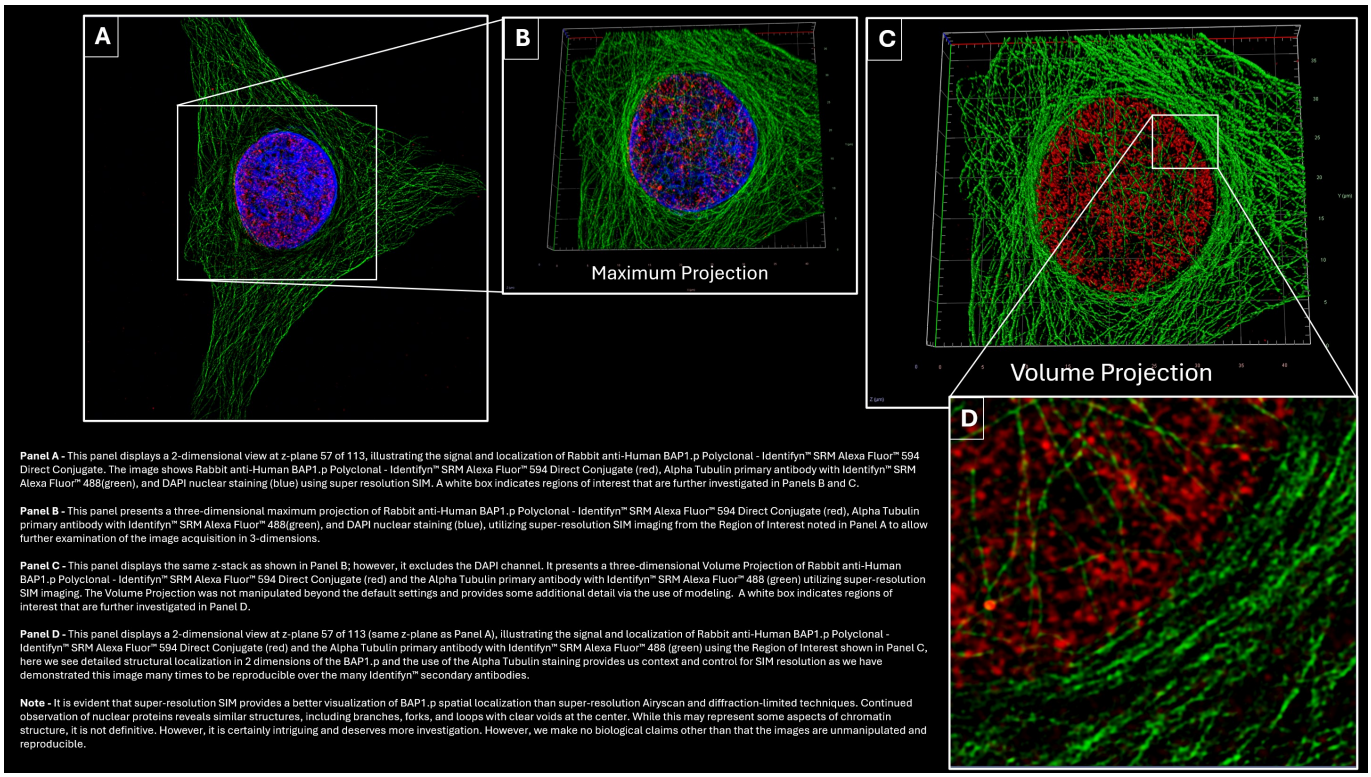
DAPI was pseudo-colored in Zen Software from blue, the default color, to dark grey to provide visual contrast.

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	46CB32367E4BA63A337FF591EB6CC3CEBA7321F698150C8B3BED6C044FFB21DD
Method PDF SHA-256	AB9A425089FB7A3FBD9108DD35A168D3F80BBE732C189123C8F468504870AC88

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	68

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
Z-Stack	113 Slices (3.36 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 2534 X 2200 | 743 X 685
Image Size (Scaled)	85.40 µm X 85.40 µm | 42.80 µm X 37.16 µm | 12.55 µm X 11.57 µm
Bit Depth	16 Bit
Image Center Position	X: 59.13 mm, Y: 39.83 mm
Stage Position	X: 59.13 mm, Y: 39.83 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 488 | 405
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	597 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	150 ms | 120 ms | 50 ms
Depth of Focus	0.92 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @5.00% | 488 nm @1.50% | 405 nm @2.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating | 32 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.4649 (594), 10.5573 (488), 8.5560 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D 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 BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

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 113 total - Panel A

Z Stack - (3D)

Z-Stack = 113 Slices (3.36 μm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 85.40 μm X 85.40 μm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 39.83 mm
Stage Position = X: 59.13 mm, Y: 39.83 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - Panel B & C

Z-Stack = 113 Slices (3.36 μm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 2534 X 2200
Image Size (Scaled) = 42.80 μm X 37.16 μm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 39.83 mm
Stage Position = X: 59.13 mm, Y: 39.83 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Single Plane (2D) - Z-plane 57 of 113 total - Panel D

Z Stack - (3D)

Z-Stack = 113 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 743 X 685
Image Size (Scaled) = 12.55 µm X 11.57 µm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 39.83 mm
Stage Position = X: 59.13 mm, Y: 39.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @5.00%
Frame Time = 1.59 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 = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32 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 = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.4649 (594), 10.5573 (488), 8.5560 (DAPI)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
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 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)

Summary

Panel A - This panel displays a 2-dimensional view at z-plane 57 of 113, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate. The image shows Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red), Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue) using super resolution SIM. A white box indicates regions of interest that are further investigated in Panels B and C.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red), Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue), utilizing super-resolution SIM imaging from the Region of Interest noted in Panel A to allow further examination of the image acquisition in 3-dimensions.

Panel C - This panel displays the same z-stack as shown in Panel B; however, it excludes the DAPI channel. It presents a three-dimensional Volume Projection of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and the Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green) utilizing super-resolution SIM imaging. The Volume Projection was not manipulated beyond the default settings and provides some additional detail via the use of modeling. A white box indicates regions of interest that are further investigated in Panel D.

Panel D - This panel displays a 2-dimensional view at z-plane 57 of 113 (same z-plane as Panel A), illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and the Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™488 (green) using the Region of Interest shown in Panel C, here we see detailed structural localization in 2 dimensions of the BAP1.p and the use of the Alpha Tubulin staining provides us context and control for SIM resolution as we have demonstrated this image many times to be reproducible over the many Identifyn® secondary antibodies.

Note - It is evident that super-resolution SIM provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. Continued observation of nuclear proteins reveals similar structures, including branches, forks, and loops with clear voids at the center. While this may represent some aspects of chromatin structure, it is not definitive. However, it is certainly intriguing and deserves more investigation. However, we make no biological claims other than that the images are unmanipulated and reproducible.

Image Corrections

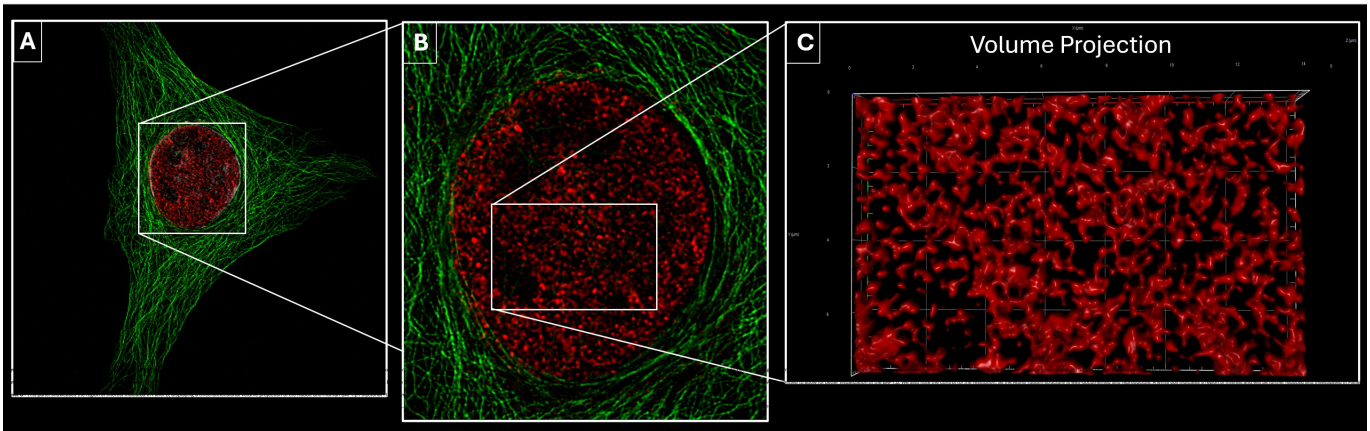
None

Image by P. Raut
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AB4B2256FCB87F2E679256A21C7F3B5B17AC7EF59330968E96D845CF8681395F
Method PDF SHA-256	68057B2A966DFD85A44F0D85B4DC446DDB663878D2128EA29E5D3B0B105E1A66

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - This panel displays a 2-dimensional view at z-plane 57 of 113, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red). Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green) and DAPI nuclear staining (blue, pseudo colored to dark grey for visual contrast) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 57 of 113 (same z-plane as previous panel), from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate (red) and the Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as Alpha Tubulin structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn™ SRM Alexa Fluor™ 594 Direct Conjugate.

Note - It is evident that super-resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super-resolution Airyscan and diffraction-limited techniques. And using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage. Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	69

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
Z-Stack	113 Slices (3.36 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1766 X 1855 | 844 X 445
Image Size (Scaled)	85.40 µm X 85.40 µm | 29.83 µm X 31.33 µm | 14.26 µm X 7.52 µm
Bit Depth	16 Bit
Image Center Position	X: 59.13 mm, Y: 59.13 mm | X: 59.13 mm, Y: 39.83 mm
Stage Position	X: 59.13 mm, Y: 59.13 mm | X: 59.13 mm, Y: 39.83 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 488 | 405
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	597 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	150 ms | 120 ms | 50 ms
Depth of Focus	0.92 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @5.00% | 488 nm @1.50% | 405 nm @2.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating | 32 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM²
SIM² Settings	Standard
Input SNR	Low
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 30°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Bennett Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG
Cat ID# - DC 000050

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

BAP1 (BRCA1-associated protein 1) is primarily recognized as a nuclear deubiquitinase (DUB) that plays a crucial role in regulating chromatin structure, transcription, DNA repair, and cell cycle progression. While extensively studied in the context of homologous recombination (HR), BAP1 is increasingly acknowledged for its important role in base excision repair (BER).

Phosphorylation of BAP1 (BAP1.p) occurs at several sites, each associated with distinct regulatory functions. The most well-characterized site is Serine 592 (S592), which is primarily phosphorylated by ATM or ATR, with ATR being the more likely kinase, especially in response to DNA damage such as single-strand breaks (SSBs), double-strand breaks (DSBs), and replication stress. Another phosphorylation site, Serine 718 (S718), is also targeted by ATM or ATR, although its functional role is less well-defined. It is believed to contribute to DNA damage response signaling and may help regulate chromatin remodeling activities. Threonine 599 (T599) has been identified as a potential ATM target based on limited phosphoproteomic data, although its exact function remains unclear. Lastly, Serine 2 (S2) is phosphorylated by casein kinase 2 (CK2) in contexts not related to DNA damage, appearing to play a role in stabilizing the BAP1 protein.

BAP1.p modulates its enzymatic activity, subcellular localization, and interaction networks, thereby influencing DNA repair processes. Although direct evidence linking phosphorylated BAP1 to base excision repair (BER) is limited compared to homologous recombination (HR) and double-strand break (DSB) repair, accumulating data suggest that it plays an indirect but functionally significant role. Phosphorylated BAP1 retains deubiquitinase activity, potentially with altered substrate specificity, and facilitates chromatin remodeling by deubiquitinating histone substrates such as H2Aub, H2AK119ub1, and H2AK15ub. This process reduces chromatin compaction and enhances accessibility for BER machinery, including OGG1, APE1, and POLβ. In this context, BAP1.p may help stabilize open chromatin near DNA lesions, promoting the recruitment of BER enzymes and ensuring the efficient excision and repair of oxidative or alkylated bases.

Furthermore, BAP1 phosphorylation may indirectly regulate PARP1 activity through interactions with PARP1 regulators, thus helping to balance PARP1 activation and prevent hyperactivation during the initiation of base excision repair (BER). Additionally, BAP1 phosphorylation is often mediated by upstream DNA damage response kinases, such as ATM and ATR, following DNA damage, including single-strand breaks (SSBs). This positions BAP1.p as a downstream effector that integrates base excision repair (BER) with broader double-strand DNA damage response (DDR) signaling and chromatin state regulation.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594, IgG was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

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 113 total - Panel A

Z Stack - (3D)

Z-Stack = 113 Slices (3.36 μm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 85.40 μm X 85.40 μm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 59.13 mm
Stage Position = X: 59.13 mm, Y: 59.13 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Single Plane (2D) - Z-plane 57 of 113 total - Panel B

Z Stack - (3D)

Z-Stack = 113 Slices (3.36 μm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 1766 X 1855
Image Size (Scaled) = 29.83 μm X 31.33 μm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 39.83 mm
Stage Position = X: 59.13 mm, Y: 39.83 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - Panel C

Z-Stack = 113 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 844 X 445
Image Size (Scaled) = 14.26 µm X 7.52 µm
Bit Depth = 16 Bit
Image Center Position = X: 59.13 mm, Y: 39.83 mm
Stage Position = X: 59.13 mm, Y: 39.83 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @5.00%
Frame Time = 1.59 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 = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32 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 - SIM² Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

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 30°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a 2-dimensional view at z-plane 57 of 113, illustrating the signal and localization of Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red . Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green) and DAPI nuclear staining (blue, pseudo colored to dark grey for visual contrast) employing super resolution SIM Squared. A white box indicates a region of interest and will further reduce the area investigated in Panel B.

Panel B - This panel presents a 2-dimensional view at z-plane 57 of 113 (same z-plane as previous panel), from the Region of Interest shown in Panel A. Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate (red) and the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). DAPI is omitted to allow for better visualization of the BAP1.p. We also note the control for the resolution, as the Alpha Tubulin structure is well-documented and reproducible. A white box indicates a region of interest and will be further reduced and investigated in Panel C.

Panel C - This panel showcases the region of interest indicated in Panel B, displaying a three-dimensional volume projection of the Rabbit anti-Human BAP1.p Polyclonal - Identifyn® SRM Alexa Fluor™ 594 Direct Conjugate.

Note - It is evident that super resolution SIM, in this case, SIM Squared, provides a better visualization of BAP1.p spatial localization than super resolution Airyscan and diffraction-limited techniques. And using the same sample as was imaged for SIM, we show here a gain in resolving the three-dimensional structural localization details of BAP1.p localization following etoposide-induced DNA damage Again, we make no claims as to what this image represents, as we are at ~40-60 nm in X, Y, but it is not hard to imagine that this is DNA damage-induced localization at or near the DNA break sites.

Image Corrections

DAPI was pseudo-colored in Zen Software from blue, the default color, to dark grey to provide visual contrast.

Image by P. Raut
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000050
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	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6BB0136D59C0B836E6FF1B1CCD6642AA4E38A5C175CF9A4BAF5EDFFC43B81A8A
Method PDF SHA-256	BE85EFC6A343B9EF460DC8DAA01C54CF893CCA3C881B1BCE9CCB0650C512CF18