BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

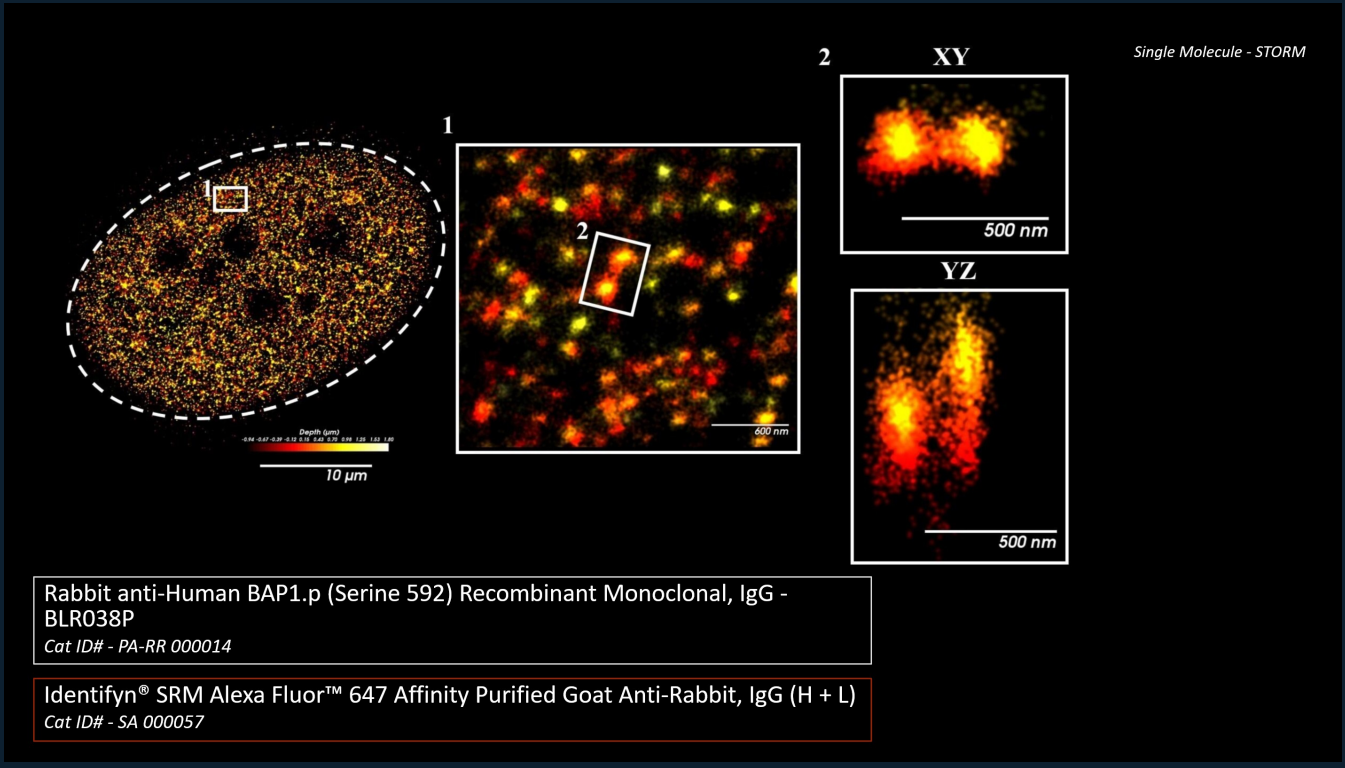
# BAP1.P

## MICROSCOPY EVIDENCE ASSESSMENT

**Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal**

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

| 8 | 1 | PENDING |
|---|---|---|
| ORDERED EVIDENCE TIERS | SUPPORTING CONTROL | SCIENTIST REVIEW |




REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

**PA-RR-000014 | PRODUCT-LEVEL EVIDENCE DOSSIER**

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

## CRO CAPABILITY DEMONSTRATED

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

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

## SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for 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 -> Single-molecule STORM -> Control

## BENNETT ASSESSMENT

The preserved PA-RR-000014 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

# BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

## OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RR-000014 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

## SETTINGS ASSESSMENT

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

## BIOLOGICAL SUPPORT

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

## CONTROL ASSESSMENT

A true preserved control record is present and appears last.

## CLAIM CEILING

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

## PROCESSING DISCLOSURE

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

## REAGENTS AND ACQUIRED CHANNELS

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

| EVIDENCE OBJECT | REAGENTS / FLUOROPHORES PRESENT | CHANNELS ACTUALLY ACQUIRED |
|---|---|---|
| Western blot | 647 | Not deterministically stated in the acquisition block |
| Widefield | 405/DAPI; 488; 647 | 3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715 |
| Widefield SIM — Apotome | 405/DAPI; 488; 647 | 3; 50 Cy 5; He 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715 |
| Confocal | 405/DAPI; 488; 647 | 3; None; 639 nm @ 0.4%; 488 nm @ 0.2%; 405 nm @ 0.4%; 653; 493; 353 |
| Airyscan | 405/DAPI; 488; 647 | 3; None; 639 nm @ 0.3%; 488 nm @ 0.3%; 405 nm @ 0.3%; 653; 493; 353 |
| SIM | 405/DAPI; 488; 647 | 2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; 640; 488 |
| SIM² | 405/DAPI; 488; 647 | 2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; 640; 488 |
| Single-molecule STORM | 647 | Both Channels |
| Control | 405/DAPI; 488; 647 | 1; None; 639 nm @ 0.4%; 639 nm @ 0.6% -; 653; 668 |

## MODALITY ASSESSMENT / PART 1 OF 9

## Western blot / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

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

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

# MODALITY ASSESSMENT / PART 2 OF 9

## Widefield / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: 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): 1126 X 954; Image Size (Pixels): 1126 X 954; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 15.00%; X-Cite Xylis Lamp Intensity @ 20.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

## MODALITY ASSESSMENT / PART 3 OF 9

## Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63x/1.25 Oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 82 Slices (8.1 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 981 X 879; 274 X 300; Image Size (Pixels): 981 X 879; 274 X 300; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 150 ms; 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 15.00%; X-Cite Xylis Lamp Intensity @ 25.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 58.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

# MODALITY ASSESSMENT / PART 4 OF 9

## Confocal / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.40 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1503 X 1503; Image Size (Pixels): 1503 X 1503; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.26 µs; Frame Time: 5.63 s. Illumination: Laser Wavelength: 639 nm @ 0.4%; 488 nm @ 0.2%. Optical/scan: Pinhole: 1.00 AU / 65 µm; 1.00 AU / 51 µm; Scan Zoom: 1.5; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 7.8 (3D, Auto). Structured source metadata values: 53.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

## MODALITY ASSESSMENT / PART 5 OF 9

## Airyscan / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.40 µm); Channels: 3; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 870 X 773; 93 X 92; Image Size (Pixels): 870 X 773; 93 X 92; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.04 µs; Frame Time: 19.77 s. Illumination: Laser Wavelength: 639 nm @ 0.3%; 488 nm @ 0.3%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 250 µm; Scan Zoom: 1.9; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution: 10.0 (3D, Auto); Super Resolution: 8.2 (3D, Auto). Structured source metadata values: 57.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

## MODALITY ASSESSMENT / PART 6 OF 9

## SIM / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 177 Slices (5.28 µm); 80 Slices (2.37 µm) - Displayed gallery content was reduced to Phospho-BAP1-specific signal.; Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1927 X 2049; 295 X 311; Image Size (Pixels): 1927 X 2049; 295 X 311; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @ 1.00%; 488 nm @ 1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 63.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

# MODALITY ASSESSMENT / PART 7 OF 9

## SIM² / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 177 Slices (5.28 µm); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1988 X 2024; 426 X 403; Image Size (Pixels): 1988 X 2024; 426 X 403; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @ 1.00%; 488 nm @ 1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 57.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

# MODALITY ASSESSMENT / PART 8 OF 9

## Single-molecule STORM / SOURCE EVIDENCE PRESERVED

### DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60x/1.30 Silicon Oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 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: 80.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### LITERATURE CORRELATION

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

### INTERPRETIVE LIMIT

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

### CLAIM CEILING

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

## MODALITY ASSESSMENT / PART 9 OF 9

## Control / SOURCE EVIDENCE PRESERVED

**DOCUMENTED SETTINGS**

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.40 µm); 161 Slices (4.80 µm); Channels: 1; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1503 X 1503; 1248 X 1248; Image Size (Pixels): 1503 X 1503; 1248 X 1248; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.26 µs; 0.27 µs; Frame Time: 5.63 s; 4.10 s. Illumination: Laser Wavelength: 639 nm @ 0.4%; 639 nm @ 0.6% -. Optical/scan: Pinhole: 1.00 AU / 65 µm; Scan Zoom: 1.5; 2.1; LSM Scan Speed: 11; 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 8.9 (3D, Auto). Structured source metadata values: 54.

**VISIBLE OBSERVATION**

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

**SOURCE-REPORTED RESULT**

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

**LITERATURE CORRELATION**

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

**INTERPRETIVE LIMIT**

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

**CLAIM CEILING**

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

## CROSS-MODALITY SYNTHESIS

The preserved PA-RR-000014 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

## BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

## REFERENCES

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

## RELEASE BOUNDARY

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

## SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

**HIGH CONFIDENCE - AUTO-RECONCILED**

Airyscan / Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm -> 0.03529 µm X 0.03529 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=870 X 773; Image Size (Scaled)=30.70 µm X 27.78 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 1.80%.

**HIGH CONFIDENCE - AUTO-RECONCILED**

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1927 X 2049; Image Size (Scaled)=40.68 µm X 43.26 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

**HIGH CONFIDENCE - AUTO-RECONCILED**

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1988 X 2024; Image Size (Scaled)=41.97 µm X 42.73 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

**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

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

# INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

# ORIGINAL RECORD BEGINS

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

## PRODUCT-LEVEL STEPDOWN MAP

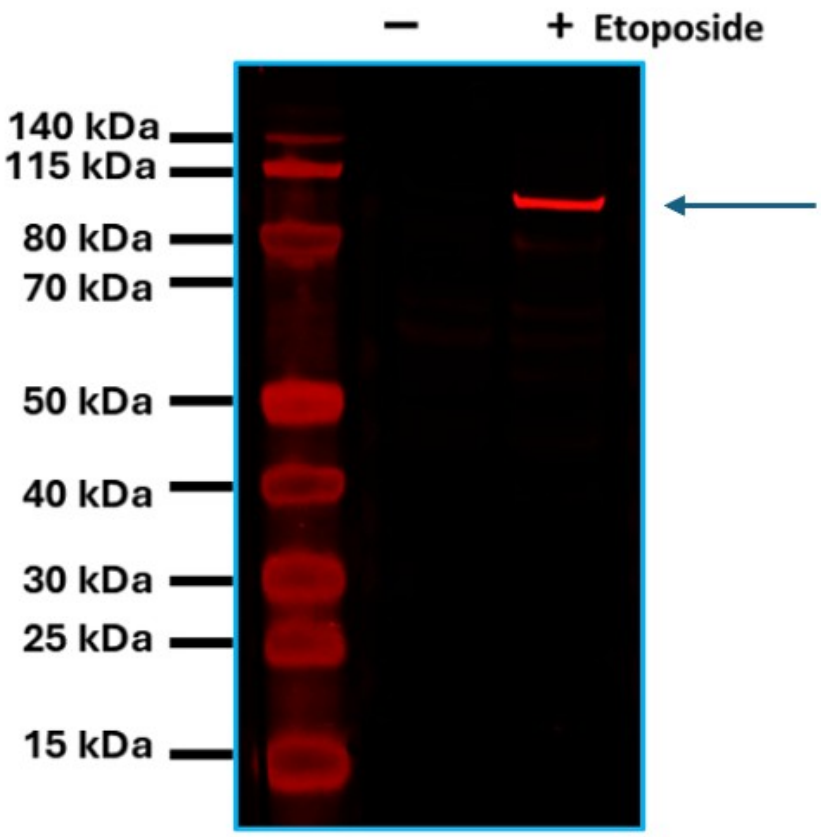
This dossier preserves the complete available evidence progression for PA-RR-000014. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

| CANONICAL STEPDOWN TIER | EVIDENCE STAGE | INSTRUMENT | STATUS |
|---|---|---|---|
| 1 | Western blot | Azure Sapphire FL | PRESERVED |
| 2 | Widefield | ZEISS Axio Imager.M1 | PRESERVED |
| 3 | Widefield SIM - Apotome | ZEISS Axio Observer.Z1/7 Apotome | PRESERVED |
| 4 | Confocal | ZEISS LSM 980 | PRESERVED |
| 5 | Airyscan | ZEISS LSM 980 | PRESERVED |
| 6 | SIM | ZEISS Elyra | PRESERVED |
| 7 | SIM² | ZEISS Elyra | PRESERVED |
| 8 | Single-molecule STORM | Bruker Vutara VXL | PRESERVED |

## INTERPRETIVE BOUNDARY

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

# ORIGINAL IMAGE EVIDENCE / WESTERN BLOT




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

## STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane 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, IgG - (BLR038P)
Cat ID# PA-RR 000014

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, 10-180 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 PBS. The blocked membrane was incubated with Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) for 20 hours, then washed 5 times with 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5 times with 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

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

Post Processing

NONE

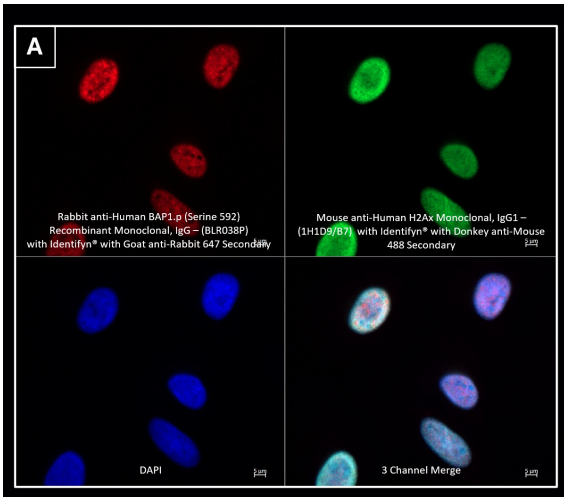
---

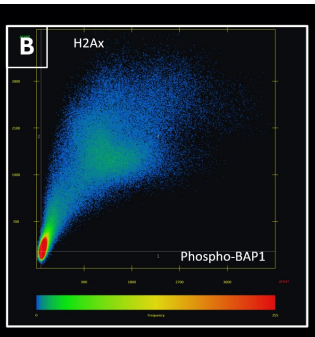
Image: S. Khanal

## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **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** | A20465A389A0CBA9185182ECE0E41BA2221A4BB407FC228E08F877B68C230CB4 |
| **Method PDF SHA-256** | F63ECEE993E5E62850B642708D17EF4CEC8458D7566A45B66D4D562AB950B63A |

# ORIGINAL IMAGE EVIDENCE / WIDEFIELD




Colocalization View – Measuring Entire Field from Panel A

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - BLR038P
*Cat ID# - PA-RR 000014*

Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)
*Cat ID# - PA-MM 000002*

**Widefield**

Panel A shows a split-view widefield acquisition following etoposide-induced DNA damage. The upper left panel shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L). The upper right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), detected using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L). The lower left panel shows DAPI nuclear staining, while the lower right panel presents the three-channel merged image. Following etoposide exposure, phospho-BAP1 demonstrates prominent intranuclear redistribution with punctate aggregate formation and enrichment within damage-associated nuclear regions. The H2Ax signal remains broadly distributed throughout the nucleus, with an overlapping organization within DNA damage-responsive chromatin territories.

Panel B presents colocalization analysis of the same acquisition, with phospho-BAP1 (Serine 592) plotted along the horizontal (X) axis and H2Ax plotted along the vertical (Y) axis. Strong correlated localization is observed between both signals, consistent with coordinated engagement within DNA damage-associated nuclear compartments following genotoxic stress.

Biologically, BAP1 (BRCA1-associated protein 1) functions as a nuclear deubiquitinase involved in chromatin remodeling, DNA damage signaling, homologous recombination repair, and transcriptional regulation. Following DNA damage, phosphorylated BAP1 is recruited to chromatin-associated repair environments where it contributes to regulation of repair complex assembly and chromatin accessibility. Etoposide-induced DNA double-strand breaks stimulate recruitment of DNA damage response machinery, including ATM/ATR-associated signaling pathways that influence BAP1 phosphorylation state and localization dynamics.

H2Ax serves as a core chromatin-associated histone variant distributed broadly throughout the nucleus and functions as the substrate pool for phosphorylation into γH2AX following DNA damage induction. As such, overlapping spatial distribution between phospho-BAP1 and total H2Ax is expected within damage-responsive chromatin regions, particularly at widefield resolution where diffraction-limited imaging conflates densely organized nuclear structures.

Importantly, while widefield microscopy effectively demonstrates global nuclear recruitment, aggregate formation, and apparent colocalization patterns, it remains insufficient to resolve nanoscale structural organization or direct molecular interaction. The strong overlap observed between phospho-BAP1 and H2Ax should therefore be interpreted as coordinated localization within shared DNA damage response territories rather than definitive molecular colocalization. Higher-resolution modalities remain necessary to resolve the underlying sub-diffraction architecture of these repair-associated signaling domains.

| | |
|---|---|
| **EVIDENCE TIER** | Widefield |
| **INSTRUMENT** | ZEISS Axio Imager.M1 |
| **CELL MODEL** | HeLa |
| **DETECTED DYES** | 405/DAPI; 488; 647 |
| **METADATA VALUES** | 40 |

# STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | 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) | 1126 X 954 |
| Image Size (Scaled) | 123.32 µm X 104.49 µm |
| Bit Depth | 14 Bit |
| Image Center Position | X: 0.00 µm, Y: 0.00 µm |
| ROI Center Offset | X: 0.00 µm, Y: 0.00 µm |
| Reflector | 50 Cy 5 \| 38 eGFP \| 49 DAPI |
| Beam Splitter | 660 \| 495 \| 395 |
| Filter Excitation Wavelength | 625 - 655 \| 450 - 490 \| 335 - 383 |
| Filter Emission Wavelength | 665 - 715 \| 500 - 550 \| 420 - 470 |
| Contrast Method | Fluorescence |
| Light Source | X-Cite Xylis Lamp Intensity @ 15.00% \| X-Cite Xylis Lamp Intensity @ 20.00% \| X-Cite Xylis Lamp Intensity @ 5.00% |
| Exposure Time | 150 ms \| 200 ms \| 20 ms |
| Excitation Wavelength | 653 \| 493 \| 353 |
| Emission Wavelength | 668 \| 517 \| 465 |
| Effective NA | 1.4 |
| Imaging Device | Axiocam 705 |
| Camera Adapter | 1X |
| Depth of Focus | 1.03 µm \| 0.80 µm \| 0.72 µm |
| Binning Mode | 2,2 |
| Image Measurement | Entire Field |

## SOURCE METHOD

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

### Identifyn Standards for Optical Microscopy Methods™

### Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

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

## Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 1126 X 954
Image Size (Scaled) = 123.32 µm X 104.49 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## 647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

## 488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 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 @ 5.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

## Split View - Panel A

The top-left panel shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L). The top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L). The lower-left panel shows DAPI nuclear staining, and the bottom-right panel shows the three-channel merged image.

## Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 95, Range Auto - Phospho-BAP1 (Serine 592)
Vertical Axis - AF488, Threshold 256, Range Auto - H2Ax

## Image Measurement = Entire Field

Colocalization analysis of Phospho-BAP1 (X-axis) and H2Ax (Y-axis) was performed across the entire image acquisition.

## Summary

Panel A shows a split-view widefield acquisition following etoposide-induced DNA damage. The upper left panel shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L). The upper right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), detected using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse IgG (H + L). The lower-left panel shows DAPI nuclear staining, while the lower-right panel shows the three-channel merged image. Following etoposide exposure, phospho-BAP1 exhibits prominent intranuclear redistribution, with punctate aggregate formation and enrichment in damage-associated nuclear regions. The H2Ax signal remains broadly distributed throughout the nucleus, with an overlapping organization within DNA damage-responsive chromatin territories.

Panel B presents colocalization analysis of the same acquisition, with phospho-BAP1 plotted along the horizontal (X) axis and H2Ax plotted along the vertical (Y) axis. Broad overlapping localization patterns are observed between the two signals, consistent with coordinated engagement of DNA damage-associated nuclear compartments following genotoxic stress.

Biologically, BAP1 (BRCA1-associated protein 1) functions as a nuclear deubiquitinase involved in chromatin remodeling, DNA damage signaling, homologous recombination repair, and transcriptional regulation. Following DNA damage, phosphorylated BAP1 is recruited to chromatin-associated repair environments where it contributes to the regulation of repair complex assembly and chromatin accessibility. Etoposide-induced DNA double-strand breaks stimulate the recruitment of the DNA damage response machinery, including ATM/ATR-associated signaling pathways, which influence BAP1 phosphorylation state and localization dynamics.

H2Ax serves as a core chromatin-associated histone variant distributed broadly throughout the nucleus and functions as the substrate pool for phosphorylation into γH2AX following DNA damage induction. As such, overlapping spatial distributions between phospho-BAP1 and total H2Ax are expected within damage-responsive chromatin regions, particularly at widefield resolution, where diffraction-limited imaging conflates densely organized nuclear structures.

Importantly, while widefield microscopy effectively demonstrates global nuclear recruitment, aggregate formation, and apparent colocalization patterns, it remains insufficient to resolve nanoscale structural organization or to directly resolve molecular interactions. The strong overlap observed between phospho-BAP1 and H2Ax should therefore be interpreted as coordinated localization within shared DNA damage response territories rather than definitive molecular colocalization. Higher-resolution modalities remain necessary to resolve the underlying sub-diffraction architecture of these repair-associated signaling domains.

## Image Correction

## NONE

**----**

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



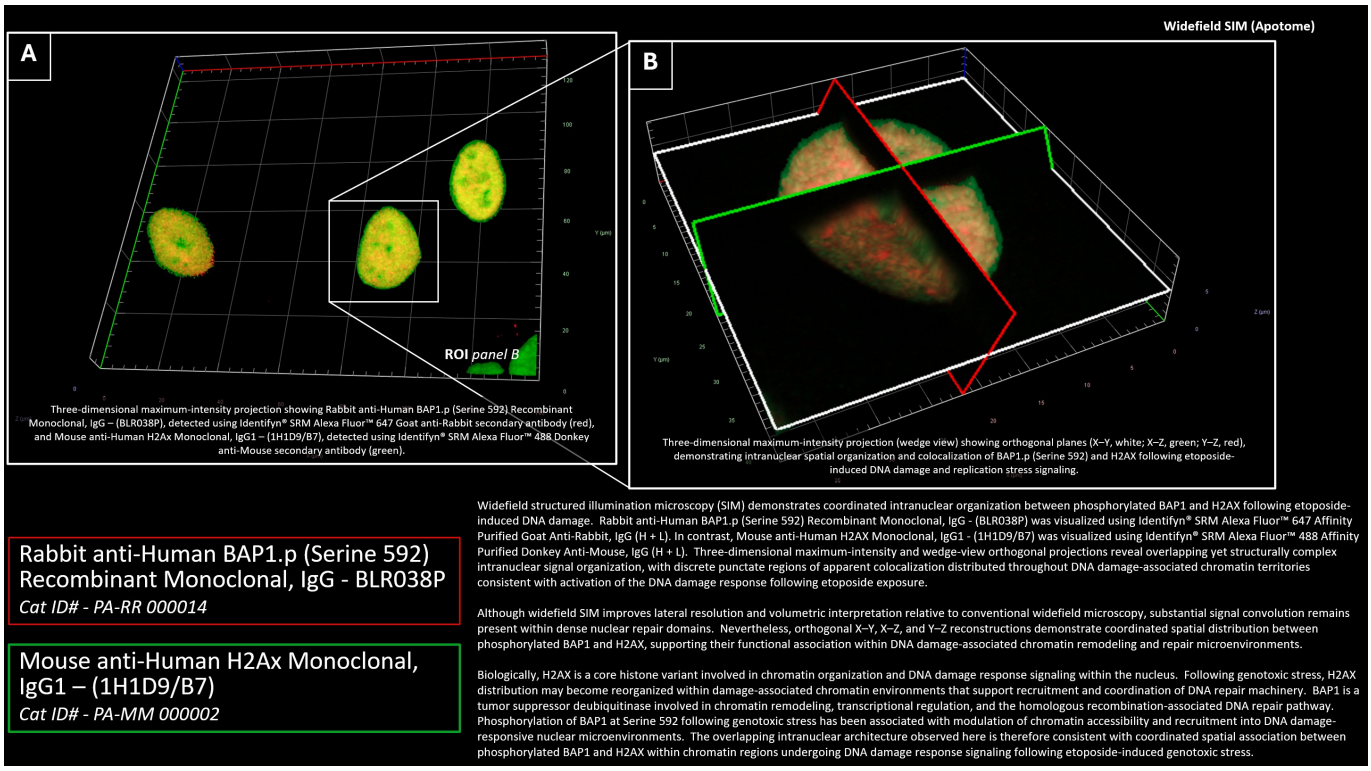
## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **Stage** | Widefield |
| **Instrument** | ZEISS Axio Imager.M1 |

## SOURCE PROVENANCE

| | |
|---|---|
| **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** | 40 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | E435174A304034E880B46187EFD00385978A7B68CBB23508F46CAA032A3889D9 |
| **Method PDF SHA-256** | 916BD576806D0E677A2E93A96883C294024CA5F5268993B613FA7310F6BB7FB7 |

# ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME




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

# STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807 Mono, 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 | 82 Slices (8.1 µm) |
| Channels | 3 |
| Scaling (Per Pixel) | 0.143 µm X 0.143 µm X 0.100 µm |
| Image Size (Pixels) | 981 X 879 \| 274 X 300 |
| Image Size (Scaled) | 140.14 µm X 125.57 µm \| 39.14 µm X 42.86 µm |
| Bit Depth | 14 Bit |
| Image Center Position | X: 57.29 mm, Y: 45.89 mm |
| Stage Position | X: 57.29 mm, Y: 45.89 mm |
| ROI Center Offset | X: 1.14 µm, Y: 0.00 µm |
| Reflector | 50 Cy 5 \| He 38 eGFP \| 49 DAPI |
| Beam Splitter | 660 \| 495 \| 395 |
| Filter Excitation Wavelength | 625 - 655 \| 450 - 490 \| 335 - 383 |
| Filter Emission Wavelength | 665 - 715 \| 500 - 550 \| 420 - 470 |
| Contrast Method | Fluorescence |
| Light Source | X-Cite Xylis Lamp Intensity @ 15.00% \| X-Cite Xylis Lamp Intensity @ 25.00% \| X-Cite Xylis Lamp Intensity @ 10.00% |
| Exposure Time | 150 ms \| 250 ms \| 25 ms |
| Excitation Wavelength | 653 \| 493 \| 353 |
| Emission Wavelength | 668 \| 517 \| 465 |
| Effective NA | 1.25 |
| Imaging Device | Axiocam 807 Mono |
| Camera Adapter | 1X |
| Depth of Focus | 1.30 µm \| 1.00 µm \| 0.72 µm |
| Binning Mode | 2,2 |
| Apotome SIM Image Processing | Display Mode "Optical Sectioning" - enabled correction selected. |
| 3D Maximum Projection | Precise |
| Appearance | Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels |
| Background | Black |
| Light | Brightness 100%, Enabled Tone Mapping |
| Projection | View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| X-Y | Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. |
| Position of Clip | -2% \| 0% |
| Clip X | 0° |
| Clip Z | 0° |
| X-Z | Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. |
| Clip Y | 30° \| -30° |
| Y-Z | Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. |

## SOURCE METHOD

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

### Identifyn Standards for Optical Microscopy Methods™

### Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

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

## Z Stack - (3D) - Panel A

## Z-Stack = 82 Slices (8.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 981 X 879
Image Size (Scaled) = 140.14 µm X 125.57 µm
Bit Depth = 14 Bit
Image Center Position = X: 57.29 mm, Y: 45.89 mm
Stage Position = X: 57.29 mm, Y: 45.89 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

## Z Stack - (3D) - Panel B, Wedge Cut, Region of Interest Panel A

## Z-Stack = 82 Slices (8.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 274 X 300
Image Size (Scaled) = 39.14 µm X 42.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 57.29 mm, Y: 45.89 mm
Stage Position = X: 57.29 mm, Y: 45.89 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

## 647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

## 488 -

Reflector = He 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 @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

## DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

## Post Processing - SIM Processing

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

## 3D Image Processing - Panel A

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

## 3D Image Processing - Panel B

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

## Clipping Image Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

### Position of Clip = -2%
### Clip X = 0°
### Clip Z = 0°

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

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

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

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

Panel B shows a wedge-cut view displayed from the top with a slight backward rotation, highlighting the region of wedge removal and revealing the full depth of signal across the z-stack. This orientation provides an alternative view of wedge geometry and confirms intranuclear localization of Phospho-BAP1 (Serine 592), as indicated by colocalization with H2Ax.

## Summary

Widefield structured illumination microscopy (SIM) demonstrates coordinated intranuclear organization between phosphorylated BAP1 and H2Ax following etoposide-induced DNA damage. Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) was visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L). In contrast, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) was visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). Maximum-intensity and wedge-cut volumetric projections reveal overlapping yet structurally complex intranuclear signal organization, with discrete punctate regions of apparent colocalization distributed throughout DNA damage-associated chromatin territories consistent with activation of the DNA damage response following etoposide exposure.

Although widefield SIM improves lateral resolution and volumetric interpretation relative to conventional widefield microscopy, substantial signal convolution remains present within dense nuclear repair domains. Nevertheless, orthogonal X-Y, X-Z, and Y-Z reconstructions demonstrate coordinated spatial distribution between phosphorylated BAP1 and H2Ax, supporting their functional association within DNA damage-associated chromatin remodeling and repair microenvironments.

Biologically, H2Ax is a core histone variant involved in chromatin organization and DNA damage response signaling within the nucleus. Following genotoxic stress, H2Ax distribution may become reorganized within damage-associated chromatin environments that support recruitment and coordination of DNA repair machinery. BAP1 is a tumor suppressor deubiquitinase involved in chromatin remodeling, transcriptional regulation, and the homologous recombination-associated DNA repair pathway. Phosphorylation of BAP1 following genotoxic stress has been associated with modulation of chromatin accessibility and recruitment into DNA damage-responsive nuclear microenvironments. The overlapping intranuclear architecture observed here is therefore consistent with coordinated spatial association between phosphorylated BAP1 and H2Ax within chromatin regions undergoing DNA damage response signaling following etoposide-induced genotoxic stress.

## Image Correction

## NONE

---

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



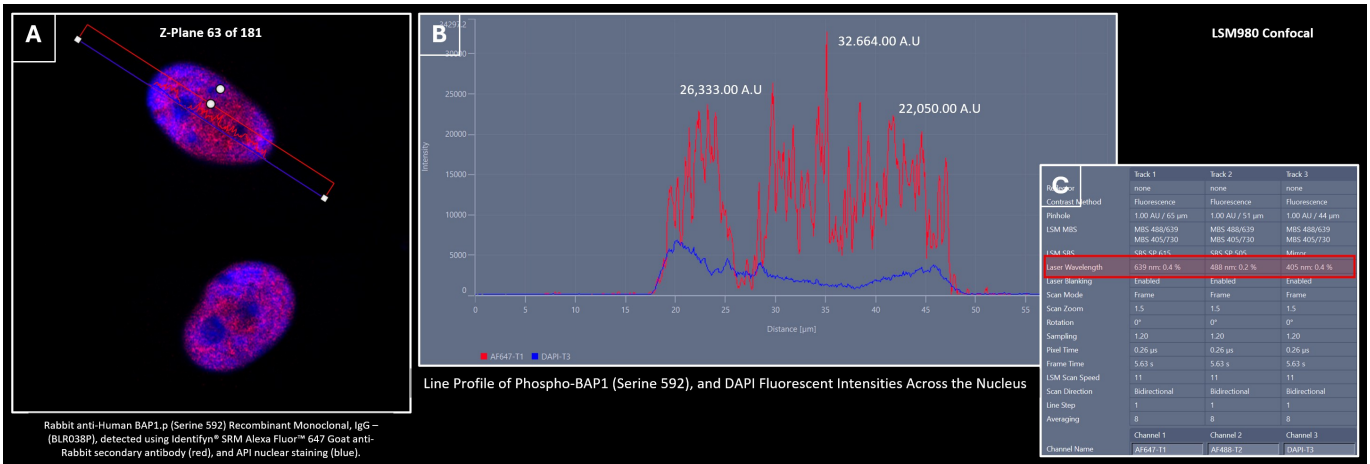
## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **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 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| **Structured source metadata values** | 58 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 62AD4164FBB27EE62971ECF03C3FE37FDD08F128D9159020330A82BB4EA9EBA8 |

## SOURCE PROVENANCE

| | |
|---|---|
| **Method PDF SHA-256** | 830CA03A83A632CD52FEA9EBC5BC02D5BD4E64F5849C142B019F37EE43FD5839 |

# ORIGINAL IMAGE EVIDENCE / CONFOCAL




Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 Goat anti-Rabbit secondary antibody (red), and API nuclear staining (blue).

Line Profile of Phospho-BAP1 (Serine 592), and DAPI Fluorescent Intensities Across the Nucleus

**Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - BLR038P**
*Cat ID# - PA-RR 000014*

**Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)**
*Cat ID# - PA-MM 000002*
*– Acquired but not Shown in Analysis*

Panel A shows an LSM980 confocal optical section acquired at Z-plane 63 of 181, demonstrating Rabbit anti-Human Phospho-BAP1 (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Goat Anti-Rabbit secondary antibody, together with DAPI nuclear staining. A line profile traversing the nucleus measures fluorescent intensity across both channels, demonstrating that the phospho-BAP1 signal remains predominantly confined to the nuclear compartment. The line profile additionally reveals multiple punctate regions of elevated phospho-BAP1 intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows histogram-based quantitative analysis of the measured fluorescent intensities from the line profile shown in Panel A. Three prominent phospho-BAP1 (Serine 592)-associated aggregate regions were identified with measured fluorescent intensities of 32,664.00, 26,333.00, and 22,050.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of phosphorylated BAP1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear-associated fluorescence. While BAP1 is constitutively present throughout the nucleus under basal conditions, genotoxic stress promotes phosphorylation-dependent spatial reorganization into punctate chromatin-associated repair microenvironments involved in DNA damage response signaling and chromatin remodeling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.4% laser power for the 639 nm channel corresponding to Alexa Fluor™ 647 and 0.4% laser power for the 405 nm DAPI channel. See Full Summary Methods Section.

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

# 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 | 181 Slices (5.40 µm) |
| Channels | 3 |
| Scaling (Per Pixel) | 0.059 µm X 0.059 µm X 0.030 µm |
| Image Size (Pixels) | 1503 X 1503 |
| Image Size (Scaled) | 88.38 µm X 88.38 µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 74.45 mm, Y: 46.33 mm |
| Stage Position | X: 74.44 mm, Y: 46.33 mm |
| ROI Center Offset | X: 0.00 µm, Y: 0.00 µm |
| Reflector | None |
| Contrast Method | Fluorescence |
| Pinhole | 1.00 AU / 65 µm \| 1.00 AU / 51 µm \| 1.00 AU / 43 µm |
| LMS MBS | MBS 488/639 & 405/730 |
| LMS SBS | SBS SP 615 \| SBS SP 505 \| Mirror |
| Laser Wavelength | 639 nm @ 0.4% \| 488 nm @ 0.2% \| 405 nm @ 0.4% |
| Laser Blanking | Enabled |
| Scan Mode | Frame |
| Scan Zoom | 1.5 |
| Rotation | 0° |
| Sampling | 1.20 |
| Pixel Time | 0.26 µs |
| Frame Time | 5.63 s |
| LSM Scan Speed | 11 |
| Scan Direction | Bidirectional |
| Line Step | 1 |
| Averaging | 8 |
| Excitation Wavelength | 653 \| 493 \| 353 |
| Emission Wavelength | 668 \| 517 \| 465 |
| Effective NA | 1.4 |
| Detection Wavelength | 640 - 732 \| 511 - 550 \| 435 - 490 |
| Imaging Device | GaAsP-Pmt3 |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Type | GaAsP-PMT |
| Detector Gain | 500 V \| 550 V |
| Detector Offset | 0 |
| Detector Digital Gain | 1.0 |
| LSM Plus Mode | Filter: 7.5 (3D, Auto) \| Filter: 7.8 (3D, Auto) \| Filter: 6.8 (3D, Auto) |
| Profile Definition | Stroke Thickness 1, Normal View, Grid Distance 1 |
| Profile View | X and Y axes were set to Auto - X (Min 0.0 and Max 63.2), Y (Min 0.0 and Max 34297) |

## 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, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

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

## Z Stack - (3D) - Panel A, Showing Z-Plane 63 of 181

## Z-Stack = 181 Slices (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1503 X 1503
Image Size (Scaled) = 88.38 µm X 88.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.45 mm, Y: 46.33 mm
Stage Position = X: 74.44 mm, Y: 46.33 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## 647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639 nm @ 0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

488 - Acquired but not Shown in Data Analysis.

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488 nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493

Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.8 (3D, Auto)

## DAPI -

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 @ 0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435 - 490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

## Two-Dimensional View - Panel A

Shows Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L), together with DAPI nuclear staining. A line profile is drawn across the nucleus for quantitative assessment of fluorescence intensity in Panel B.

## Profile View Display - Panel B

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 63.2), Y (Min 0.0 and Max 34297)

## Measured at Z-plane 63 of 181

## Zen Acquisition Info Tab - Panel C

Shown are the acquisition parameters for the 647 channel corresponding to BAP1.p (Serine 592) and the DAPI channel, collected using the 639 nm and 405 nm lasers at 0.4% power, respectively. Scan zoom, scan speed, and line-averaging settings are provided to ensure transparency, reproducibility, and consistency of the fluorescence line-profile measurements acquired from the BAP1.p (Serine 592) nuclear aggregates.

The 488 channel (H2Ax) is also shown at 0.2% but was not used in the data analysis.

## Summary

Panel A shows an LSM 980 confocal optical section acquired at Z-plane 63 of 181, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit secondary antibody, together with DAPI nuclear staining. A line profile traversing the nucleus measures fluorescent intensity across both channels, demonstrating that the BAP1.p (Serine 592) signal remains predominantly confined to the nuclear compartment. The line profile additionally reveals multiple punctate regions of elevated BAP1.p (Serine 592) intensity associated with intranuclear aggregate formation following etoposide-induced DNA damage.

Panel B shows quantitative analysis of the fluorescence intensities measured from the line profile shown in Panel A. Three prominent BAP1.p (Serine 592)-associated aggregate regions were identified with measured fluorescent intensities of 32,664.00, 26,333.00, and 22,050.00 arbitrary units (A.U.), respectively. These findings support localized redistribution and concentration of phosphorylated BAP1 into discrete intranuclear signaling domains rather than a uniform increase in total nuclear-associated fluorescence. While BAP1 is constitutively present throughout the nucleus under basal conditions, genotoxic stress promotes phosphorylation-dependent spatial reorganization into punctate, chromatin-associated repair microenvironments that are involved in DNA damage response signaling and chromatin remodeling.

Panel C provides acquisition transparency by including the Zeiss ZEN imaging parameters used to collect the dataset. Recorded excitation inputs included 0.4% laser power for the 639 nm channel corresponding to Alexa Fluor™ 647 and 0.4% laser power for the 405 nm DAPI channel. See Full Summary Methods Section.

## Image Correction

## NONE

**----**

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



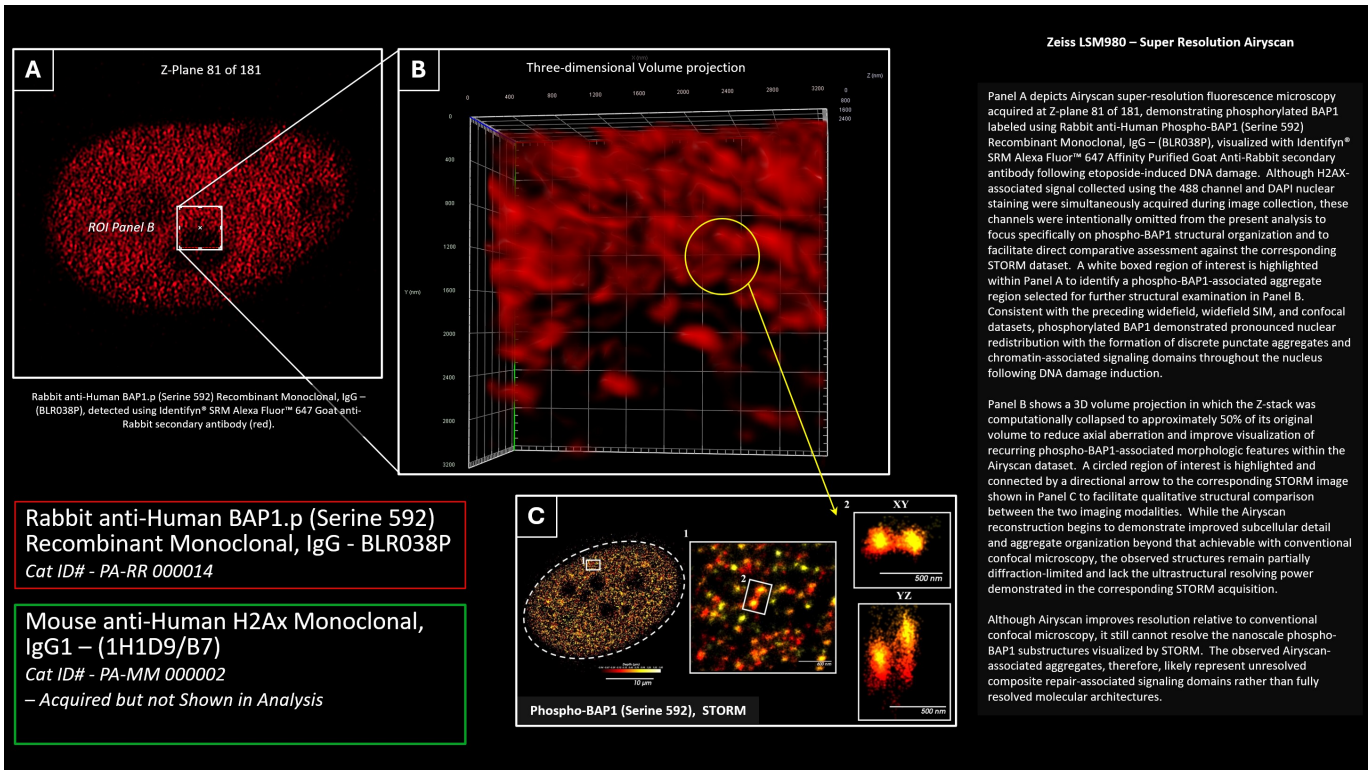
## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |

## SOURCE PROVENANCE

| | |
|---|---|
| **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** | 53 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | E95BA27DDD7695DDA6716C4A9BB5626200F97EBA5D051F261FC018BC1D58B552 |
| **Method PDF SHA-256** | 2734884F2EB35FFBB1C56EF1D8B7710CBF5565CF0F59A1729B96976C172A8A2D |

# ORIGINAL IMAGE EVIDENCE / AIRYSCAN




| | |
|---|---|
| **EVIDENCE TIER** | Airyscan |
| **INSTRUMENT** | ZEISS LSM 980 |
| **CELL MODEL** | HeLa |
| **DETECTED DYES** | 405/DAPI; 488; 647 |
| **METADATA VALUES** | 57 |

# 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 | 181 Slices (5.40 µm) |
| Channels | 3 |
| Scaling (Per Pixel) | 0.03529 µm X 0.03529 µm X 0.03000 µm |
| Image Size (Pixels) | 870 X 773 \| 93 X 92 |
| Image Size (Scaled) | 30.70 µm X 27.78 µm \| 3.28 µm X 3.25 µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 68.96 mm, Y: 49.41 mm |
| Stage Position | X: 68.96 mm, Y: 49.41 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 / 250 µm \| 5.00 AU / 214 µm |
| LMS MBS | MBS 488/639 & 405/730 |
| LMS SBS | SBS LP 640 \| SBS SP 550 \| SBS SP 505 |
| Laser Wavelength | 639 nm @ 0.3% \| 488 nm @ 0.3% \| 405 nm @ 0.3% |
| Laser Blanking | Enabled |
| Scan Mode | Frame |
| Scan Zoom | 1.9 |
| Rotation | 0° |
| Sampling | 2.00 |
| Pixel Time | 1.04 µs |
| Frame Time | 19.77 s |
| LSM Scan Speed | 6 |
| Scan Direction | Bidirectional |
| Line Step | 1 |
| Averaging | 4 |
| Excitation Wavelength | 653 \| 493 \| 353 |
| Emission Wavelength | 668 \| 517 \| 465 |
| Effective NA | 1.4 |
| Detection Wavelength | 643 - 735 \| 499 - 548 \| 422 - 477 |
| Imaging Device | Airyscan |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Type | GaAsP-PMT |
| Detector Gain | 800 V \| 500 V |
| Detector Offset | 0 |
| Detector Digital Gain | 1.0 |
| Airyscan Mode | Super Resolution: 10.0 (3D, Auto) \| Super Resolution: 8.2 (3D, Auto) |
| 3D Volume Projection | Precise |
| Appearance | Threshold 2%, Ramp 98%, Maximum 100% |
| 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, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

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

## Z Stack - (3D) - Panel A, Z-Plane 81 of 181

## Z-Stack = 181 Slices (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 870 X 773
Image Size (Scaled) = 30.70 µm X 27.78 µm
Bit Depth = 16 Bit
Image Center Position = X: 68.96 mm, Y: 49.41 mm
Stage Position = X: 68.96 mm, Y: 49.41 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## Z Stack - (3D) - Panel B

## Z-Stack = 181 Slices (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 93 X 92
Image Size (Scaled) = 3.28 µm X 3.25 µm
Bit Depth = 16 Bit
Image Center Position = X: 68.96 mm, Y: 49.41 mm
Stage Position = X: 68.96 mm, Y: 49.41 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 @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

488 - Acquired but not Shown in Data Analysis.

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493

Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

DAPI - Acquired but not Shown in Data Analysis.

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 8.2 (3D, Auto)

## 2D View - Panel A

Airyscan optical section acquired at Z-plane 81 of 181 showing Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified Goat Anti-Rabbit, IgG (H + L) (red). A white box denotes the region of interest (ROI) selected for further structural comparison in Panels B and C.

## 3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
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

The corresponding STORM dataset was generated from the same BAP1.p (Serine 592) and is shown to demonstrate the resolution limitations of Airyscan while also highlighting its improved structural detail and localization capability relative to conventional confocal microscopy. Although Airyscan provides enhanced visualization of phospho-BAP1-associated aggregate organization, the observed structures remain partially diffraction-limited compared with the nanoscale resolution achieved by single-molecule localization microscopy using STORM.

## Summary

Panel A depicts Airyscan super-resolution fluorescence microscopy acquired at Z-plane 81 of 181, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit secondary antibody following etoposide-induced DNA damage. Although H2Ax-associated signal collected using the 488 channel and DAPI nuclear staining were simultaneously acquired during image collection, these channels were intentionally omitted from the present analysis to focus specifically on phospho-BAP1 structural organization and to facilitate direct comparative assessment against the corresponding STORM dataset. A white boxed region of interest is highlighted within Panel A to identify a phospho-BAP1-associated aggregate region selected for further structural examination in Panel B. Consistent with the preceding widefield, widefield SIM, and confocal datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and chromatin-associated signaling domains throughout the nucleus following DNA damage induction.

Panel B shows a processed 3D volume rendering in which the Z-stack was computationally collapsed to approximately 50% of its original volume to reduce axial aberration and improve visualization of recurring phospho-BAP1-associated morphologic features within the Airyscan dataset. A circled region of interest is highlighted and connected by a directional arrow to the corresponding STORM image shown in Panel C to facilitate qualitative structural comparison between the two imaging modalities. While the Airyscan reconstruction begins to demonstrate improved subcellular detail and aggregate organization beyond that achievable with conventional confocal microscopy, the observed structures remain partially diffraction-limited and lack the nanoscale structural resolving power demonstrated in the corresponding STORM acquisition.

Importantly, although Airyscan approximately doubles lateral resolution relative to traditional confocal imaging through detector-array reassignment and computational deconvolution approaches, the modality remains fundamentally constrained in its ability to resolve the nanoscale substructures and aggregate-associated architectural organization visualized using STORM. As such, while broad morphologic similarities between the Airyscan and STORM datasets may be appreciated within selected regions, direct one-to-one structural interpretation should be approached cautiously, as the apparent Airyscan-associated aggregate morphology likely represents unresolved composite signal originating from multiple underlying nanoscale phospho-BAP1-associated repair domains. These findings remain highly consistent with the progressive resolution hierarchy observed throughout the preceding stepdown datasets, collectively demonstrating how increasing optical resolving power incrementally reveals additional levels of phospho-BAP1 spatial organization within DNA damage-associated chromatin microenvironments.

## Image Correction

## NONE

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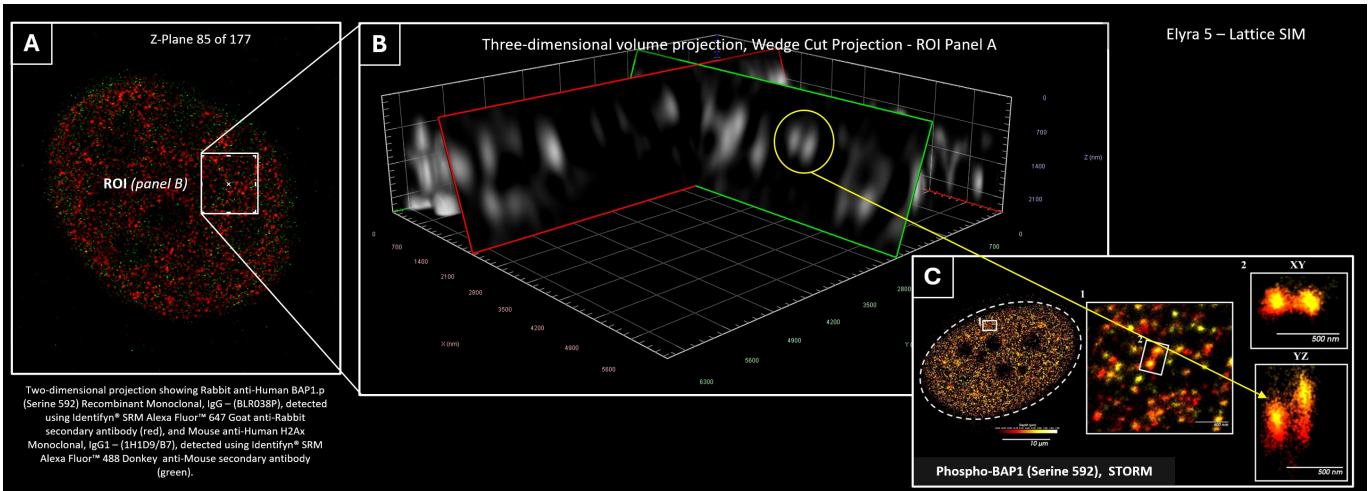
Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett



## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **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** | 57 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | B93C475FE1C6B41FD0DF720B0A5EC32CFA05AFA68C8CC7B33F01B2E499FF5BEC |
| **Method PDF SHA-256** | 867352FA167FE9642B8A3769741B12D1812207FB7D6197FCE649573C2295224B |

# ORIGINAL IMAGE EVIDENCE / SIM




Two-dimensional projection showing Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 Goat anti-Rabbit secondary antibody (red), and Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7), detected using Identifyn® SRM Alexa Fluor™ 488 Donkey anti-Mouse secondary antibody (green).

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - BLR038P
*Cat ID# - PA-RR 000014*

Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)
*Cat ID# - PA-MM 000002*

Panel A shows Lattice SIM super-resolution fluorescence microscopy acquired at Z-plane 85 of 177 demonstrating phosphorylated BAP1 detected using Rabbit anti-Human Phospho-BAP1 (Serine 592) Recombinant Monoclonal, IgG – (BLR038P), visualized with Identifyn® SRM Alexa Fluor™ 647 affinity-purified Goat Anti-Rabbit $F(ab')_2$ (H + L). H2Ax was simultaneously acquired using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey Anti-Mouse $F(ab')_2$ (H + L). A white box denotes the region of interest (ROI) selected for further examination in Panel B.

Panel B shows a processed three-dimensional volume-rendered reconstruction of the ROI identified in Panel A. The phospho-BAP1-associated 647 signal was pseudo colored white to enhance visual contrast, while the H2Ax channel was omitted from the processed rendering. Wedge-view processing was performed using X-Z and Y-Z clipping planes pseudocolored green and red, respectively, positioned at approximately 15-degree angles to expose internal Z-axis structural organization and facilitate comparison with the corresponding STORM dataset shown in Panel C.Consistent with the corresponding STORM dataset, Lattice SIM demonstrated recurring phospho-BAP1-associated morphologies within both the XY and YZ subsets, providing substantially improved structural visualization relative to preceding imaging modalities while remaining below the nanoscale resolving capability achieved using STORM.

For a More Thorough Summary see Accompanying Methods Section.

| | |
|---|---|
| **EVIDENCE TIER** | SIM |
| **INSTRUMENT** | ZEISS Elyra |
| **CELL MODEL** | HeLa |
| **DETECTED DYES** | 405/DAPI; 488; 647 |
| **METADATA VALUES** | 63 |

## STRUCTURED ACQUISITION METADATA / SIM

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye: |
| Objective | Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens |
| Z-Stack | 177 Slices (5.28 µm) \| 80 Slices (2.37 µm) - Displayed gallery content was reduced to Phospho-BAP1-specific signal. |
| Channels | 2 |
| Scaling (Per Pixel) | 0.02111 µm X 0.02111 µm X 0.03000 µm |
| Image Size (Pixels) | 1927 X 2049 \| 295 X 311 |
| Image Size (Scaled) | 40.68 µm X 43.26 µm \| 6.23 µm X 6.57 µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 66.86 mm, Y: 36.62 mm |
| Stage Position | X: 66.86 mm, Y: 36.62 mm |
| ROI Center Offset | X: 0.00 µm, Y: 0.00 µm |
| Reflectors | SR HE LBF 405/488/561/642 |
| Beam Splitter | 405, 488, 561, 642 |
| Filter Excitation Wavelength | 200 - 405, 200 - 488, 200 - 561, 200 - 642 |
| Filter Emission Wavelength | 419 - 470, 503 - 546, 576 - 617, 657 - 800 |
| Contrast Method | Fluorescence |
| Illumination Wavelength | 640 \| 488 |
| Channel Name | T1-Axiocam 820-SR \| T2-Axiocam 820-SR |
| Excitation Wavelength | 640 \| 488 |
| Emission Wavelength | 729 \| 525 |
| Effective NA | 1.4 |
| Detection Wavelength | 419 - 470, 503 - 546, 576 - 617, 657 - 800 \| 503 - 546, 657 - 800 |
| Imaging Device | Axiocam 820 |
| Camera Adapter | 1x |
| Exposure Time | 100 ms \| 120 ms |
| Depth of Focus | 1.13 µm \| 0.81 µm |
| Binning Mode | 2,2 |
| Laser Wavelength | 640 nm @ 1.00% \| 488 nm @ 1.00% |
| Frame Time | 1.33 s \| 1.59 s |
| Scan Direction | Unidirectional |
| Grating | 36.5 µm grating \| 27.5 µm grating |
| Processing Dimension | 3D |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Result Sampling | 4 |
| Process Sampling | N/A |
| Algorithm | SIM |
| SIM Settings | Standard |
| Auto Sharpness | On |
| Sharpness | 10.3458 (647), 11.5304 (488) |
| Fitting | Fast Fit |
| Normalization | Auto |
| 3D Volume Projection | Precise |
| Appearance | Threshold 2%, Ramp 98%, Maximum 100% |
| Background | Black |
| Light | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping |
| Projection | View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) |
| 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 | -30% \| -36% |
| Clip Y | -15° \| 15° |
| Clip Z | 0° |
| Y-Z | Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. |

## SOURCE METHOD

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

### Identifyn Standards for Optical Microscopy Methods™

### Identifyn® Primary Antibody

Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

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

## Z Stack - (3D) - Panel A, Z-Plane 85 of 177

## Z-Stack = 177 Slices (5.28 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1927 X 2049
Image Size (Scaled) = 40.68 µm X 43.26 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.86 mm, Y: 36.62 mm
Stage Position = X: 66.86 mm, Y: 36.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 80 Slices (2.37 µm) - Displayed gallery content was reduced to Phospho-BAP1-specific signal.

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 295 X 311
Image Size (Scaled) = 6.23 µm X 6.57 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.86 mm, Y: 36.62 mm
Stage Position = X: 66.86 mm, Y: 36.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## 647 -

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

## 488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 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 @ 1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

## Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.3458 (647), 11.5304 (488)
Fitting = Fast Fit
Normalization = Auto

## 2 Dimensional View - Panel A

Shows an Elyra 5 Lattice SIM optical section acquired at Z-plane 85 of 177, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Goat anti-Rabbit secondary antibody, and Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Donkey anti-Mouse secondary antibody. A white box denotes the region of interest (ROI) selected for further examination in Panel B.

## 3D Image Processing - Panel B, Showing Phospho-BAP1/647 only

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Clipping Plane Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P) within the nuclear compartment by adjusting or cutting away portions of the image using wedge cut processing, removing parts of the image in the X-Z and Y-Z planes.

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 = -30%
## Clip Y = -15°
## Clip Z = 0°

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

## Position of Clip = -36%
## Clip Y = 15°
## Clip Z = 0°

Panel B shows a wedge-cut volume-rendered view from the top with a slight backward rotation, highlighting the wedge-removal region and revealing BAP1.p (Serine 592)-associated signal distribution throughout the z-stack. This orientation provides XZ and YZ visualization planes for structural comparison against the corresponding STORM dataset.

## STORM - Panel C

The corresponding STORM dataset was generated from the same BAP1.p (Serine 592) and is shown to demonstrate the enhanced nanoscale structural resolution achieved relative to the corresponding Lattice SIM dataset, while also highlighting the overall consistency in BAP1.p-associated aggregate morphology and spatial organization observed between the two imaging modalities.

## Summary

Panel A depicts Lattice SIM super-resolution fluorescence microscopy acquired at Z-plane 85 of 177, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified Goat Anti-Rabbit, IgG (H + L), together with H2Ax detected using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey Anti-Mouse, IgG (H + L). A white box denotes the region of interest (ROI) selected for further structural examination in Panel B. Consistent with the preceding widefield, widefield SIM, confocal, and Airyscan datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and DNA damage-associated chromatin signaling domains following etoposide-induced DNA damage.

Panel B shows a processed three-dimensional volume-rendered reconstruction of the ROI identified in Panel A. For visualization purposes, the phospho-BAP1-associated 647 signal was pseudocolored white rather than the default red to enhance visual contrast and improve assessment of aggregate-associated structural organization. The H2Ax-associated channel was omitted from the processed rendering to focus specifically on phospho-BAP1 morphology and facilitate direct comparison with the corresponding STORM dataset shown in Panel C. Wedge-view processing was employed using both X-Z and Y-Z clipping planes, with the X-Z plane pseudocolored green and the Y-Z plane pseudocolored red. The cut planes were positioned at approximately 15-degree angles to expose the internal Z-axis structural organization and to generate a volumetric perspective that closely matches the Y-Z subset visualization shown in the lower-right region of the corresponding STORM image.

Importantly, Lattice SIM demonstrated substantial improvements in evaluating phospho-BAP1 localization, aggregate organization, and volumetric structural distribution compared with prior imaging modalities. While the modality does not fully achieve the nanoscale resolution of STORM, both the XY and YZ subsets demonstrated recurring morphological similarities and consistent aggregate-associated organizational patterns across the two imaging platforms. These findings suggest that Lattice SIM provides a biologically consistent intermediate-resolution representation of phospho-BAP1-associated repair domains, enabling substantially improved structural interpretation while preserving broader contextual nuclear architecture that may become fragmented or abstracted in single-molecule localization microscopy datasets such as STORM.

## Image Correction

## NONE

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



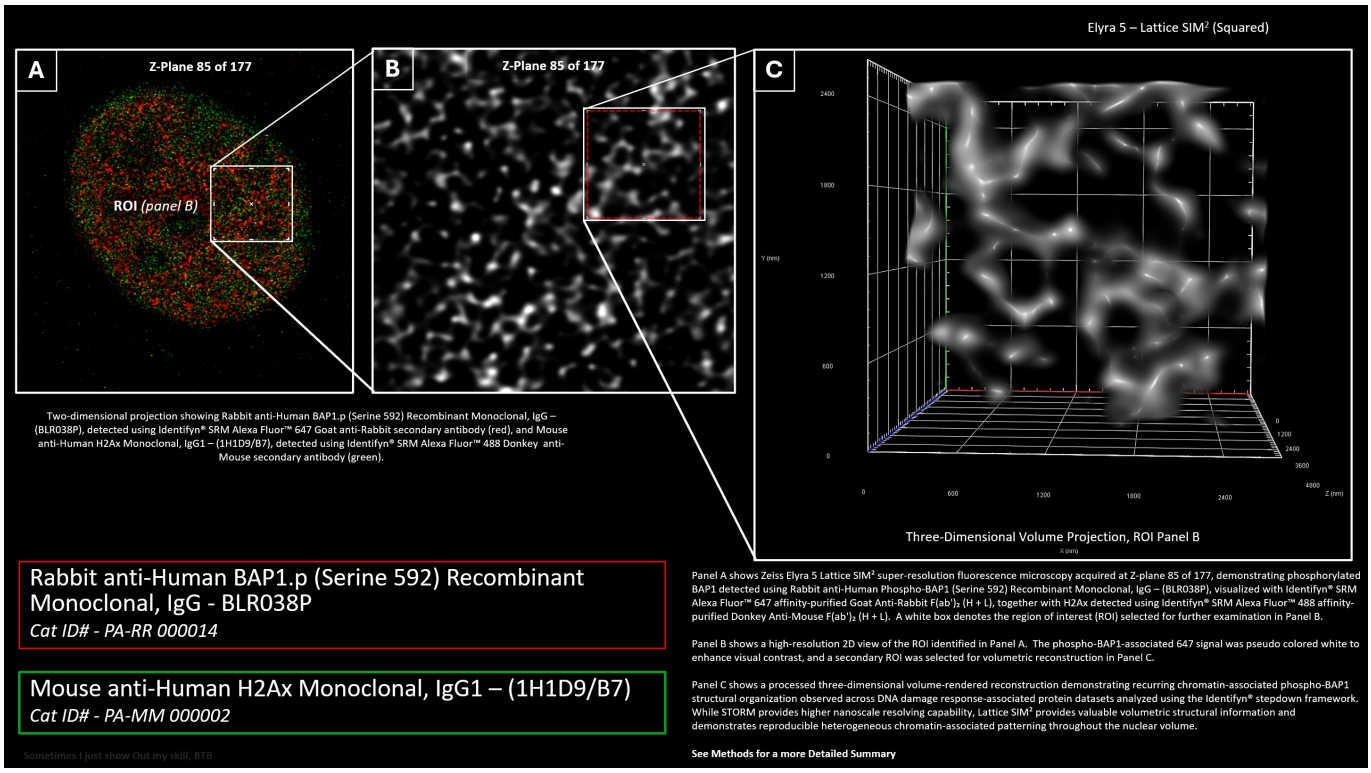
## SOURCE PROVENANCE

| | |
|---|---|
| Catalog | PA-RR-000014 |
| Stage | SIM |

## SOURCE PROVENANCE

| | |
|---|---|
| **Instrument** | ZEISS Elyra |
| **Microscope configuration** | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye: |
| **Structured source metadata values** | 63 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 70A21EB3F8335490587C233AFF73F7E15BF0C60A58AAD20C582BFA25328609A8 |
| **Method PDF SHA-256** | 970D7D633978DCEDCA92A482DB5D6F1E0ECE119F2D60219AD1BF4AB4CA2BD3E2 |

# ORIGINAL IMAGE EVIDENCE / SIM²




| | |
|---|---|
| **EVIDENCE TIER** | SIM² |
| **INSTRUMENT** | ZEISS Elyra |
| **CELL MODEL** | HeLa |
| **DETECTED DYES** | 405/DAPI; 488; 647 |
| **METADATA VALUES** | 57 |

## STRUCTURED ACQUISITION METADATA / SIM²

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye: |
| Objective | Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens |
| Z-Stack | 177 Slices (5.28 µm) |
| Channels | 2 |
| Scaling (Per Pixel) | 0.02111 µm X 0.02111 µm X 0.03000 µm |
| Image Size (Pixels) | 1988 X 2024 \| 426 X 403 \| 132 X 125 |
| Image Size (Scaled) | 41.97 µm X 42.73 µm \| 8.99 µm X 8.51 µm \| 2.79 µm X 2.64 µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 66.86 mm, Y: 36.62 mm |
| Stage Position | X: 66.86 mm, Y: 36.62 mm |
| ROI Center Offset | X: 0.00 µm, Y: 0.00 µm |
| Reflectors | SR HE LBF 405/488/561/642 |
| Beam Splitter | 405, 488, 561, 642 |
| Filter Excitation Wavelength | 200 - 405, 200 - 488, 200 - 561, 200 - 642 |
| Filter Emission Wavelength | 419 - 470, 503 - 546, 576 - 617, 657 - 800 |
| Contrast Method | Fluorescence |
| Illumination Wavelength | 640 \| 488 |
| Channel Name | T1-Axiocam 820-SR \| T2-Axiocam 820-SR |
| Excitation Wavelength | 640 \| 488 |
| Emission Wavelength | 729 \| 525 |
| Effective NA | 1.4 |
| Detection Wavelength | 419 - 470, 503 - 546, 576 - 617, 657 - 800 \| 503 - 546, 657 - 800 |
| Imaging Device | Axiocam 820 |
| Camera Adapter | 1X |
| Exposure Time | 100 ms \| 120 ms |
| Depth of Focus | 1.13 µm \| 0.81 µm |
| Binning Mode | 2,2 |
| Laser Wavelength | 640 nm @ 1.00% \| 488 nm @ 1.00% |
| Frame Time | 1.33 s \| 1.59 s |
| Scan Direction | Unidirectional |
| Grating | 36.5 µm grating \| 27.5 µm grating |
| Result Sampling | 4 |
| Process Sampling | 4 |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Algorithm | SIM2 |
| Input SNR | Low |
| Regularization Weight | 0.0000 |
| Iterations | 3 |
| Filter | No Filter |
| Fitting | Fast Fit |
| Normalization | Auto |
| 3D Volume Projection | Precise |
| Appearance | Threshold 2%, Ramp 98%, Maximum 100% |
| 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, IgG - (BLR038P)
Cat ID# PA-RR 000014

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

### Identifyn® Secondary Antibodies

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

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker cat#37527X3.
The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), 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 Donkey Anti-Mouse, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

## Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:
Z Stack - (3D) - Panel A, Z-Plane 85 of 177

## Z-Stack = 177 Slices (5.28 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1988 X 2024
Image Size (Scaled) = 41.97 µm X 42.73 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.86 mm, Y: 36.62 mm
Stage Position = X: 66.86 mm, Y: 36.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## Z Stack - (3D) - Panel B, Z-Plane 85 of 177

## Z-Stack = 177 Slices (5.28 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 426 X 403
Image Size (Scaled) = 8.99 µm X 8.51 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.86 mm, Y: 36.62 mm
Stage Position = X: 66.86 mm, Y: 36.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## Z Stack - (3D) - Panel C, Three Dimensional Volume View

## Z-Stack = 177 Slices (5.28 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 132 X 125
Image Size (Scaled) = 2.79 µm X 2.64 µm
Bit Depth = 16 Bit
Image Center Position = X: 66.86 mm, Y: 36.62 mm
Stage Position = X: 66.86 mm, Y: 36.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## 647 -

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

### 488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 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 @ 1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

## Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

## 2 Dimensional View - Panel A

Shows an Elyra 5 Lattice SIM2 optical section acquired at Z-plane 85 of 177, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), visualized using Identifyn® SRM Alexa Fluor™ 647 Goat anti-Rabbit secondary antibody, and Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Donkey anti-Mouse secondary antibody. A white box denotes the region of interest (ROI) selected for further examination in Panel B.

## 2 Dimensional View - Panel B

Region of interest from Panel A showing phospho-BAP1/647 pseudocolored white to enhance structural contrast. A white box denotes an additional ROI for further examination in Panel C.

## 3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
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)

## Summary

Panel A depicts Zeiss Elyra 5 Lattice SIM² super-resolution fluorescence microscopy acquired at Z-plane 85 of 177, demonstrating Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), detected using Identifyn® SRM Alexa Fluor™ 647 affinity-purified Goat Anti-Rabbit, IgG (H + L), together with H2Ax detected using Identifyn® SRM Alexa Fluor™ 488 affinity-purified Donkey Anti-Mouse, IgG (H + L). A white box denotes the region of interest (ROI) selected for further structural examination in Panel B. Consistent with the preceding widefield, widefield SIM, confocal, Airyscan, and Lattice SIM datasets, phosphorylated BAP1 demonstrated pronounced nuclear redistribution with the formation of discrete punctate aggregates and chromatin-associated DNA damage response domains following etoposide-induced DNA damage.

Panel B shows a high-resolution 2D view of the ROI identified in Panel A. For visualization purposes, the phospho-BAP1-associated 647 signal was pseudocolored white rather than the default red to improve visual contrast and facilitate assessment of fine structural organization. Within this region, a secondary ROI highlighting a representative phospho-BAP1-associated structural assembly was selected for further volumetric reconstruction and examination in Panel C.

Panel C shows a processed three-dimensional volume-rendered reconstruction of the ROI identified in Panel B. Unlike the preceding Airyscan and Lattice SIM datasets, direct comparison with STORM was intentionally omitted in this dataset because the observed volumetric structural organization demonstrated highly recurring and reproducible morphologic patterns consistently identified across thousands of DNA damage response-associated protein datasets analyzed using the Identifyn® stepdown framework. At the resolution achieved using Lattice SIM², chromatin-associated structural assemblies become readily visible throughout the nuclear volume, demonstrating reproducible heterogeneous chromatin-associated patterning across phospho-BAP1-associated repair domains.

Importantly, while STORM continues to provide the highest structural resolution and approximate ground-truth localization performance at ~20 nm resolution, Lattice SIM² offers substantial advantages in volumetric structural interpretation, achieving ~40 nm lateral resolution in the X and Y axes while preserving highly coherent three-dimensional contextual architecture across extended Z-stack acquisitions. As such, Lattice SIM² provides a complementary super-resolution perspective distinct from STORM, enabling improved visualization of chromatin-associated volumetric repair structures and heterogeneous nuclear organizational patterning that may be less readily appreciated within highly localized single-molecule reconstruction datasets alone.

## Image Correction

## NONE

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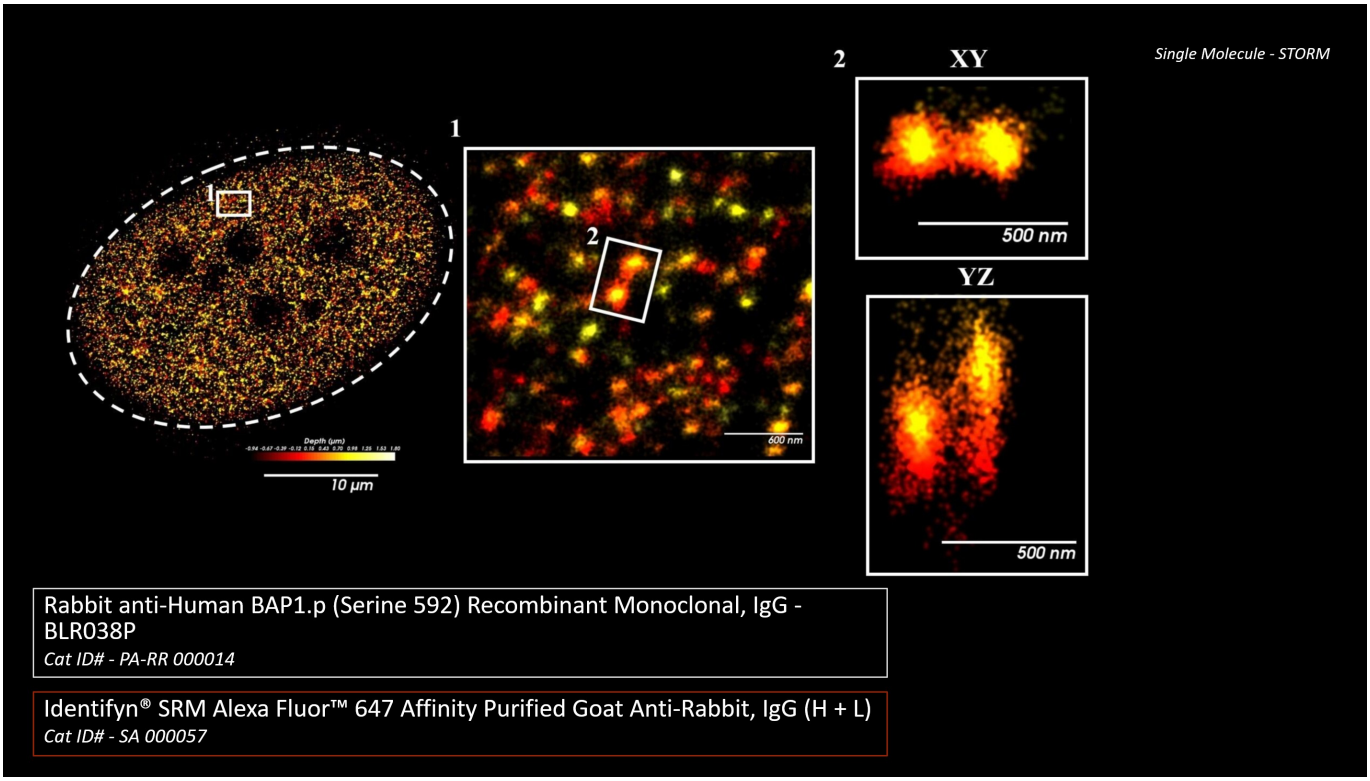
Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **Stage** | SIM² |
| **Instrument** | ZEISS Elyra |
| **Microscope configuration** | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye: |
| **Structured source metadata values** | 57 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 575645AD61C38025E603D6A798D7A8532541938D67579BE7472AC3B233A55BCE |
| **Method PDF SHA-256** | 701F9AEE8064BA50D130459E4FB01077600C07695D79A6200CBEA2369ACF557C |

# ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM




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

# STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters: |
| Objective | Olympus 60x/1.30 Silicon Oil objective |
| Number of Probes | 1 |
| Number of Simultaneous Probes | 1 |
| Number of Reference Probes | 1 |
| Camera | Both Cameras |
| Color Channel | Both Channels |
| Probe Capture Mode | Interleaved |
| Z -Stack Capture Mode | Interleaved |
| Multiple Location Capture | Not selected |
| Post Record Reference | Not Selected |
| Fluidics | Not Selected |
| Autofocus Drift Correction | On |
| Biplane Module | 635 nm Dichroic |
| Objective | Silicon Selected |
| Stage Insert | Rectangular Selected |
| Coarse Focus Position | 8-Well Chamber Selected |
| Dichroic | SuperRes |
| PSF | (Red, Orange) |
| Heater | Current: 26 |
| Recording Vertical Field of View | 100% |
| Widefield Camera Exposure Time | 100 ms \| 20 ms |
| Super Resolution Camera Exposure Time | 20 ms |
| Widefield Camera Binning | 1 |
| Per-Probe Exposure Time | Selected |
| Predefined Probe | First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic) |
| Photoactivation/Imaging Laser | 0% Selected |
| Exposure Time | 20 ms Selected |
| Record Workflow | Selected Options |
| View Mode | Widefield 200 µm |
| Capture Mode | Live |
| Piezo Current | 184 µm |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Widefield 640nm | 0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input |
| Reference Probe 1 Laser control 640 nm (635 nm Dichroic) | Not Selected |
| Widefield camera Exposure Time | 100 ms |
| Widefield Laser | "Widefield 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 | 50 µm is selected |
| Cell(s) or Cellular Structure Localization | Positioning of the target Cell or Subsection of the Target Cell is optimized and focused. |
| Piezo Record | Begin: 184; End: 185 |
| SuperRes 640nm | 0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input |
| Record Workflow - Record Tab | Capture Parameters |
| Frames | Probe 1: 200 |
| Z Positions | 5 |
| Cycles | 30 |
| Time Points | 1 |
| Pause Between Cycles | Not Selected |
| Automatic Photoactivation Laser Control | Not Selected |
| Laser Power | 30 |
| 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: 15 |
| Sizing Mode | Constant |
| Particle size | 20 |
| Color by | Depth |
| Color Table | Single |
| Opacity Mode | Depth; Opacity 1: 0.4 |
| Front-to-back Attenuation | Not Selected |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| 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, IgG - (BLR038P)
Cat ID# - PA-RR 000014

### Identifyn® Secondary Antibody

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## 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 20 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal - (BLR038P) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3 times in Identifyn®'s proprietary Wash Buffer, and Identifyn®'s 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber enabling image acquisition.

## Microscope

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

## Calibration -

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

## https://identifyn.com/storm-calibration

## Image Acquisition -

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

## Configuration Tab - Capture Configuration

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

## Microscope Configuration

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

## Camera Configuration

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

## Probe Configuration

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

## Experiment Configuration

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

## Stage and Piezo Selection

## Examination of the Sample

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

## Piezo Current: 184 µm

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

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

## Advanced Tools Option

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

Widefield Laser: "Widefield 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: 50 µm is selected

### Capture Mode

### Live

### Stage and Piezo Selection

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

### Piezo Record: Begin: 184; End: 185

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

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

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

### Not Selected

### Advanced Tools

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

### Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 5
Cycles: 30
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: 30

### 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: 15

## Region of Interest -

## Not selected

## Display -

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

## Record Workflow - View Tab

## Region of Interest

## Default values

## Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 20
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.4
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

---

## Image by P. Raut

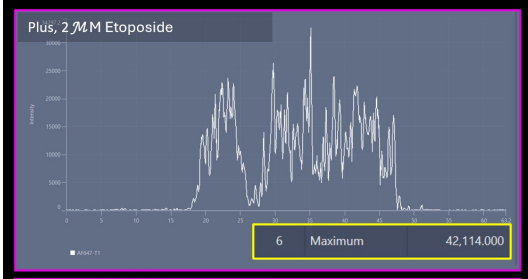


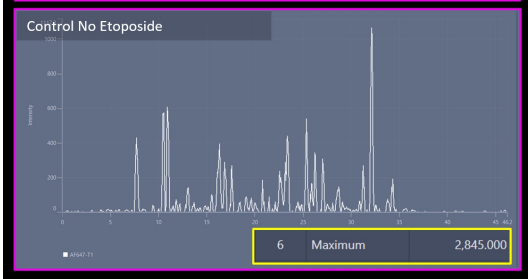
## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **Stage** | Single-molecule STORM |
| **Instrument** | Bruker Vutara VXL |
| **Microscope configuration** | Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters: |
| **Structured source metadata values** | 80 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | BDA2E22CA611F9E3A5B46D0DFC3B1FF0E8300A7BF27C0596D5891060724BCE80 |
| **Method PDF SHA-256** | FFF9D210B35BFAC4666EB4FEBFAE6AA046685904764325B822482C12A8EBDB2E |

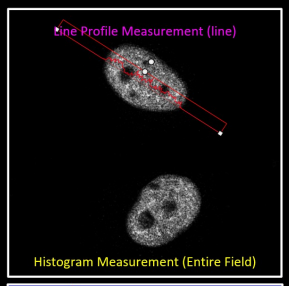
# ORIGINAL IMAGE EVIDENCE / CONTROL

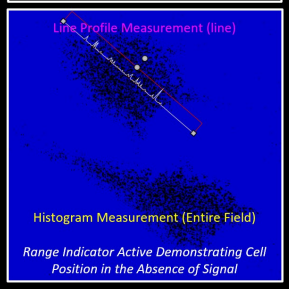
Line Profile of Foci/Aggregate Fluorescent Intensities Across the Nucleus




Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - BLR038P
*Cat ID# - PA-RR 000014*




*Zeiss LSM980 NIR - Confocal*

*Laser power, line step, averaging, detector voltage, and detector gain were identical across all conditions (see Methods).*

Histogram Measurement Mean Fluorescent Intensity

| No. | Description | AF647-T1 |
|---|---|---|
| 1 | Arithmetic mean intensity | 1,400.456 |

Maximum and Mean Fluorescence Intensity of Phospho-BAP1 (Serine 592)/647 Following Etoposide-Induced DNA Damage (Arbitrary Units, A.U.). In the Presence of Etoposide, the Maximum Foci/Aggregate Signal observed was 42,114.000

Histogram Measurement Mean Fluorescent Intensity

| No. | Description | AF647-T1 |
|---|---|---|
| 1 | Arithmetic mean intensity | 9.616 |

Maximum and Mean Fluorescence Intensity of Phospho-BAP1 (Serine 592)/647 in the absence of DNA Damage (Arbitrary Units, A.U.). In the absence of Etoposide, the Maximum Foci/Aggregate Signal observed was 9.616

Untreated cells demonstrated minimal phosphorylated BAP1 (Serine 592)-associated fluorescence with no meaningful punctate nuclear aggregates observed, consistent with the absence of DNA damage-induced signaling activity. Due to the near-complete absence of a detectable signal in control cells, the range indicator was activated in the lower line-profile image to demonstrate cellular positioning. Following etoposide-induced DNA damage, phosphorylated BAP1 showed marked nuclear signal enrichment and discrete aggregate formation consistent with DNA damage-associated chromatin signaling domains. Quantitative analysis demonstrated an arithmetic mean increase in fluorescent intensity from 9.616 A.U. in untreated cells to 1,400.456 A.U. following damage induction, representing an approximate 145.6-fold increase. Maximal aggregate intensity increased from 2,845.000 A.U. to 42,144.000 A.U., corresponding to an approximate 14.8-fold increase in peak focal signal intensity. For more See Accompanying Methods Document.

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

## STRUCTURED ACQUISITION METADATA / CONTROL

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye: |
| Objective | Plan-Apochromat 63x/1.40 Oil DIC M27 |
| Z-Stack | 181 Slices (5.40 µm) \| 161 Slices (4.80 µm) |
| Channels | 1 |
| Scaling (Per Pixel) | 0.059 µm X 0.059 µm X 0.030 µm |
| Image Size (Pixels) | 1503 X 1503 \| 1248 X 1248 |
| Image Size (Scaled) | 88.38 µm X 88.38 µm \| 73.41 µm X 73.41 µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 74.45 mm, Y: 46.33 mm \| X: 70.82 mm, Y: 52.34 mm |
| Stage Position | X: 74.44 mm, Y: 46.33 mm \| X: 70.82 mm, Y: 52.34 mm |
| ROI Center Offset | X: 0.00 µm, Y: 0.00 µm |
| Reflector | None |
| Contrast Method | Fluorescence |
| Pinhole | 1.00 AU / 65 µm |
| LMS MBS | MBS 488/639 & 405/730 |
| LMS SBS | SBS SP 615 |
| Laser Wavelength | 639 nm @ 0.4% \| 639 nm @ 0.6% - |
| Laser Blanking | Enabled |
| Scan Mode | Frame |
| Scan Zoom | 1.5 \| 2.1 |
| Rotation | 0° |
| Sampling | 1.20 |
| Pixel Time | 0.26 µs \| 0.27 µs |
| Frame Time | 5.63 s \| 4.10 s |
| LSM Scan Speed | 11 \| 12 |
| Scan Direction | Bidirectional |
| Line Step | 1 |
| Averaging | 8 |
| Excitation Wavelength | 653 |
| Emission Wavelength | 668 |
| Effective NA | 1.4 |
| Detection Wavelength | 640 - 732 |
| Imaging Device | GaAsP-Pmt3 |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Type | GaAsP-PMT |
| Detector Gain | 500 V |
| Detector Offset | 0 |
| Detector Digital Gain | 1.0 |
| LSM Plus Mode | Filter: 7.5 (3D, Auto) \| Filter: 8.9 (3D, Auto) |
| Profile Definition | Stroke Thickness 1.00, Normal View, Grid Distance 1 |
| Profile View | X and Y axes were set to Auto - X (Min 0.0 and Max 63.2), Y (Min 0.0 and Max 34297) \| X and Y axes were set to Auto - X (Min 0.0 and Max 46.2), Y (Min 0.0 and Max 1117) |
| Histogram Definition | Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535 |
| Histogram View | X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 2000000) |
| Arithmetic Mean intensity | 1,400.456 \| 9.616 |

## 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, IgG - (BLR038P)
Cat ID# PA-RR 000014

### Identifyn® Secondary Antibodies

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

BAP1 (BRCA1-associated protein 1) is a nuclear deubiquitinase (DUB) that plays central roles in chromatin remodeling, transcriptional regulation, DNA repair, and maintenance of genomic stability. While classically associated with homologous recombination (HR) and double-strand break (DSB) repair pathways, increasing evidence suggests that phosphorylated BAP1 (BAP1.p) also contributes functionally to base excision repair (BER) and broader chromatin-associated DNA damage response signaling.

Phosphorylation of BAP1 occurs at multiple regulatory residues, with Serine 592 (S592) representing the most well-characterized DNA damage-associated phosphorylation site. S592 phosphorylation is mediated primarily through ATM and ATR signaling pathways following replication stress, single-strand breaks (SSBs), and double-strand breaks (DSBs), with ATR likely serving as the dominant kinase under replication-associated stress conditions. Additional phosphorylation sites, including Serine 718 (S718) and the putative ATM-responsive Threonine 599 (T599), may further regulate chromatin remodeling activity and the integration of DNA damage signaling. These phosphorylation events alter BAP1 localization, substrate accessibility, and protein interaction dynamics within damage-responsive chromatin environments.

Functionally, BAP1.p retains deubiquitinase activity and contributes to chromatin relaxation through the removal of ubiquitin marks from histone substrates, including H2Aub, H2AK119ub1, and H2AK15ub. This reduction in chromatin compaction likely enhances accessibility for DNA repair machinery, including BER-associated proteins such as OGG1, APE1, and POLβ. In this context, phosphorylated BAP1 may stabilize open chromatin domains surrounding DNA lesions, facilitating efficient lesion processing and repair. Emerging evidence also suggests that BAP1.p indirectly influences PARP1-associated signaling dynamics, potentially modulating PARP1 activity during early BER initiation and preventing excessive PARylation-dependent chromatin stress.

Importantly, BAP1 phosphorylation positions the protein as an integrative downstream effector linking BER, chromatin state regulation, and broader ATM/ATR-mediated DNA damage response pathways. Following etoposide-induced genotoxic stress, the observed nuclear redistribution and aggregate formation of BAP1.p are therefore biologically consistent with recruitment into DNA damage-associated chromatin microenvironments involved in repair coordination, chromatin accessibility regulation, and checkpoint-associated signaling.

## Method

### Plus Damage -

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 20 hours, followed by -20°C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

### Minus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human BAP1.p (Serine 592) Recombinant Monoclonal, IgG - (BLR038P), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

### Microscope

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

### Z Stack - (3D) - Panel A, Showing Z-Plane 63 of 181

### Z-Stack = 181 Slices (5.40 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1503 X 1503
Image Size (Scaled) = 88.38 µm X 88.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 74.45 mm, Y: 46.33 mm
Stage Position = X: 74.44 mm, Y: 46.33 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

### Z-Stack = 161 Slices (4.80 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1248 X 1248
Image Size (Scaled) = 73.41 µm X 73.41 µm
Bit Depth = 16 Bit
Image Center Position = X: 70.82 mm, Y: 52.34 mm
Stage Position = X: 70.82 mm, Y: 52.34 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

## 647 - Plus Etoposide, Upper Panel

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639 nm @ 0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26 µs
Frame Time = 5.63 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

## 647 - No Etoposide, Lower Panel - Control

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615

## Laser Wavelength = 639 nm @ 0.6% -

A higher image input setting was required in the untreated control sample to visualize the absence of a detectable phospho-BAP1-associated signal.

Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27 µs
Frame Time = 4.10 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 = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.9 (3D, Auto)

## Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1.00, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 63.2), Y (Min 0.0 and Max 34297)

This panel measures Phospho-BAP1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 40K A.U. at its maximum.

Measured at the Z-plane, 68 of 181.

## Profile View Display - Minus DNA Damage, Bottom

## Profile Definition = Stroke Thickness 1.00, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 46.2), Y (Min 0.0 and Max 1117)

This panel measures Phospho-BAP1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~3K A.U. at its maximum.

Measured at the Z-plane, 81 of 161.

## Histogram Display - Plus DNA Damage, Top

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 2000000)

## Arithmetic Mean intensity = 1,400.456

The histogram was generated from the entire image field to quantify the overall Phospho-BAP1 response following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

## Histogram Display - Minus DNA Damage, Bottom

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 2000000)

## Arithmetic Mean intensity = 9.616

The histogram was generated from the entire image field to quantify the overall Phospho-BAP1 protein level in the absence of DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

## Summary

In untreated cells, the phosphorylated BAP1 signal was effectively absent, with only minimal background-associated fluorescence observed throughout the nucleus. This finding is consistent with the tightly regulated nature of BAP1 phosphorylation and supports the expectation that phospho-BAP1-associated signaling events occur predominantly following genotoxic stress and activation of the DNA damage pathway. In the untreated control population, no meaningful punctate nuclear aggregates or damage-associated signaling domains were identified, and the residual low-level signal appeared diffuse and non-structured in distribution. Due to the near-complete absence of detectable phospho-BAP1 signal in these control cells, the range indicator was activated in the lower line-profile image to demonstrate cellular positioning and confirm that the absence of signal corresponded to true biological signal loss rather than the absence of cellular material within the sampled region.

Following etoposide-induced DNA damage, phosphorylated BAP1 showed a pronounced increase in both overall nuclear-associated fluorescence intensity and focal signal enrichment, with the formation of discrete nuclear aggregates consistent with DNA damage-associated chromatin signaling domains. Quantitative analysis showed an arithmetic mean fluorescent intensity of 1,400.456 A.U. in the damaged population, compared with 9.616 A.U. in untreated cells, representing an approximate 145.64-fold increase in the phosphorylated BAP1-associated signal following DNA damage induction. Line-profile and histogram analysis further supported this marked redistribution and enrichment response, with maximal aggregate intensity increasing from 2,845.000 A.U. in untreated cells to 42,144.000 A.U. following etoposide exposure, corresponding to an approximate 14.8-fold increase in peak focal signal intensity.

Biologically, these findings are consistent with phosphorylation-dependent recruitment and stabilization of BAP1 within DNA damage response-associated chromatin microenvironments following double-strand break induction and replication-associated stress signaling. The observed punctate enrichment pattern likely reflects clustered repair-associated signaling domains and localized chromatin-remodeling events rather than isolated molecular assemblies. At diffraction-limited resolution, focal overlap with additional DNA damage response-associated proteins should be interpreted as coordinated pathway engagement within shared nuclear repair microenvironments rather than as direct evidence of molecular interaction.

## Image Correction

The phospho-BAP1 channel (647) was displayed in grayscale to enhance visual contrast.

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


## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | PA-RR-000014 |
| **Stage** | Control |
| **Instrument** | ZEISS LSM 980 |

## SOURCE PROVENANCE

| | |
|---|---|
| **Microscope configuration** | Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye: |
| **Structured source metadata values** | 54 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 62A771A84F75BBB2846A3B5D81316BEEDBC3DB8E38AAC31B05E7517EEB48E523 |
| **Method PDF SHA-256** | 8C8BD0116EC0A8F058C1BC3E65A071F62015E4A08804E01FC018E2460D30A604 |