BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

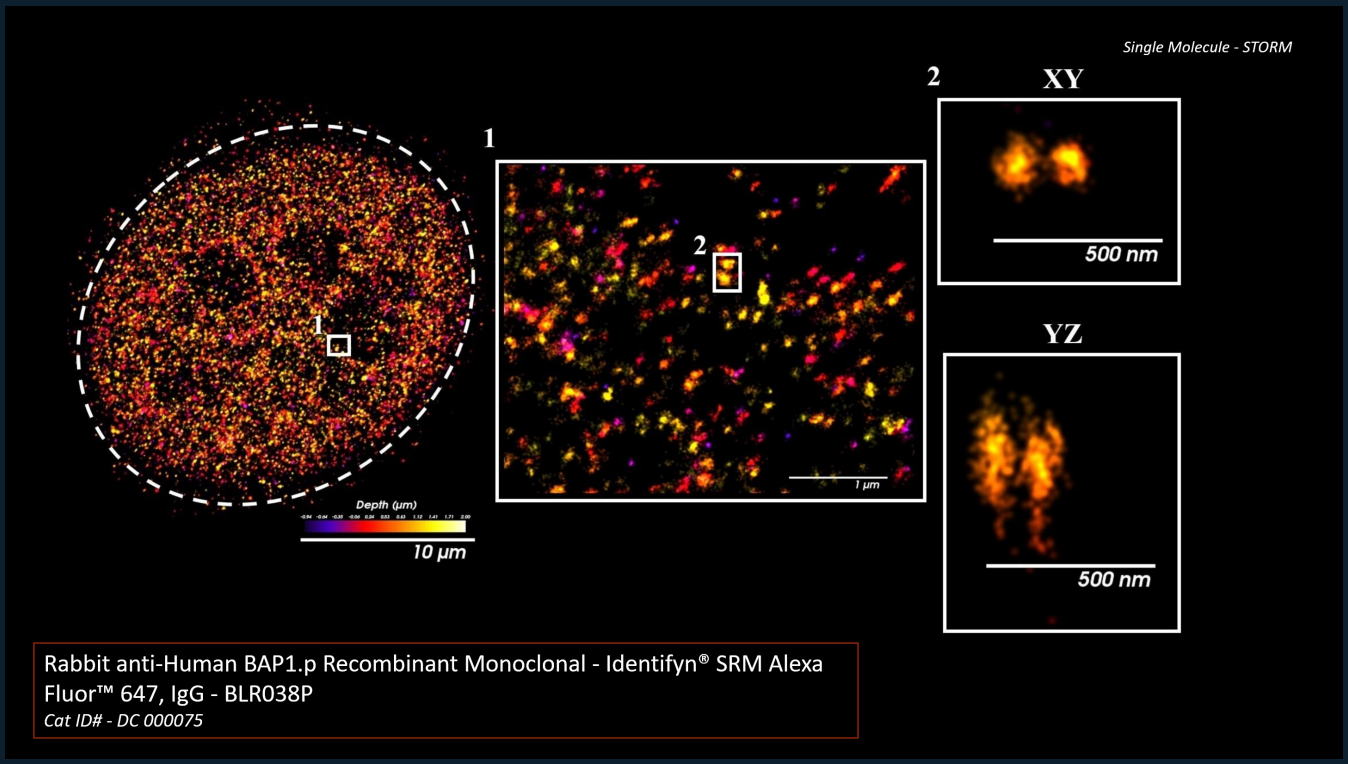
BAP1.P

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN -> SINGLE-MOLECULE STORM

5	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human BAP1.p Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - BLR038P
Cat ID# - DC 000075

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000075 | 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
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Single-molecule STORM presentation 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 -> Confocal -> Airyscan -> Single-molecule STORM

BENNETT ASSESSMENT

The preserved DC-000075 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. Missing modalities are not represented or inferred.

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 / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000075 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 5 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	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 647	2; 50 Cy 5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Confocal	405/DAPI; 488; 647	2; None; 639 nm @ 1.80%; 405 nm @ 0.3%; 653; 353; 668; 465
Airyscan	405/DAPI; 488; 647	2; None; 639 nm @ 2.80%; 405 nm @ 0.05%; 653; 353; 668; 465
Single-molecule STORM	647	Both Channels

MODALITY ASSESSMENT / PART 1 OF 5

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 5

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: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 668 X 569; Image Size (Pixels): 668 X 569; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250 ms; 30 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 25.00%; X-Cite Xylis Lamp Intensity @ 15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 5

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM980 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: 151 Slices (4.50 µm) - Panel A, Z-Plane 53 of 151; Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1121 X 1121; Image Size (Pixels): 1121 X 1121; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.30 µs; Frame Time: 3.69 s. Illumination: Laser Wavelength: 639 nm @ 1.80%; 405 nm @ 0.3%. Optical/scan: Pinhole: 1.00 AU / 66 µm; 1.00 AU / 43 µm; Scan Zoom: 2.0; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.3 (3D, Auto); Filter: 6.5 (3D, Auto). Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 5

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: 91 Slices (2.7 µm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 903 X 687; 142 X 134; Image Size (Pixels): 903 X 687; 142 X 134; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 10.99 µs; Frame Time: 20.84 s. Illumination: Laser Wavelength: 639 nm @ 2.80%; 405 nm @ 0.05%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 215 µm; Scan Zoom: 1.8; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution: 10.0 (3D, Auto); Super Resolution: 9.8 (3D, Auto). Structured source metadata values: 51.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 5

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60x/1.30 Silicon Oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 81.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000075 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. Missing modalities are not represented or inferred.

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)=903 X 687; Image Size (Scaled)=31.87 µm X 24.25 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

UNRESOLVED - PRESERVED AS CONFLICT

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

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) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647

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

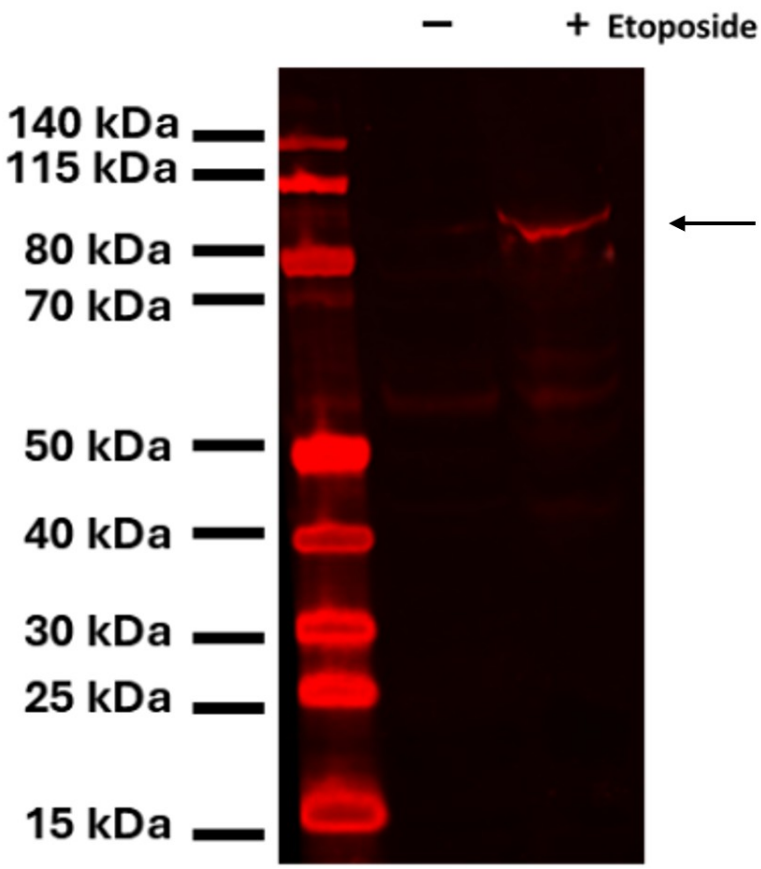
This dossier preserves the complete available evidence progression for DC-000075. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



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

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

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

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P)
Cat ID# - DC 000075

Expected MW: ~95 kDa

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis on NuPAGE™ 4-12% Bis-Tris, 1.0-1.5 mm Mini Protein Gels in a Mini Gel Tank. Thermo Fisher™ 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, then washed 5 times with 1X PBS. The blocked membrane was incubated with Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), for 20 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

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

Post Processing

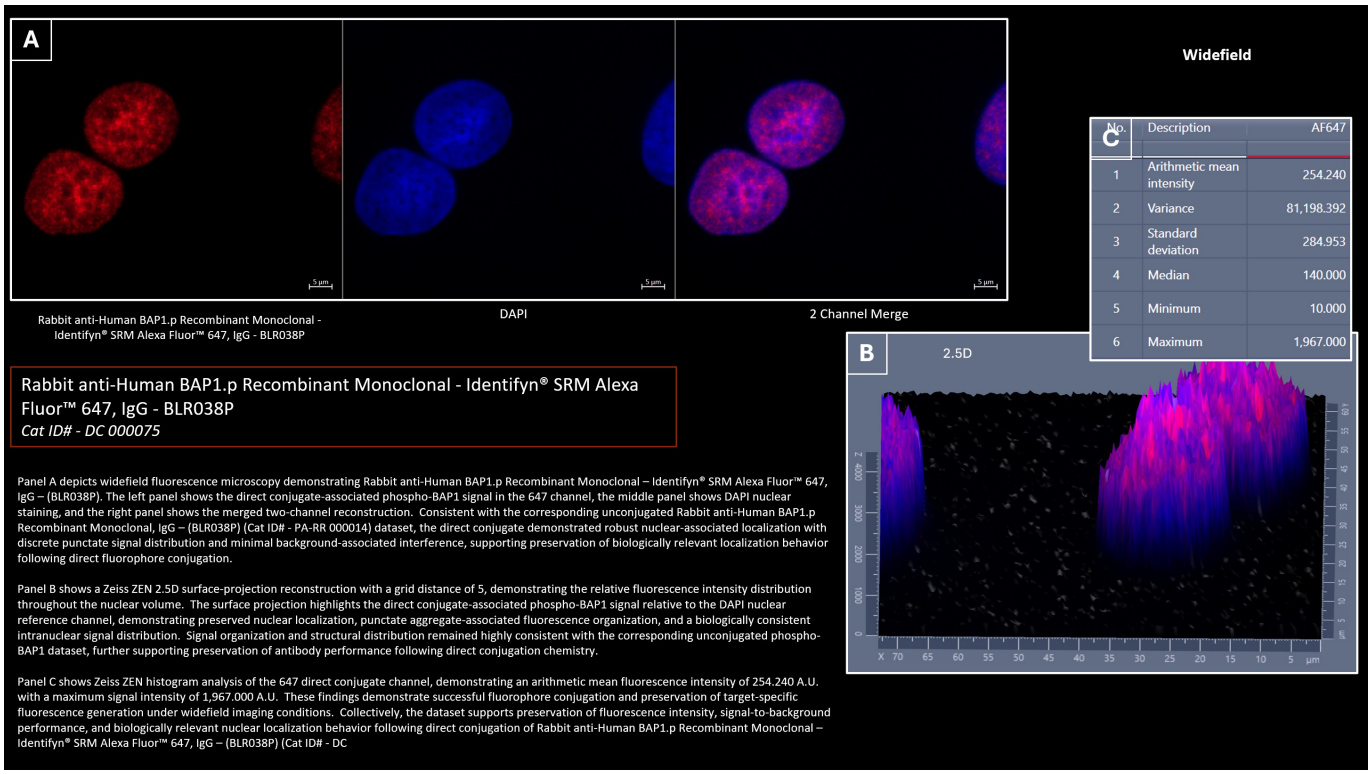
NONE

Image: S. Khanal

SOURCE PROVENANCE

Catalog	DC-000075
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6F328E9AD7D1EE573C7615DDE32BE4A4780A707FA5920FB44696C2A784452269
Method PDF SHA-256	336CF408F85BC9C1B22C5E63897AC6B7DC530F2E642712DF96A1C97E6699D833

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	39

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	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image Size (Pixels)	668 X 569
Image Size (Scaled)	73.16 µm X 62.32 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 49 DAPI
Beam Splitter	660 | 395
Filter Excitation Wavelength	625 - 655 | 335 - 383
Filter Emission Wavelength	665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 25.00% | X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time	250 ms | 30 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	51
Angle Y	-180
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

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 (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P)
Cat ID# - DC 000075

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 by treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3).
The primary antibody is Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times 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 = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 668 X 569
Image Size (Scaled) = 73.16 µm X 62.32 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

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

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 @ 15.00%
Exposure Time = 30 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 left panel shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear-associated localization with discrete punctate BAP1.p-associated signal distribution, supporting effective direct fluorophore conjugation and preservation of biologically consistent target localization under widefield fluorescence microscopy conditions.

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface
Grid distance = 5
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 51
Angle Y = -180
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

ZEN Acquisition Histogram Data - Panel C

Shown is the Zeiss ZEN Histogram analysis for Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), demonstrating an arithmetic mean fluorescence intensity of 254.240 A.U. and a maximum signal intensity of 1,967.000 A.U. in the 647 channel. The direct conjugate dataset was acquired using the 647 X-Cite Xylis Lamp at 25% intensity with an exposure time of 250 ms, supporting successful fluorophore conjugation, robust signal generation, and preservation of biologically consistent nuclear localization characteristics under standard widefield fluorescence microscopy acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P). The left panel shows the direct conjugate-associated BAP1.p signal in the 647 channel, the middle panel shows DAPI nuclear staining, and the right panel shows the merged two-channel reconstruction. Consistent with the corresponding unconjugated Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (PA-RR 000038) dataset, the direct conjugate demonstrated robust nuclear-associated localization with discrete punctate BAP1.p-associated signal distribution and minimal background-associated interference, supporting preservation of biologically relevant localization behavior following direct fluorophore conjugation.

Panel B shows a Zeiss ZEN 2.5D surface-projection reconstruction with a grid distance of 5, demonstrating the relative fluorescence intensity distribution throughout the nuclear compartment. The surface projection highlights the direct conjugate-associated BAP1.p signal relative to the DAPI nuclear reference channel, demonstrating preserved nuclear localization, punctate aggregate-associated fluorescence organization, and biologically coherent intranuclear signal distribution. Signal organization and structural distribution remained highly consistent with the corresponding unconjugated BAP1.p dataset, further supporting preservation of antibody performance following direct conjugation chemistry.

Panel C shows Zeiss ZEN histogram analysis of the 647 direct conjugate channel, demonstrating an arithmetic mean fluorescence intensity of 254.240 A.U. with a maximum signal intensity of 1,967.000 A.U. These findings support successful fluorophore conjugation and the preservation of target-specific fluorescence under widefield imaging conditions. Collectively, the dataset supports preservation of fluorescence intensity, signal-to-background performance, and biologically relevant nuclear localization behavior following direct conjugation of Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) (Cat ID# - DC 000075).

Image Correction

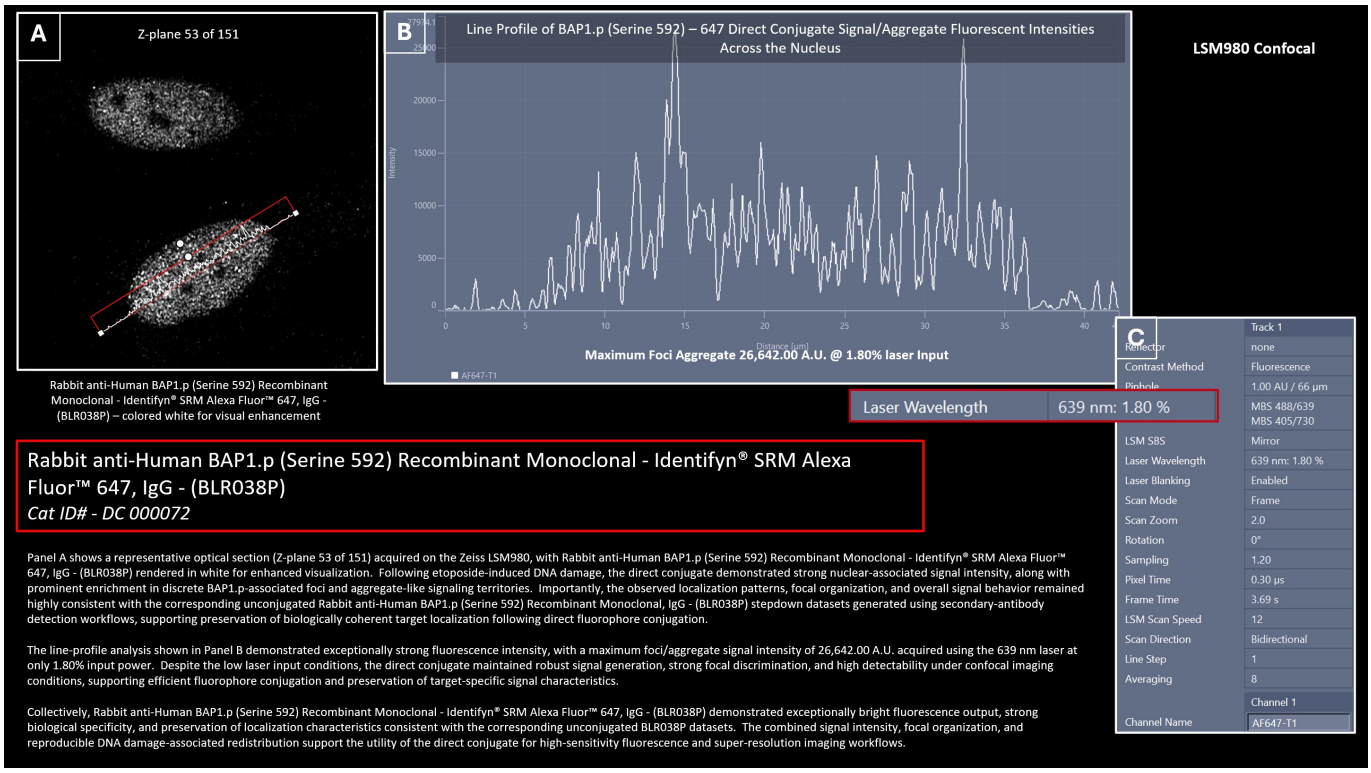
NONE

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

SOURCE PROVENANCE

Catalog	DC-000075
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	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D0AACB82A412412589B1EE459B859C42AA6C0788B31B5E67E86592CE55F36DF2
Method PDF SHA-256	9C9B54263928EC7FC389C8BBA92CC6CCCCA95025EA94390BDEDECF8F69707E40

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	45

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 LSM980 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	151 Slices (4.50 µm) - Panel A, Z-Plane 53 of 151
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1121 X 1121
Image Size (Scaled)	65.93 µm X 65.93 µm
Bit Depth	16 Bit
Image Center Position	X: 88.41 mm, Y: 51.43 mm
Stage Position	X: 88.41 mm, Y: 51.43 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 66 µm | 1.00 AU / 43 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	Mirror
Laser Wavelength	639 nm @ 1.80% | 405 nm @ 0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	1.20
Pixel Time	0.30 µs
Frame Time	3.69 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Detection Wavelength	635 - 735 | 430 - 480
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.3 (3D, Auto) | Filter: 6.5 (3D, Auto)
Profile Definition	Stroke Thickness 1.0, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 42.2), Y (Min 0.0 and Max 27974)

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 (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P)
Cat ID# - DC 000075

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3).
The primary antibody is Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM980 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)

Z-Stack = 151 Slices (4.50 µm) - Panel A, Z-Plane 53 of 151

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1121 X 1121
Image Size (Scaled) = 65.93 µm X 65.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.41 mm, Y: 51.43 mm
Stage Position = X: 88.41 mm, Y: 51.43 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 66 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 639 nm @ 1.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30 µs
Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 635 - 735
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.3 (3D, Auto)

DAPI - Acquired but not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30 µs
Frame Time = 3.69 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 = 430 - 480
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.5 (3D, Auto)

2-Dimensional View - Panel A

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) acquired on the Zeiss LSM980 Confocal microscope at Z-plane 53 of 151 following DNA damage induction. The 647 channel is rendered in white to enhance visualization of signal distribution and fluorescence intensity along the selected line profile. Shown in the image is the graphical overlay identifying the region where the line profile was drawn for examination in Panel B.

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1.0, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 42.2), Y (Min 0.0 and Max 27974)

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel for Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) using the 639 nm laser at 1.80%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Collectively, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) demonstrated exceptionally strong fluorescence intensity, robust detectability, and biologically coherent nuclear-associated redistribution following DNA damage induction. Importantly, the direct conjugate preserved localization characteristics, focal organization, and aggregate-like signaling behavior, which were highly consistent with those of the corresponding unconjugated Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (PA-RR 000038) datasets generated using secondary-antibody detection workflows. The observed preservation of signal distribution and DNA damage-associated structural behavior supports maintenance of target specificity and biological performance following direct fluorophore conjugation, while simultaneously providing exceptionally bright signal generation under low-input confocal acquisition conditions.

Image Correction

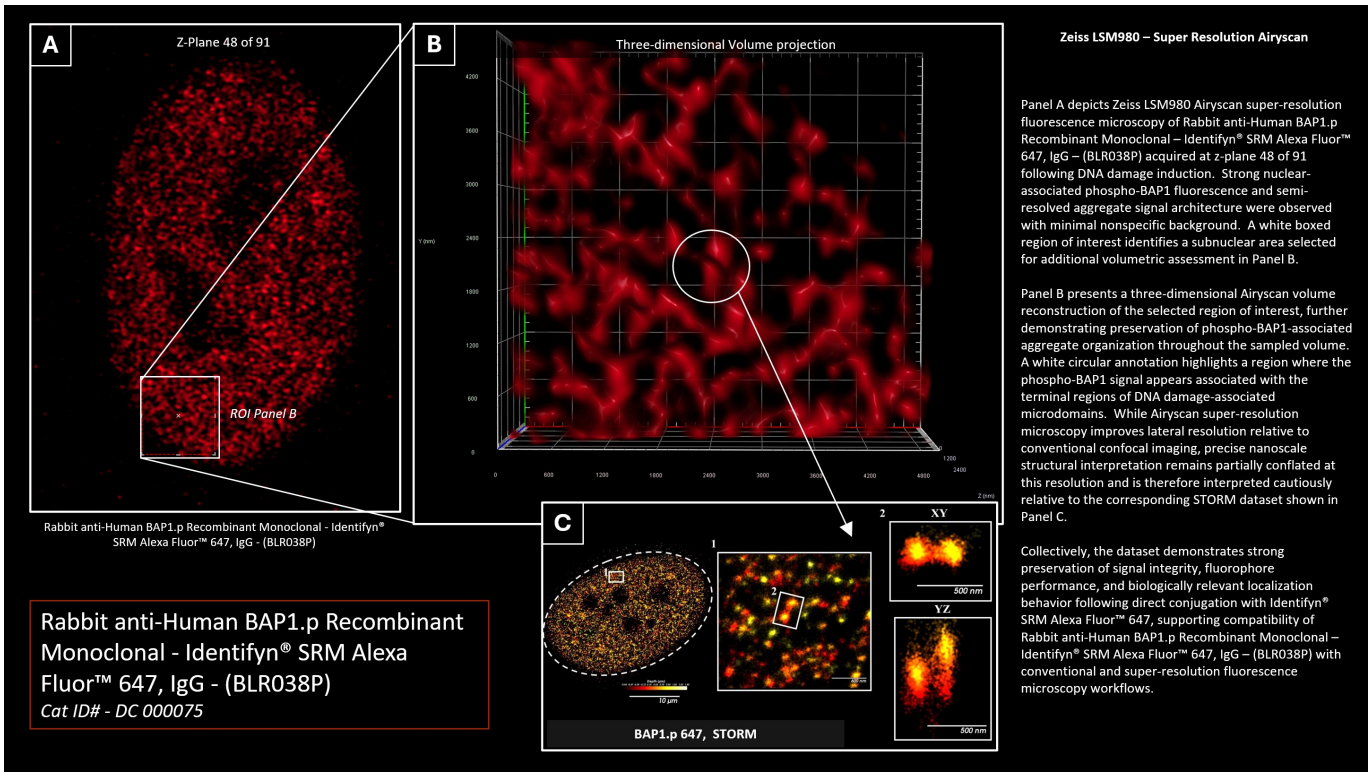
NONE

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

SOURCE PROVENANCE

Catalog	DC-000075
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM980 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	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CE767184D0B05972681C12AA01CA1587CFB6731DA67ED11A02FA96846ED34F8D
Method PDF SHA-256	A8C07C2A85CA164604028288F723FF6753B6A0C9A3CFA2A76114B1D9438AF1DD

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	51

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	91 Slices (2.7 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	903 X 687 | 142 X 134
Image Size (Scaled)	31.87 µm X 24.25 µm | 5.01 µm X 4.73 µm
Bit Depth	16 Bit
Image Center Position	X: 85.94 mm, Y: 51.29 mm
Stage Position	X: 85.94 mm, Y: 51.29 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 215 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 505
Laser Wavelength	639 nm @ 2.80% | 405 nm @ 0.05%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	2.00
Pixel Time	10.99 µs
Frame Time	20.84 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Detection Wavelength	643 - 745 | 350 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	Super Resolution: 10.0 (3D, Auto) | Super Resolution: 9.8 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P)
Cat ID# - DC 000075

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 by treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3).
The primary antibody is Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times 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-Plane 48 of 91 & Panel B, 3-Dimensional Volume View

Z-Stack = 91 Slices (2.7 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 903 X 687
Image Size (Scaled) = 31.87 µm X 24.25 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.94 mm, Y: 51.29 mm
Stage Position = X: 85.94 mm, Y: 51.29 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 3-Dimensional Volume View

Z-Stack = 91 Slices (2.7 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 142 X 134
Image Size (Scaled) = 5.01 µm X 4.73 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.94 mm, Y: 51.29 mm
Stage Position = X: 85.94 mm, Y: 51.29 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639 nm @ 2.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 2.00
Pixel Time = 10.99 µs
Frame Time = 20.84 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 745
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

DAPI - Acquired in Image but not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 215 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 2.00
Pixel Time = 10.99 µs
Frame Time = 20.84 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 = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 9.8 (3D, Auto)

2-Dimensional View - Panel A

Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) acquired at z-plane 48 of 91 following DNA damage induction. The BAP1.p signal demonstrates strong nuclear localization, discrete BAP1.p-associated punctate aggregate formation, and robust signal integrity, consistent with the corresponding unconjugated, widefield, and confocal datasets within this conjugation product set.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

STORM imaging from the same product dataset is shown in Panel C to provide a structural comparison between Airyscan super-resolution microscopy and single-molecule localization imaging resolution. Compared with conventional confocal imaging, Airyscan provides improved visualization of BAP1.p-associated punctate aggregate organization; however, important nanoscale structural details remain partially unresolved at the Airyscan resolution scale. In contrast, STORM imaging further resolves discrete nanoscale fluorescence territories and heterogeneous sub-diffraction-scale structural organization that are not fully distinguishable in the Airyscan dataset. Although overall localization patterns remain highly consistent between modalities, interpretation of precise molecular positioning within the Airyscan dataset remains inferential, supporting the role of Airyscan as a transitional modality within the Identifyn® Stepdown Methodology™.

Summary

Panel A depicts Zeiss LSM980 Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) acquired at Z-plane 48 of 91 following DNA damage induction. The BAP1.p 647 signal is displayed in its native fluorescence pseudocolor to facilitate assessment of fluorescence distribution, signal intensity, and subnuclear localization behavior throughout the nucleus. Compared with conventional confocal imaging, Airyscan microscopy demonstrated improved resolution of BAP1.p-associated punctate aggregate organization and enhanced structural discrimination of DNA damage-associated fluorescence territories. Strong nuclear-associated signal intensity, minimal nonspecific background, and preservation of biologically coherent localization behavior were observed throughout the dataset, supporting robust fluorophore performance and preservation of antibody functionality following direct conjugation. Signal distribution and aggregate-associated structural organization remained highly consistent with the corresponding unconjugated Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (PA-RR 000038) stepdown dataset, supporting preservation of localization fidelity under super-resolution imaging conditions.

Panel B presents a three-dimensional Airyscan volume reconstruction of the selected region of interest shown in Panel A. Orthogonal volumetric visualization further demonstrates the preservation of BAP1.p-associated signal enrichment and semi-resolved aggregate organization throughout the sampled nuclear volume. While Airyscan substantially improves structural discrimination relative to conventional confocal microscopy, nanoscale organizational interpretation remains partially resolution-limited at this imaging scale compared to single-molecule localization microscopy approaches. Importantly, comparison with the corresponding STORM dataset shown in Panel C further demonstrates that the direct conjugate maintained strong signal integrity, preserved the aggregate-associated fluorescence architecture, exhibited high signal-to-noise performance, and remained compatible with increasingly demanding super-resolution imaging workflows.

Collectively, the Airyscan dataset supports the performance, stability, and structural imaging compatibility of Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) under high-resolution fluorescence microscopy conditions. The preservation of biologically coherent localization behavior, robust fluorescence intensity, minimal background-associated interference, and consistent super-resolution signal architecture further supports the Identifyn® direct conjugation platform's ability to maintain antibody functionality and fluorophore integrity under demanding super-resolution imaging conditions.

Image Correction

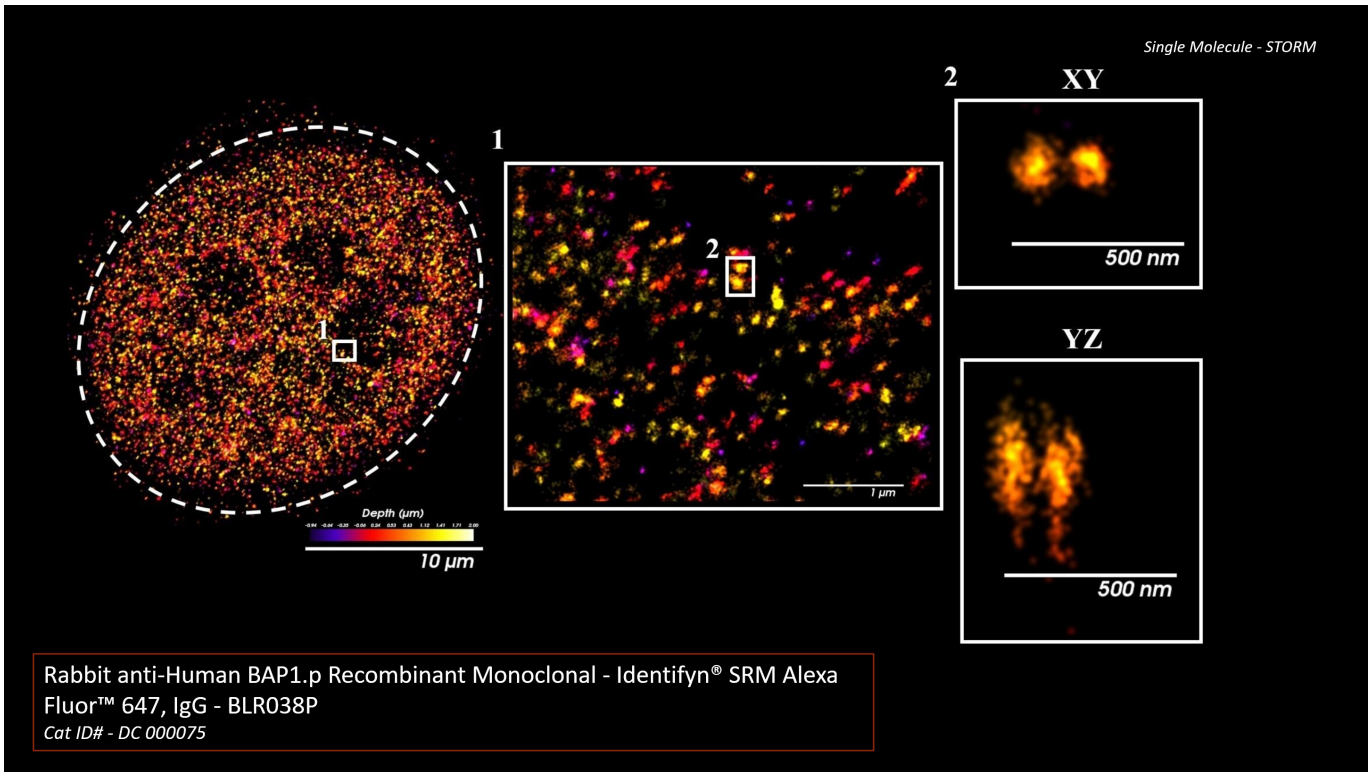
NONE

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

SOURCE PROVENANCE

Catalog	DC-000075
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	51
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0A8B80C7FA887EA42912778AA051FADE9CC9B09E883C7D2AF32EFA9463FB294B
Method PDF SHA-256	600E4BBC8DAE2302C2EFB03E451F2AFAF29D28FF0DA83E47EB53F4CD37CE4B06

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	81

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

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

FIELD	SOURCE-DOCUMENTED VALUE
Cell(s) or Cellular Structure Localization	The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused. | Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Current	184.6 µm
Widefield 640 nm	0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 183.4; End: 184.6
SuperRes 640 nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	6
Cycles	25
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	15
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 10
Sizing Mode	Radial Precision
Particle size	4 nm
Color by	Depth
Color Table	Single

FIELD	SOURCE-DOCUMENTED VALUE
Opacity Mode	Depth; Opacity 1: 0.15
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	35
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Compute Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P)
Cat ID# - DC 000075

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 µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody is Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5 times in Identifyn®'s proprietary wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Control Data - Primary Unconjugated Antibody

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope Configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 184.6 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

Advanced Tools Option

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

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

Autofocus - Calibration Values

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

View Mode

SuperRes: 50µm is Selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 183.4; End: 184.6

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

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

Not Selected

Advanced Tools

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 6
Cycles: 25
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 15

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 10

Region of Interest -

Not selected

Display -

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Radial Precision
Particle size: 4 nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.15
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

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

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

Radial precision: 35
Axial precision: 70

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

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

Summary

Collectively, the STORM dataset demonstrates that Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) maintained strong fluorescence performance, localization fidelity, and structural signal integrity under demanding single-molecule localization microscopy conditions. STORM imaging fundamentally relies on the ability of fluorophores to undergo repeated stochastic photoswitching ("blinking") cycles between fluorescent and dark states while simultaneously maintaining sufficient photon output for precise single-molecule localization. As a result, fluorophore brightness, photoswitching efficiency, blink-rate stability, resistance to photobleaching, and preservation of biologically coherent localization behavior represent critical limitations for many conventional direct conjugates under STORM acquisition workflows.

Importantly, the Identifyn® direct conjugate maintained robust signal intensity, reproducible blinking behavior, strong signal-to-noise characteristics, and stable localization performance throughout volumetric STORM acquisition, drift correction, particle filtering, and DBSCAN cluster-analysis workflows. Unlike many conventional direct conjugates that exhibit diminished photon output, unstable photoswitching kinetics, elevated background fluorescence, rapid photobleaching, or localization artifacts under single-molecule imaging conditions, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR038P) preserved discrete nanoscale fluorescence organization and biologically coherent BAP1.p-associated structural architectures throughout the STORM pipeline.

Furthermore, localization behavior and nanoscale fluorescence organization remained highly consistent with the corresponding unconjugated Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (PA-RR 000038) stepdown datasets, supporting preservation of antibody functionality following direct fluorophore conjugation. Collectively, these findings support the Identifyn® direct conjugation platform's ability to overcome many traditional limitations associated with direct-conjugate STORM imaging, including insufficient fluorophore brightness, unstable blinking kinetics, poor photoswitching efficiency, elevated background signal, and loss of structural localization fidelity under demanding super-resolution acquisition conditions.

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

SOURCE PROVENANCE

Catalog	DC-000075
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Structured source metadata values	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DA21F99DFA5549F769F1DC81DE2EA1FB2E454030DED1323E06750FA88214CA09
Method PDF SHA-256	2B696EDCC112A17182FAF0FEAC7842FC8627F593DA5674117FB980C2584512BD