BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

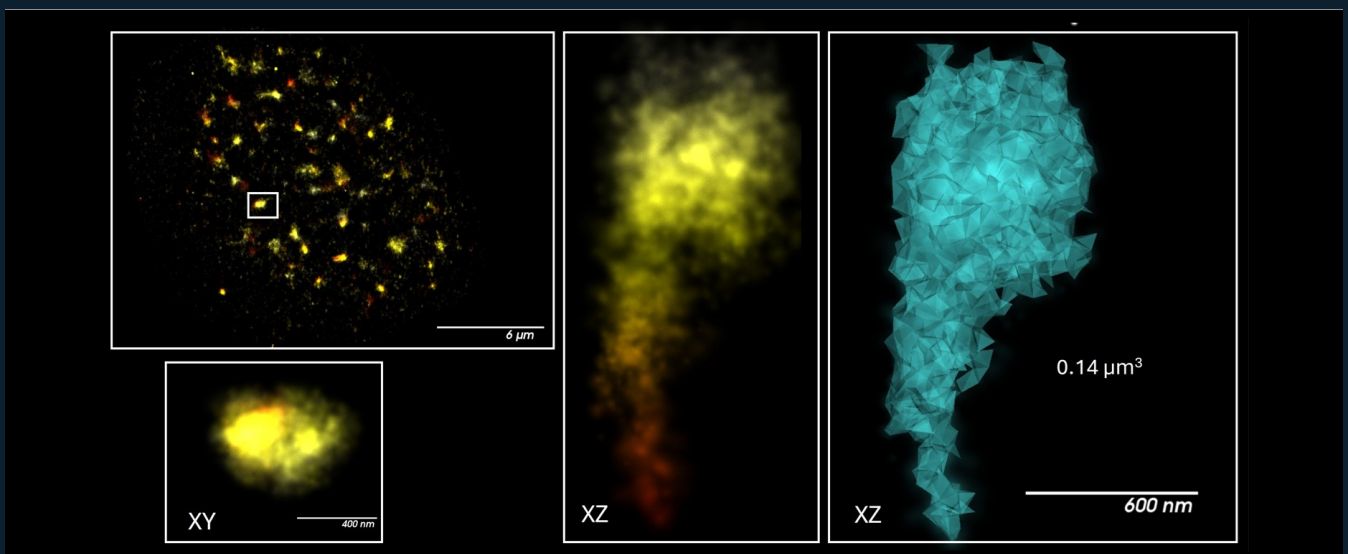
H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc

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

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



The upper left panel displays the entire nucleus captured using STORM imaging methodology. The lower left image shows the X-Y projection, which resembles what is typically seen in diffraction-limited microscopy and is commonly referred to as a single foci. However, our data reveal the presence of two foci, which we refer to as a "doublet."

The center panel presents the Y-Z projections, illustrating the structure that appears to be a single foci or termination point involved in the damage-induced phosphorylation event. Additionally, it suggests further phosphorylation extending along the chromatin from this focus or termination point.

The right panel demonstrates the volume rendering of the single foci in three dimensions by calculating convex hulls, resulting in a total signal volume of 0.14 cubic microns (140 cubic nanometers).

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000004 | 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 H2AX, 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-000004 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-000004 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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 680	3; 38 eGFP; 50 Cy5; 49 DAPI; 450-490; 625-655; 335-383; 500-550
Widefield SIM — Apotome	405/DAPI; 532; 555; 647	3; AF532-49014; 50 Cy5; 49 DAPI; 515 - 545; 625 - 655; 335-383; 555 - 595
Confocal	405/DAPI; 488	2; None; 488nm @1.0%; 405nm @0.3%; 493; 353; 517; 465
Airyscan	405/DAPI; 488	2; None; 488nm @0.3%; 405nm @1.5%; 493; 353; 517; 465
SIM	405/DAPI; 488; 647; 680	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T2-Axiocam 820-SR; T2-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 647; 680	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T2-Axiocam 820-SR; T2-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 555; 647	2; 50 Cy 5; AF532-49014; 49 DAPI; 625 - 655; 515 - 545; 335 - 383; 665 -715

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 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 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 940 X 696; Image Size (Pixels): 940 X 696; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms; 500ms. Illumination: Light source: X-Cite Xylis Lamp Intensity 20%; X-Cite Xylis Lamp Intensity @25%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 23 Slices (4.4µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 1118 X 1011; Image Size (Pixels): 1118 X 1011; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 50ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @40%. 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: 47.

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; 532; 555; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 39 Slices (1.52µm); Channels: 2; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.071µm; Image size (Pixels): 730 X 586; Image Size (Pixels): 730 X 586; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-Pmt1; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.51µs; Frame Time: 10.15s. Illumination: Laser Wavelength: 488nm @1.0%; 405nm @0.3%. Optical/scan: Pinhole: 1.00 AU / 45µm; 1.00 AU / 37µm; Scan Zoom: 1.4; LSM Scan Speed: 9; Scan Direction: Bidirectional; Averaging: 16; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 47.

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 900, and records detected dye/channel descriptors as 405/DAPI; 488.

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 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 32 Slices (1.55µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 50nm; Image size (Pixels): 910 X 766; Image Size (Pixels): 910 X 766; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.59µs; Frame Time: 4.44s. Illumination: Laser Wavelength: 488nm @0.3%; 405nm @1.5%. Optical/scan: Pinhole: 5.00 AU / 219µm; 5.00 AU / 181µm; Scan Zoom: 1.6; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 27°, Scale Z 60%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.3 (3D, Auto); Super Resolution 7.8 (3D, Auto). Structured source metadata values: 46.

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 900, and records detected dye/channel descriptors as 405/DAPI; 488.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 35 Slices (1.7µm); Channels: 2; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm; Image size (Pixels): 3087 X 3590; Image Size (Pixels): 3087 X 3590; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820#2. Timing: Exposure Time: 150ms; 50ms; Frame Time: 1.98s; 676.00ms. Illumination: Laser Wavelength: 640nm @9.00%; 405nm @6.0%; Illumination Wavelength: 640; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 65°, Scale Z 39%, 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 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; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 43 Slices (1.68µm); Channels: 2; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 40.00nm; Image size (Pixels): 1058 X 1352; Image Size (Pixels): 1058 X 1352; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820#2. Timing: Exposure Time: 150ms; 50ms; Frame Time: 1.98s; 676.00ms. Illumination: Laser Wavelength: 640nm @9.00%; 405nm @8.0%; Illumination Wavelength: 640; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 51°, Scale Z 39%, 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; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 80.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1069 X 920; Image Size (Pixels): 1069 X 920; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 40ms; 35ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @6.0%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 39.

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 Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 555; 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-000004 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): 35.29nm X 35.29nm X 50nm -> 0.03529 µm X 0.03529 µm X 0.05000 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)=910 X 766; Image Size (Scaled)=32.11µm X 27.03µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm -> 0.01320 µm X 0.01320 µm X 0.05000 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)=3087 X 3590; Image Size (Scaled)=40.73µm X 47.37µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.05%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 40.00nm -> 0.01320 µm X 0.01320 µm X 0.04000 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)=1058 X 1352; Image Size (Scaled)=13.96µm X 17.84µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 139. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc

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

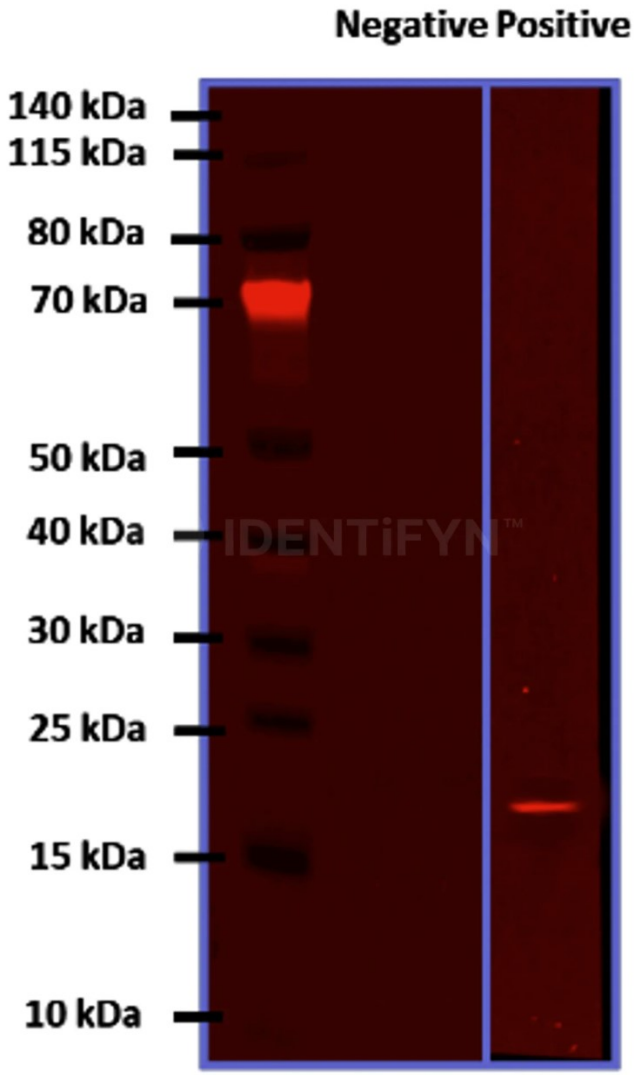
This dossier preserves the complete available evidence progression for PA-RR-000004. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 900	PRESERVED
5	Airyscan	ZEISS LSM 900	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	594
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	624±40nm
Detector	Avalanche Photodiode
Intensity	6
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Kyle Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Etoposide-treated (2 µM) HeLa cells were homogenized using an ultrasonication probe and centrifuged to pellet the cell debris. The lysate supernatant was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank The ThermFisher's's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Identifyn® Rabbit Anti-Human γH2AX chimeric antibody (Human Fc) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat anti-Human IgG was incubated for 1 hour, followed by washing 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence
Laser = 532nm
Emission Filter = 624±40nm
Detector = Avalanche Photodiode
Intensity = 6
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Kyle Small

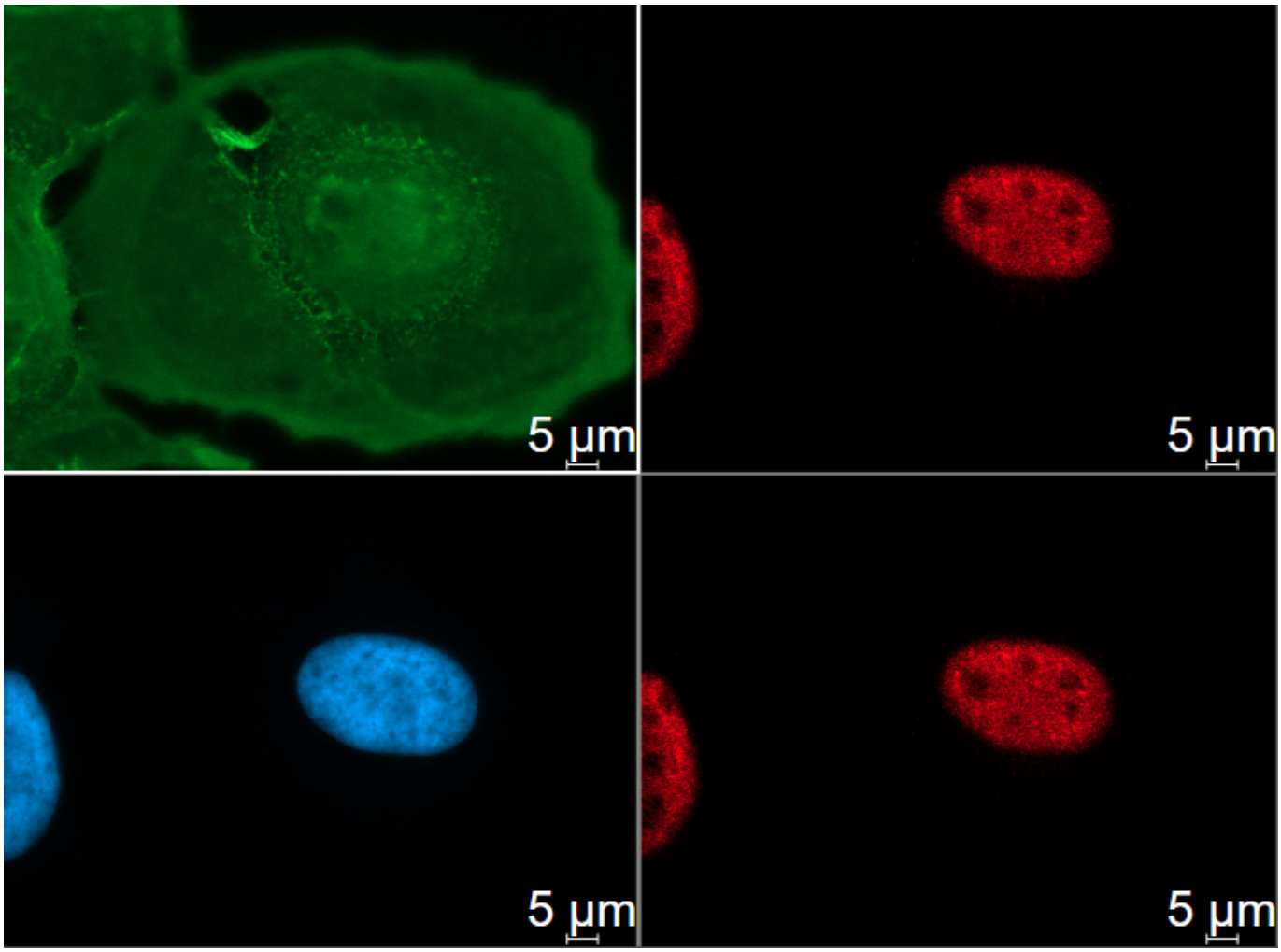
SOURCE PROVENANCE

Catalog	PA-RR-000004

SOURCE PROVENANCE

Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7AEEABC448D59D6C82CB70847B5C961EC2E678A1E74B5DB968B726AD206E0B34
Method PDF SHA-256	D58D3C529181E54162E4EFF9A0424F2C82ED9D9CAA7BD1EFB5F062C39FFA3CE4

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 mono, 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)	0.110µm X 0.110µm
Image size (Pixels)	940 X 696
Image Size (Scaled)	102.95µm X 76.23µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	38 eGFP | 50 Cy5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450-490 | 625-655 | 335-383
Filter Emission Wavelength	500-550 | 665-715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity 20% | X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms | 500ms | 50ms
Excitation Wavelength	493 | 679 | 353
Emission Wavelength	517 | 702 | 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.80µm | 1.09µm | 0.72µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000069

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

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn™488 secondary antibody 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 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110μm X 0.110μm
Image size (Pixels) = 940 X 696
Image Size (Scaled) = 102.95μm X 76.23μm
Bit Depth = 12 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

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%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.80µm
Binning Mode = 2,2

680 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength =625-655
Filter Emission Wavelength = 665-715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 500ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 1.09µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335-383
Filter Emission Wavelength = 420-470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 50ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA =1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Post Processing

None

Image Correction

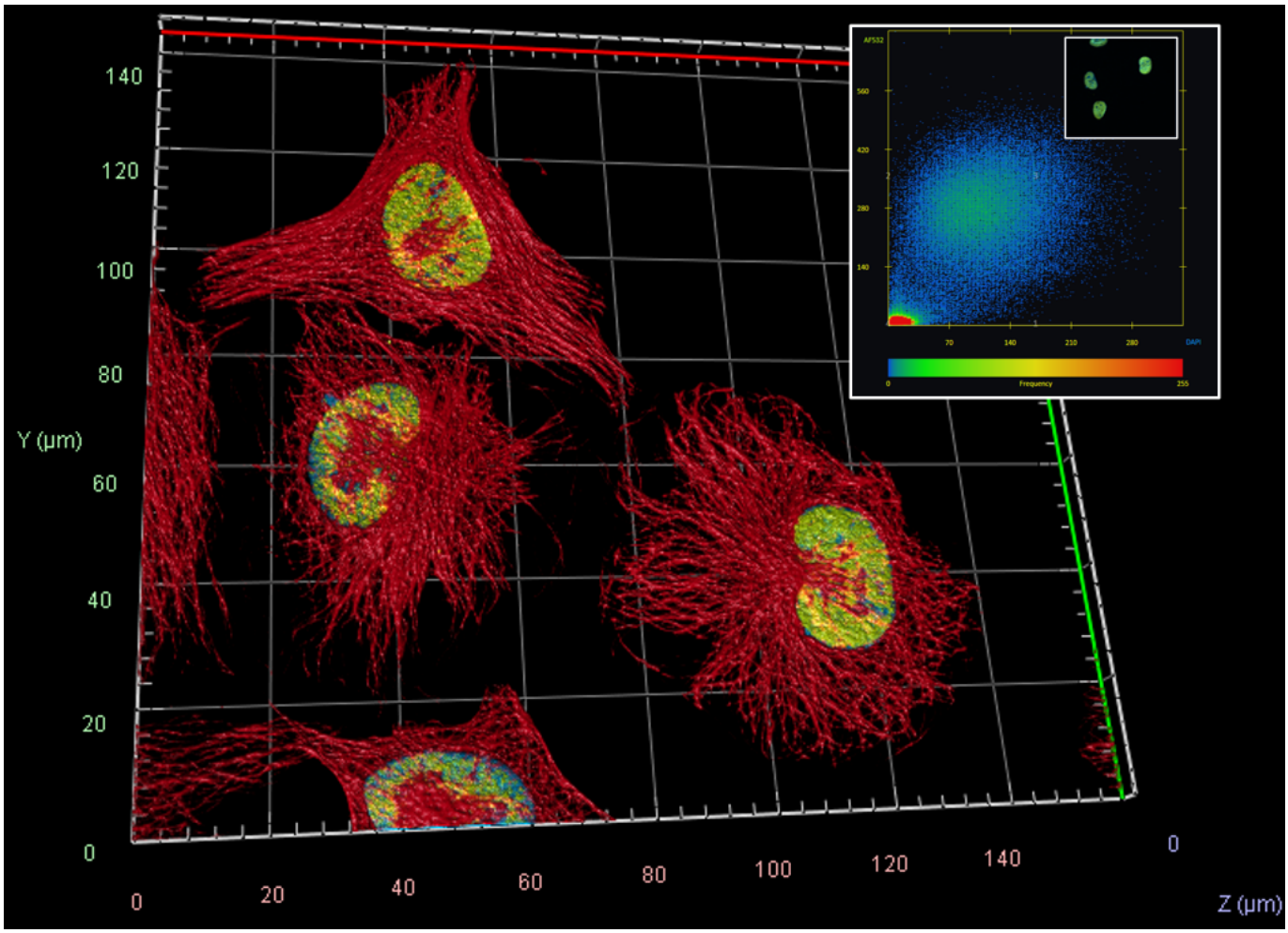
None

Image by K. Shivanna
Analysis and Presentation by B. T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A35F58850784158AF62E756481DAE57B751205DE95D67A7EE5E76F9AA8A59C62
Method PDF SHA-256	FB25C3D483D88A6B86AEEC9F12E6FABEDB518D45D2476C8E010EC692AFFACCE8

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; 532; 555; 647
METADATA VALUES	47

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	23 Slices (4.4µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	1118 X 1011
Image Size (Scaled)	159.71µm X 144.43µm
Bit Depth	14 Bit
Image Center Position	X: 87.50mm, Y: 48.39mm
Stage Position	X: 87.50mm, Y: 48.39mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF532-49014 | 50 Cy5 | 49 DAPI
Beam Splitter	550 | 660 | 395
Filter Excitation Wavelength	515 - 545 | 625 - 655 | 335-383
Filter Emission Wavelength	555 - 595 | 665 - 715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @40% | X-Cite Xylis Lamp Intensity = @8%
Exposure Time	50ms | 200ms | 20ms
Excitation Wavelength	528 | 653 | 353
Emission Wavelength	553 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.07µm | 1.30µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Human, IgG (H + L)
Cat ID# - SA 000028

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 23 Slices (4.4µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.200µm
Image Size (Pixels) = 1118 X 1011
Image Size (Scaled) = 159.71µm X 144.43µm
Bit Depth = 14 Bit
Image Center Position = X: 87.50mm, Y: 48.39mm
Stage Position = X: 87.50mm, Y: 48.39mm
ROI Center Offset = X: 1.14, Y: 0.00µm

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 50ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.07µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @40%
Exposure Time = 200ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335-383
Filter Emission Wavelength = 420-470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity = @8%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Colocalization View

Horizontal Axis - DAPI, Threshold 0, Range Auto
Vertical Axis - 532 (gamma-H2AX), Threshold 0, Range Auto
2D

Summary

The main image and colocalization graphic, with an inset image minus the alpha-tubulin signal, demonstrate the nuclear localization of gamma-H2AX within the nuclear compartment.

Image Corrections

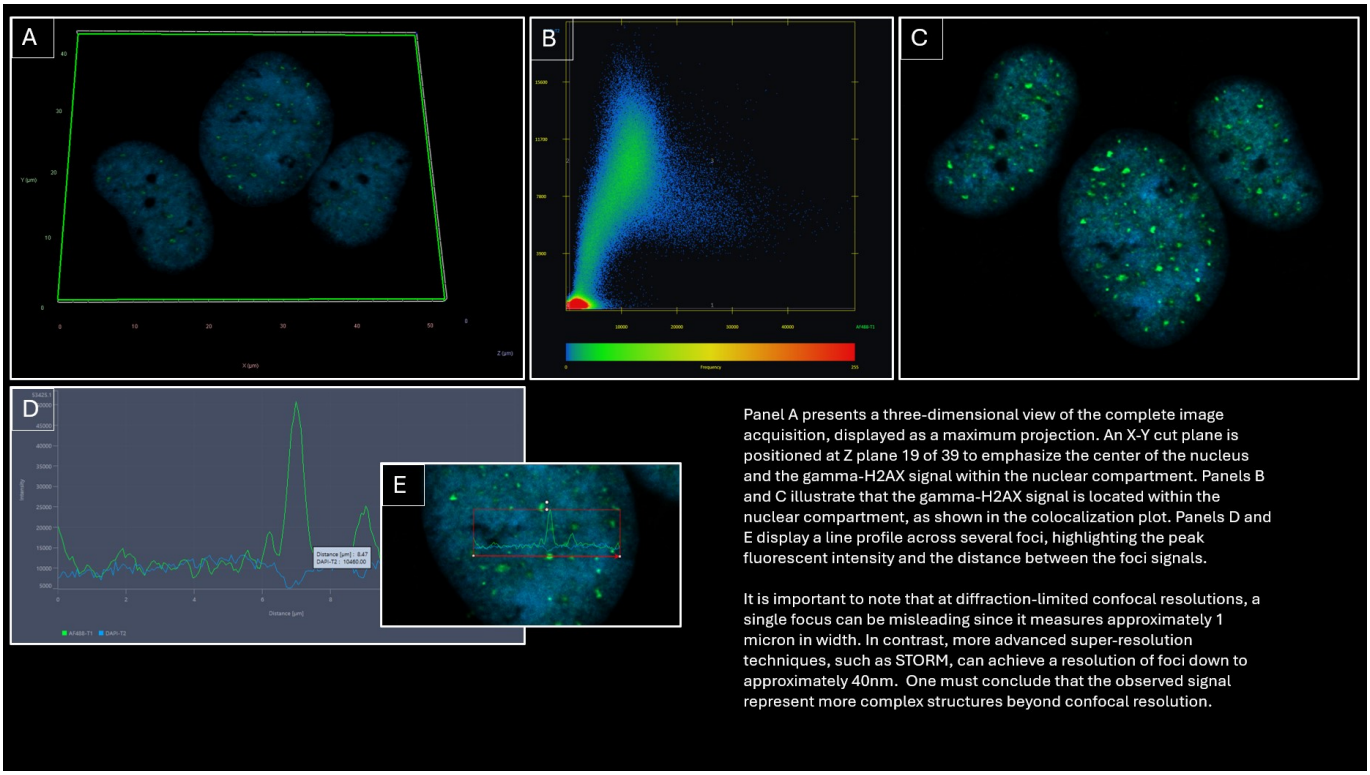
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	95EF08DCE8E482ADCB5E3E8C577E4CA3DCF287675B3670FB8355CC0E7FA8C8FA
Method PDF SHA-256	5D4BE72AB2DDA311DA1EDE307AA97989C03DFD68F4A2C77DE16EF1C13D1730D5

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	47

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 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	39 Slices (1.52µm)
Channels	2
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.071µm
Image Size (Pixels)	730 X 586
Image Size (Scaled)	51.54µm X 41.37µm
Bit Depth	16 Bit
Image Center Position	X: 77.00mm, Y: 53.10mm
Stage Position	X: 77.00mm, Y: 53.10mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 45µm | 1.00 AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @1.0% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.4
Rotation	0°
Sampling	1.00
Pixel Time	0.51µs
Frame Time	10.15s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Averaging	16
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	484-590 | 410-470
Imaging Device	Airyscan | GaAsP-Pmt1
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	650 V
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (488), Threshold 1% (405), Ramp 0.0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 13.4), Y (Min 4854 and Max 53425)

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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 39 Slices (1.52μm)

Channels = 2
Scaling (Per Pixel) = 0.071μm X 0.071μm X 0.071μm
Image Size (Pixels) = 730 X 586
Image Size (Scaled) = 51.54μm X 41.37μm
Bit Depth = 16 Bit
Image Center Position = X: 77.00mm, Y: 53.10mm
Stage Position = X: 77.00mm, Y: 53.10mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 45µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 10.15s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 16
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 484-590
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 10.15s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 16
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsP-Pmt1
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (488), Threshold 1% (405), Ramp 0.0% 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)

Colocalization View - Panels B and C

Horizontal Axis - 488, Threshold 1475, Range Auto
Vertical Axis - DAPI, Threshold 384, Range Auto

Profile View Display

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 13.4), Y (Min 4854 and Max 53425)

Summary

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. An X-Y cut plane is positioned at Z plane 19 of 39 to emphasize the center of the nucleus and the gamma-H2AX signal within the nuclear compartment. Panels B and C demonstrate that the gamma-H2AX signal is localized within the nuclear compartment, as indicated in the colocalization plot. Panels D and E display a line profile across several foci, highlighting the peak fluorescent intensity and the distance between the foci signals.

It is important to note that a single Foci can be misleading at diffraction-limited confocal resolutions since it measures approximately 1 micron in width. In contrast, more advanced super-resolution techniques, such as STORM, can achieve a resolution of foci down to approximately 40 nm. This suggests our observed signal may represent more complex structures beyond our current resolution.

Image Corrections -

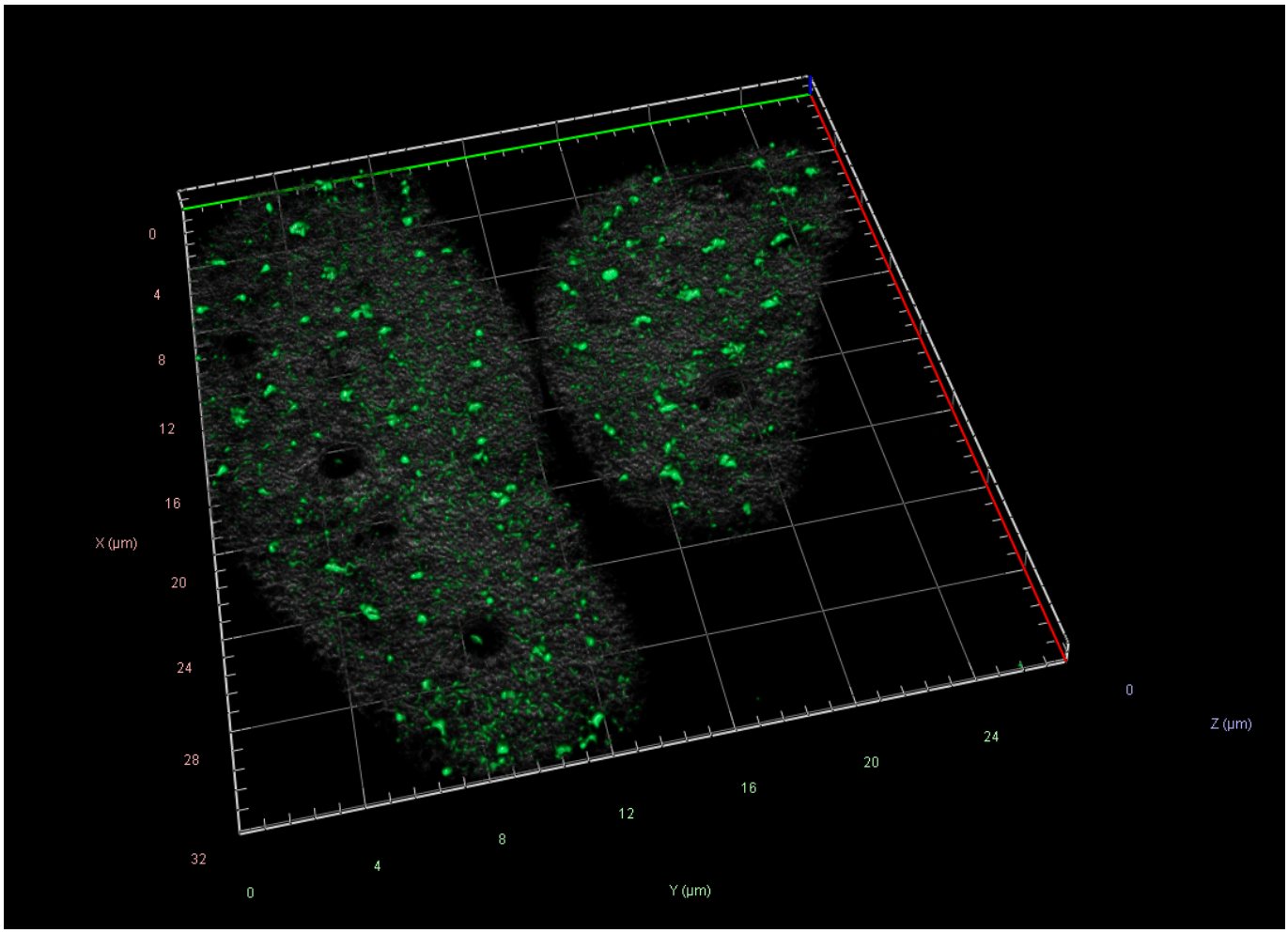
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0E9D9D5BF8D793A7C798FC41F02AA25DD0B2D8216B4D4CF2C96423CB37390C0F
Method PDF SHA-256	0A1067D22D91D569E2AEBAE81B3EB90D32402FD4B3B1918CA0AD3D21EDBF7C76

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	46

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 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	32 Slices (1.55µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.05000 µm
Image Size (Pixels)	910 X 766
Image Size (Scaled)	32.11µm X 27.03µm
Bit Depth	16 Bit
Image Center Position	X: 72.12mm, Y: 53.02mm
Stage Position	X: 72.12mm, Y: 53.02mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 219µm | 5.00 AU / 181µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @0.3% | 405nm @1.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	2.00
Pixel Time	0.59µs
Frame Time	4.44s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	480-560 | 400-460
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.3 (3D, Auto) | Super Resolution 7.8 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 4% (488), Threshold 4% (405), Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 139%, Azimuth 56°, Elongation -97°, Enabled Directional Light and Tone Mapping
Projection	View Angle 27°, Scale Z 60%, 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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 32 Slices (1.55μm)

Channels = 2
Scaling (Per Pixel) = 35.29nm X 35.29nm X 50nm
Image Size (Pixels) = 910 X 766
Image Size (Scaled) = 32.11μm X 27.03μm
Bit Depth = 16 Bit
Image Center Position = X: 72.12mm, Y: 53.02mm
Stage Position = X: 72.12mm, Y: 53.02mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 219µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 4.44s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480-560
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.3 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 181µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 4.44s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-460
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 7.8 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode""“SR"
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 4% (488), Threshold 4% (405), Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 139%, Azimuth 56°, Elongation -97°, Enabled Directional Light and Tone Mapping
Projection = View Angle 27°, Scale Z 60%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

DAPI is represented as dark grey instead of its default blue coloring to demonstrate contrast and the specificity and brightness of the Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG primary antibody

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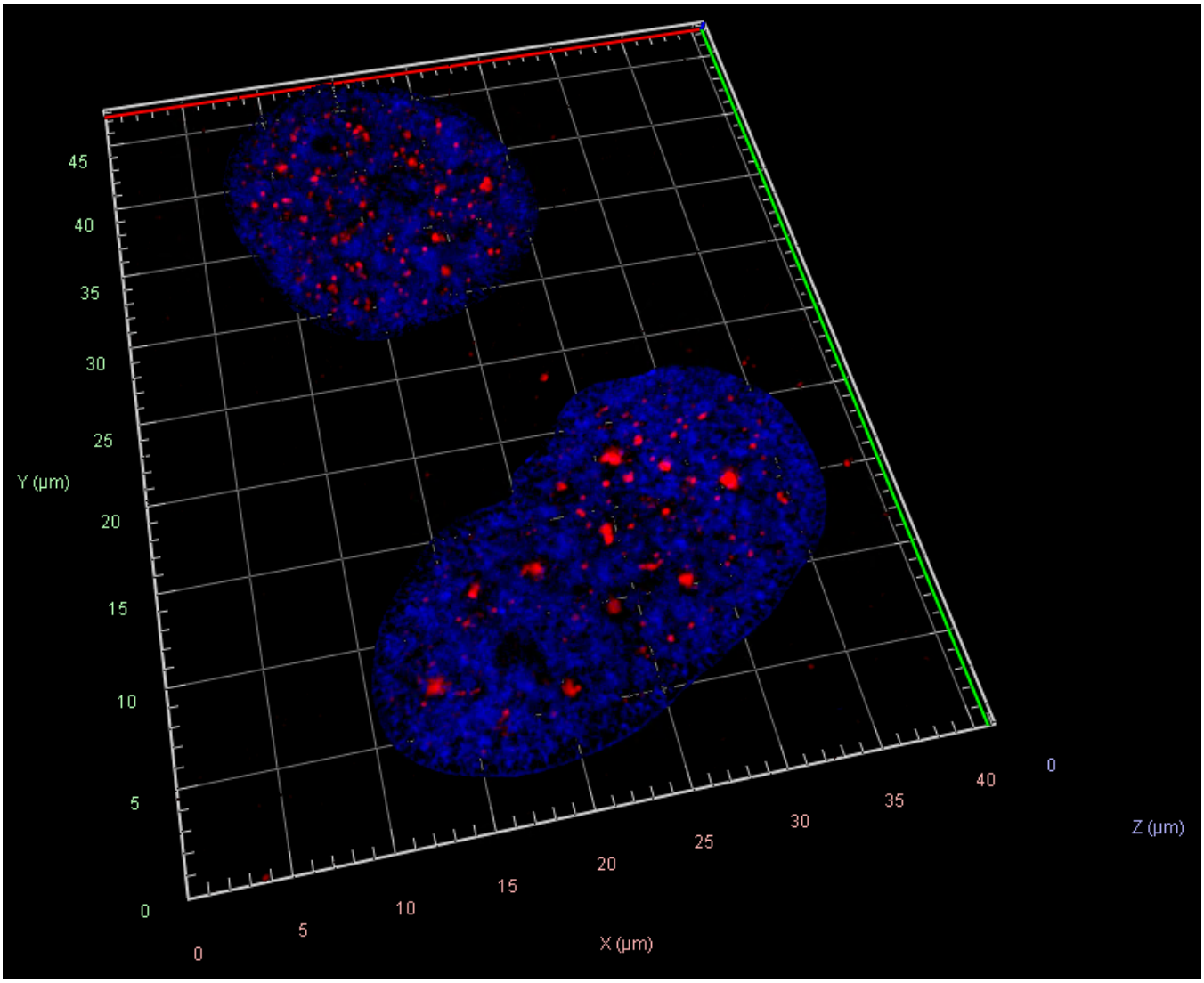
Image by P. Raut
Analysis and Presentation by B. T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8953DD53DFBE63C61A0A648F450AC51B1F5E9AB6D31B5A194F77FC4504A923E1
Method PDF SHA-256	FEBE2D1D5B49E3D582DC4B34638F94008EF599EC87BBD968B2790E91E38FE654

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 680
METADATA VALUES	57

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	35 Slices (1.7µm)
Channels	2 | 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.05000 µm
Image Size (Pixels)	3087 X 3590
Image Size (Scaled)	40.73µm X 47.37µm
Bit Depth	16 Bit
Image Center Position	X: 6.46mm, Y: 255.40mm
Stage Position	X: 6.46mm, Y: 255.40mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
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, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 405
Channel Name	T2-Axiocam 820-SR | T2-Axiocam 820 #2-SR
Excitation Wavelength	640 | 405
Emission Wavelength	729 | 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 | 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820#2
Camera Adapter	1X
Exposure Time	150ms | 50ms
Depth of Focus	1.13µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @9.00% | 405nm @6.0%
Frame Time	1.98s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.3886
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Maximum Projection	Precise
Appearance	Threshold 2% (488), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 85%, Enabled Tone Mapping
Projection	View Angle 65°, Scale Z 39%, 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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000069

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 35 Slices (1.7μm)

Channels = 2
Scaling (Per Pixel) = 13.20nm X 13.20nm X 50.00nm
Image Size (Pixels) = 3087 X 3590
Image Size (Scaled) = 40.73μm X 47.37μm
Bit Depth = 16 Bit
Image Center Position = X: 6.46mm, Y: 255.40mm
Stage Position = X: 6.46mm, Y: 255.40mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

680 -

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, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T2-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 = 150ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @9.00%
Frame Time = 1.98s
Scan Direction = Unidirectional
Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T2-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @6.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 2
Channels = 1
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.3886
Input SNR = N/A
Regularization Weight = N/A
Iterations = N/A
Filter = N/A
Fitting = N/A
Normalization = Automatic
Experimental PSF = N/A

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 2% (488), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 85%, Enabled Tone Mapping
Projection = View Angle 65°, Scale Z 39%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

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

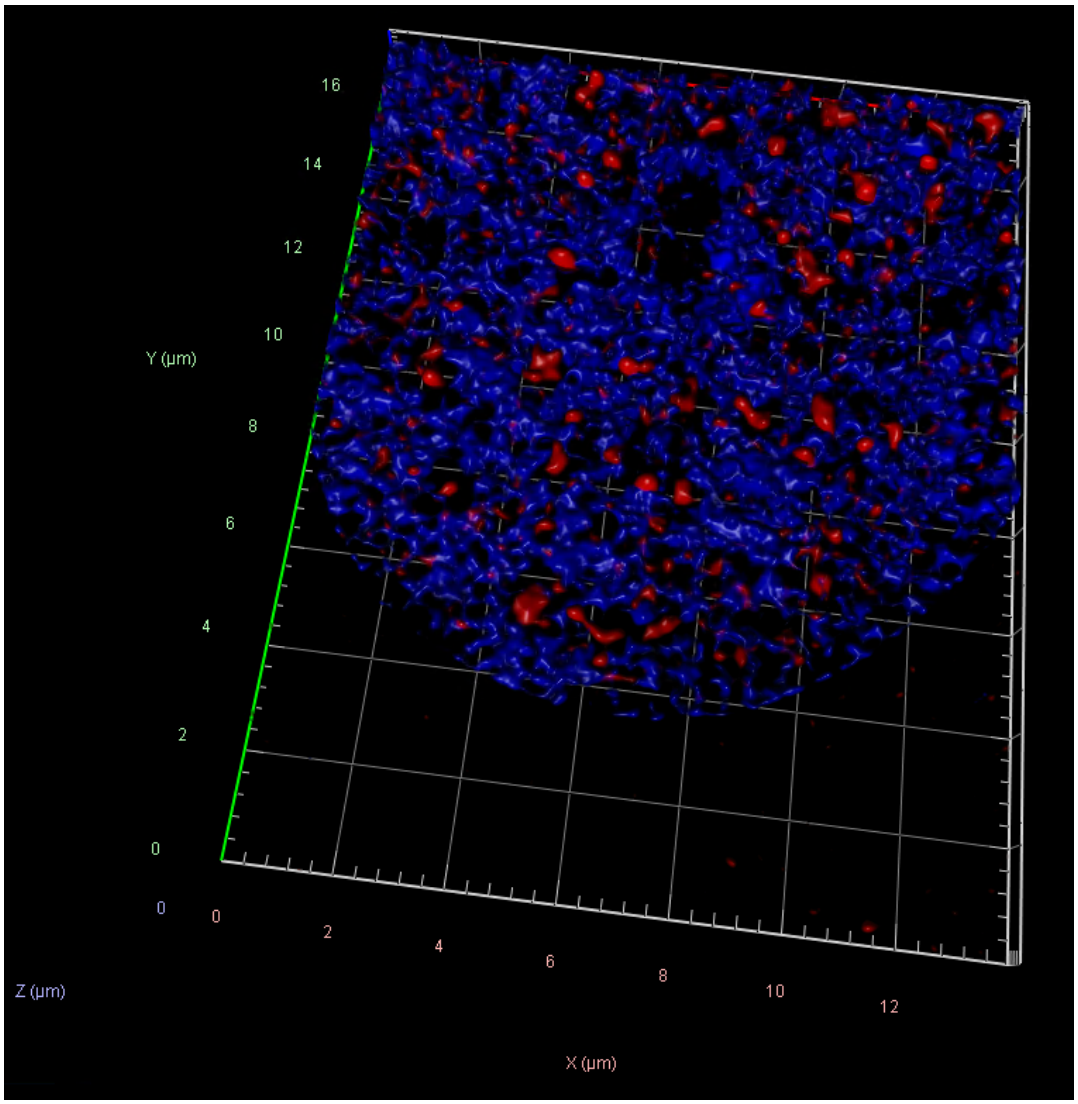
SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X 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	7BEB745CED49E30F65C43998A374BCF6C75177F2187E95BB829F866D5BF72DB9

SOURCE PROVENANCE

Method PDF SHA-256	8C94EA9DEE85E11F893FE59596859F0B688CAE9FFB9048A20B5B55E26CD2544F

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 680
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 Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	43 Slices (1.68µm)
Channels	2 | 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.04000 µm
Image Size (Pixels)	1058 X 1352
Image Size (Scaled)	13.96µm X 17.84µm
Bit Depth	16 Bit
Image Center Position	X: 3.09mm, Y: -2.13mm
Stage Position	X: 3.09mm, Y: -2.13mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
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, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 405
Channel Name	T2-Axiocam 820-SR | T2-Axiocam 820 #2-SR
Excitation Wavelength	640 | 405
Emission Wavelength	729 | 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 | 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820#2
Camera Adapter	1X
Exposure Time	150ms | 50ms
Depth of Focus	1.13µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @9.00% | 405nm @8.0%
Frame Time	1.98s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.3886
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Volume Projection	Precise
Appearance	Threshold 4% (488), Threshold 2% (DAPI), Ramp 96% (680), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 57°, Elongation -126°, Enabled Tone Mapping
Projection	View Angle 51°, Scale Z 39%, 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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000069

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 43 Slices (1.68μm)

Channels = 2
Scaling (Per Pixel) = 13.20nm X 13.20nm X 40.00nm
Image Size (Pixels) = 1058 X 1352
Image Size (Scaled) = 13.96μm X 17.84μm
Bit Depth = 16 Bit
Image Center Position = X: 3.09mm, Y: -2.13mm
Stage Position = X: 3.09mm, Y: -2.13mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

680 -

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, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T2-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 = 150ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @9.00%
Frame Time = 1.98s
Scan Direction = Unidirectional
Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 647 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T2-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @8.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 1
Algorithm = SIM2
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.3886
Input SNR = N/A
Regularization Weight = N/A
Iterations = N/A
Filter = N/A
Fitting = N/A
Normalization = Automatic
Experimental PSF = N/A

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 4% (488), Threshold 2% (DAPI), Ramp 96% (680), Ramp 98% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 57°, Elongation -126°, Enabled Tone Mapping
Projection = View Angle 51°, Scale Z 39%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

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

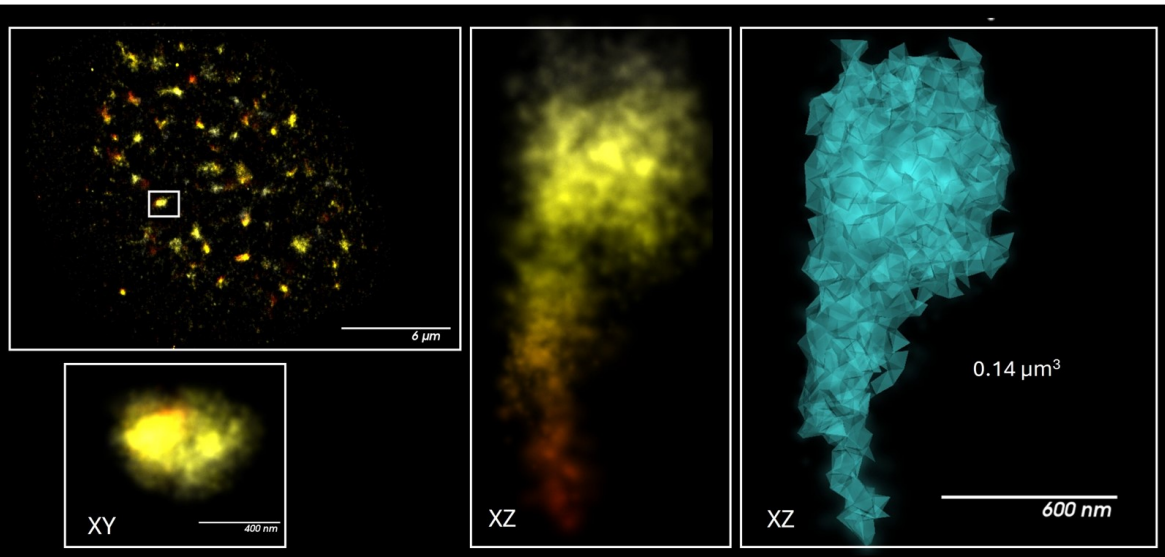
SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X 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

SOURCE PROVENANCE

Image SHA-256	485A1849A3400D29375C0AA120190AAF2594B17B358433E7DF4BE82A75C90D56
Method PDF SHA-256	9E4A5EB61B49C9739C3739FEB208F2932FAB5D6FD8949476C8B60B87995C4FDD

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



The upper left panel displays the entire nucleus captured using STORM imaging methodology. The lower left image shows the X-Y projection, which resembles what is typically seen in diffraction-limited microscopy and is commonly referred to as a single foci. However, our data reveal the presence of two foci, which we refer to as a "doublet."

The center panel presents the Y-Z projections, illustrating the structure that appears to be a single foci or termination point involved in the damage-induced phosphorylation event. Additionally, it suggests further phosphorylation extending along the chromatin from this focus or termination point.

The right panel demonstrates the volume rendering of the single foci in three dimensions by calculating convex hulls, resulting in a total signal volume of 0.14 cubic microns (140 cubic nanometers).

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

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	182.0 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 180.1; End: 181.3
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 500
Z Positions	6 using Steps: 0.2µm (200nm)
Cycles	10
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	30%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 20
Sizing Mode	Constant
Particle size	20nm
Color by	Depth
Color Table	Single
Opacity Mode	Constant; Opacity 1: 0.3
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-450 to +450
Radial precision	25
Axial precision	60
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.05µm (500nm)
Maximum Particle Count to Form Cluster	20
Computer Hulls	Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	600nm
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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000058

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

HeLa cells were grown on a µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:100 Cells were washed 3X in Identifyn's™ proprietary Wash Buffer and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:5 . Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 182.0 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 (640 nm Dichroic): Not Selected

Advanced Tools Option

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

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

Autofocus - Calibration Values

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

View Mode

SuperRes: 50µm is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 180.1; End: 181.3

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

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

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

Not Selected

Advanced Tools

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 500
Z Positions: 6 using Steps: 0.2µm (200nm)
Cycles: 10
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 30%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 20

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: 20nm
Color by: Depth
Color Table: Single
Opacity Mode: Constant; Opacity 1: 0.3
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

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

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

PSF Z offset: -450 to +450
Radial precision: 25
Axial precision: 60

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: 0.05µm (500nm)
Maximum Particle Count to Form Cluster: 20
Computer Hulls: Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Hull Alpha shape Radius: 600nm
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Summary

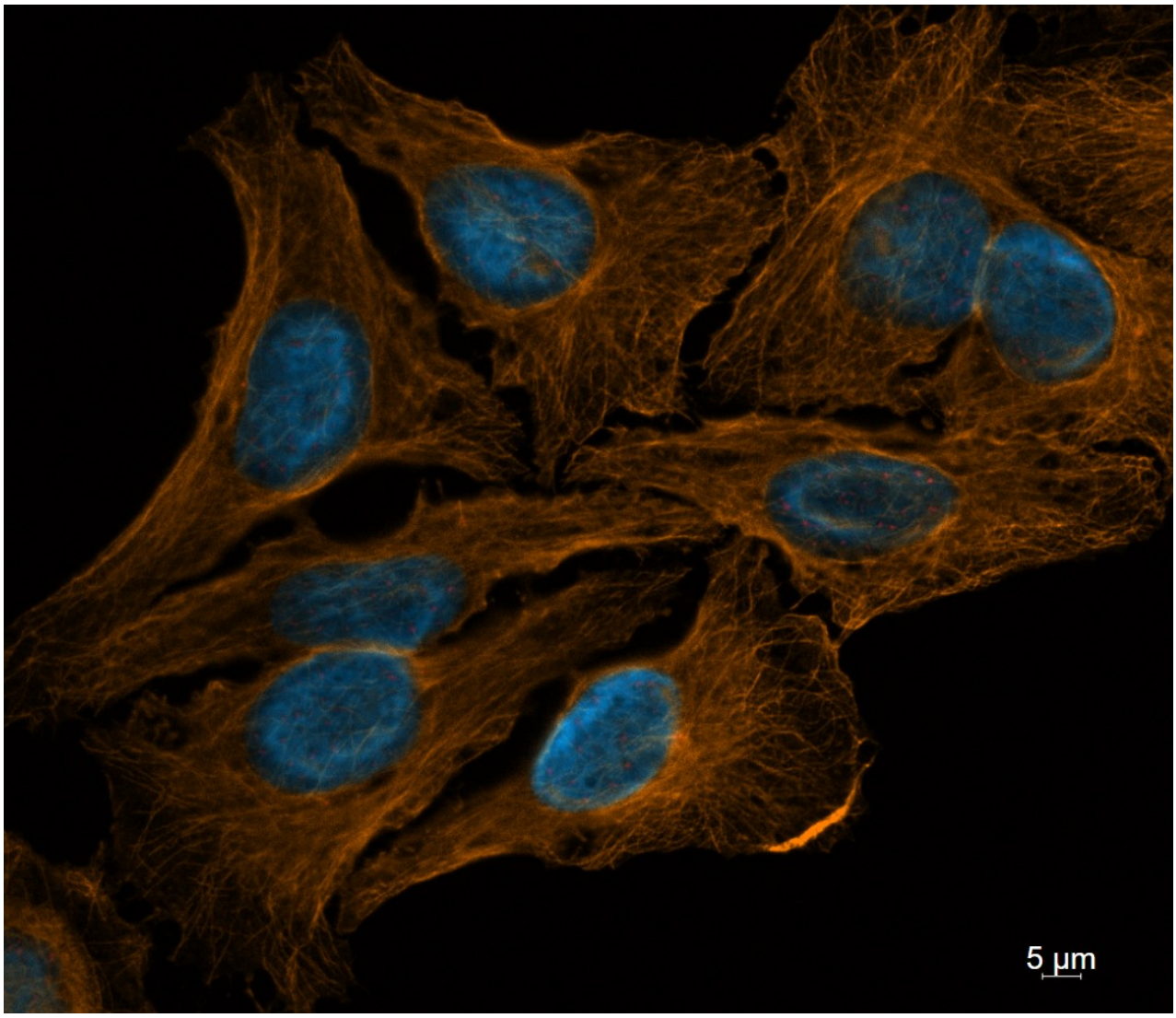
The upper left panel displays the entire nucleus captured using STORM imaging methodology. The lower left image shows the X-Y projection, which resembles what is typically seen in diffraction-limited microscopy and is commonly referred to as a single foci. However, our data reveal the presence of two foci, which we refer to as a "doublet."
The center panel presents the Y-Z projections, illustrating the structure that appears to be a single foci or termination point involved in the damage-induced phosphorylation even. Additionally, it suggests further phosphorylation extending along the chromatin from this focus or termination point.
The right panel demonstrates the volume rendering of the single foci in three dimensions by calculating convex hulls, resulting in a total signal volume of 0.14 cubic microns (140 cubic nanometers).

Image by S. Thakur

SOURCE PROVENANCE

Catalog	PA-RR-000004
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	2ECC9B994F41376A9B744D2BC822E56DB1F21789DC0DB201798BFE9E482CE2D3
Method PDF SHA-256	CDFEF8C95FE6793BD6EBB081F0A27E39E16D9B5BD1E9E62B1A0CC5E9CFDFBD9E

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 647
METADATA VALUES	39

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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1069 X 920
Image Size (Scaled)	152.71µm X 131.43µm
Bit Depth	14 Bit
Image Center Position	X: 80.42mm, Y: 46.38mm
Stage Position	X: 80.42mm, Y: 46.38mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50 Cy 5 | AF532-49014 | 49 DAPI
Beam Splitter	660 | 550 | 395
Filter Excitation Wavelength	625 - 655 | 515 - 545 | 335 - 383
Filter Emission Wavelength	665 -715 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @6.0%
Exposure Time	40ms | 35ms | 20ms
Excitation Wavelength	653 | 553 | 353
Emission Wavelength	668 | 568 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.30µm | 1.10µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction 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 γH2Ax (Serine 139) Recombinant Monoclonal, Human Fc, IgG (BLR014M)
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000065

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000040

Gamma H2AX (γH2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of double-strand breaks (DSBs). When DSBs occur in chromatin, γH2AX is rapidly phosphorylated specifically at the serine 139 residue (referred to as Ser139 phosphorylation or γ-phosphorylation) near the site of the break. This phosphorylation event forms γH2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

DNA damage was NOT induced.

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed three times in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 1069 X 920
Image Size (Scaled) = 152.71µm X 131.43µm
Bit Depth = 14 Bit
Image Center Position = X: 80.42mm, Y: 46.38mm
Stage Position = X: 80.42mm, Y: 46.38mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

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 @6.0%
Exposure Time = 40ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.0%
Exposure Time = 35ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10µm
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 @6.0%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

Summary

The control images indicate that, in the absence of Etoposide-induced DNA damage, there is a significant reduction in the presence of γH2Ax Human Fc in the nucleus. In contrast, the complete dataset for γH2Ax, Human Fc demonstrates a strong nuclear aggregate signal after Etoposide induces DNA damage.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RR-000004
Stage	Control
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	1EEA4B590BF443023E90D6B9973F2B0B4AD7FB153817F3204CA9CC0190BD4CC7
Method PDF SHA-256	7E7D01E1F2620678A96AF9C610C52DFDDA95DA623058C915707A05384FA36AF5