BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

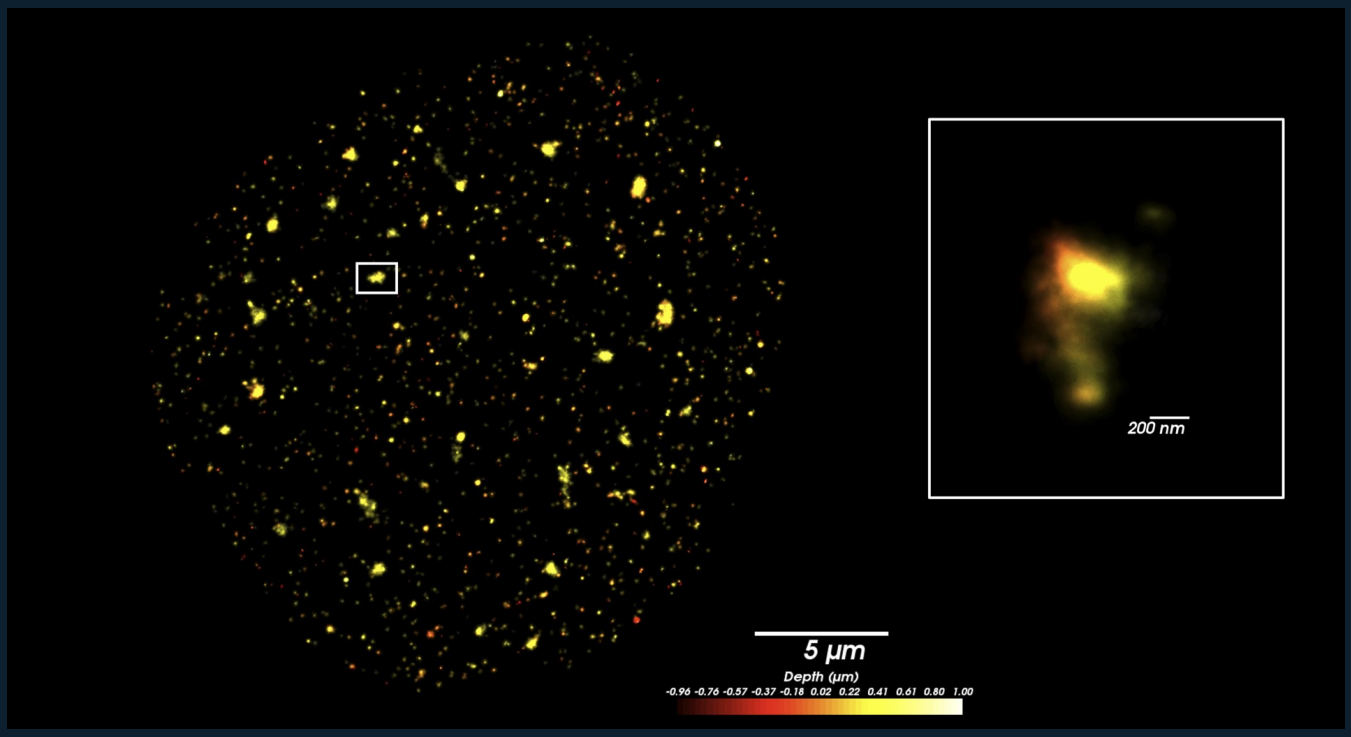
H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680

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

8	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000022 | 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
- 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

BENNETT ASSESSMENT

The preserved DC-000022 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 DC-000022 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

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 680	3; 50 Cy5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 680	3; 50 Cy5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 680	3; None; 640nm @0.8%; 488nm @0.5%; 405nm @0.2%; 679; 493; 353
Airyscan	405/DAPI; 488; 680; 750	3; None; 639nm @0.08%; 488nm @0.01%; 405nm @0.2%; 679; 493; 353
SIM	405/DAPI; 488; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 680	3; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR
Single-molecule STORM	680	Both Channels

MODALITY ASSESSMENT / PART 1 OF 8

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 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

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 8

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 sCMOS, 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): 108.87 X 77.98; Image Size (Pixels): 108.87 X 77.98; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @7.0%. Optical/scan: Effective NA: 1.4; .5. Structured source metadata values: 40.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 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 8

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: 82 Slices (8.81µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 1126 X 1068; Image Size (Pixels): 1126 X 1068; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 300ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30%; X-Cite Xylis Lamp Intensity @15%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 125%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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 8

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 Confocal, 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: 151 Slices (4.50µm); Channels: 3; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.030µm; Image size (Pixels): 958 X 958; Image Size (Pixels): 958 X 958; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt2; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.55µs; Frame Time: 2.37s. Illumination: Laser Wavelength: 640nm @0.8%; 488nm @0.5%. Optical/scan: Pinhole: 1.00AU / 57µm; 1.00AU / 43µm; Scan Zoom: 1.5; LSM Scan Speed: 9; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

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; 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

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 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 171 Slices (5.10µm); Channels: 3; Scaling (Per Pixel): 35.30nm X 35.30nm X 30.00nm; Image size (Pixels): 1884 X 1884; 715 X 627; Image Size (Pixels): 1884 X 1884; 715 X 627; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.10µs; Frame Time: 18.77s. Illumination: Laser Wavelength: 639nm @0.08%; 488nm @0.01%. Optical/scan: Pinhole: 5.00AU / 328µm; 5.00AU / 247µm; Scan Zoom: 2.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 10.0 (3D, Auto); Super Resolution 9.4 (3D, Auto). Structured source metadata values: 63.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 680; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

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: 121 Slices (3.60µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; Image Size (Pixels): 5056 X 5056; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 100ms; Frame Time: 1.59s; 1.33s. Illumination: Laser Wavelength: 640nm @3.0%; 488nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 63.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 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 8

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: 153 Slices (4.56µm); Channels: 3; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 413 X 401; Image Size (Pixels): 5056 X 5056; 413 X 401; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 100ms; Frame Time: 1.59s; 1.33s. Illumination: Laser Wavelength: 640nm @3.0%; 488nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 70.

VISIBLE OBSERVATION

A preserved presentation image is available for 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; 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 8

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: 200ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 79.

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 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000022 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): 35.30nm X 35.30nm X 30.00nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1884 X 1884; Image Size (Scaled)=66.50µm X 66.50µm; 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 30.00nm -> 0.01320 µm X 0.01320 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=66.72µm X 66.72µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm -> 0.01320 µm X 0.01320 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=66.72µm X 66.72µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "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 - Identifyn® SRM Alexa Fluor™ 680

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

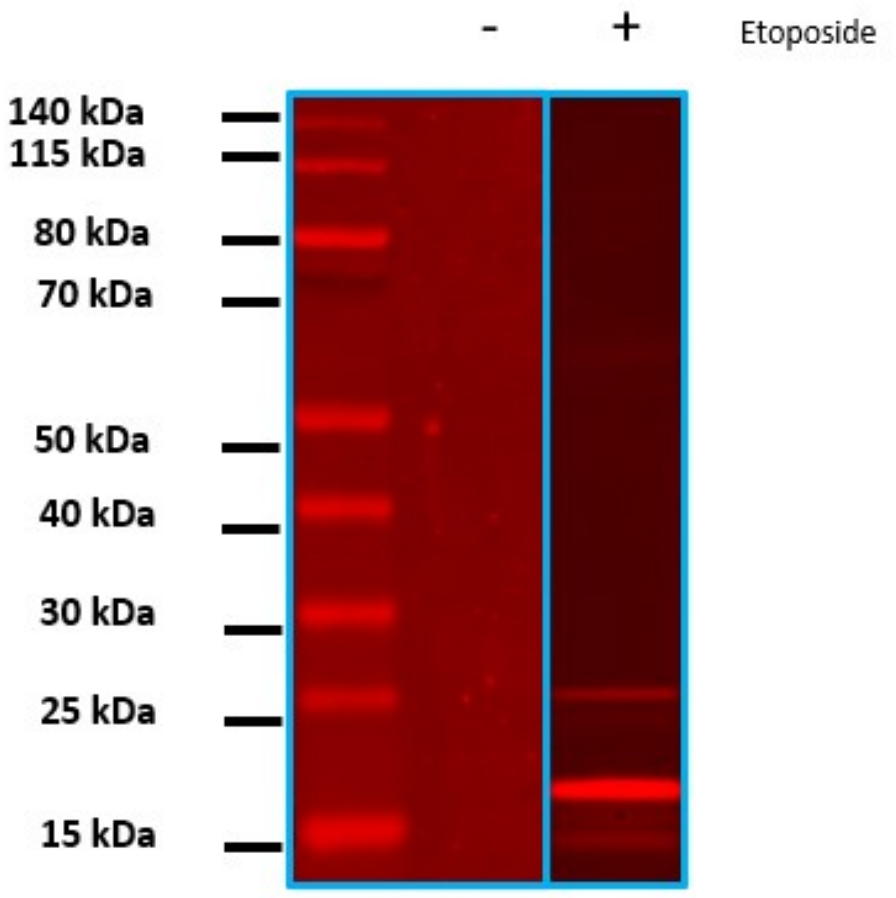
This dossier preserves the complete available evidence progression for DC-000022. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HEK293
DETECTED DYES	680
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	685nm
Emission Filter	720±24 nm
Detector	PMT
Intensity	2
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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

HEK 293 cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down the cell debris The lysate supernatant (100ug) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12%, Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate for 2 hours, followed by 5X PBS wash. The membrane was dried for 10 minutes at room temperature before imaging.

Control Data - Primary Unconjugated Antibody

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

Scan Settings

Detection mode = Fluorescence
Laser = 685nm
Emission Filter = 720±24 nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

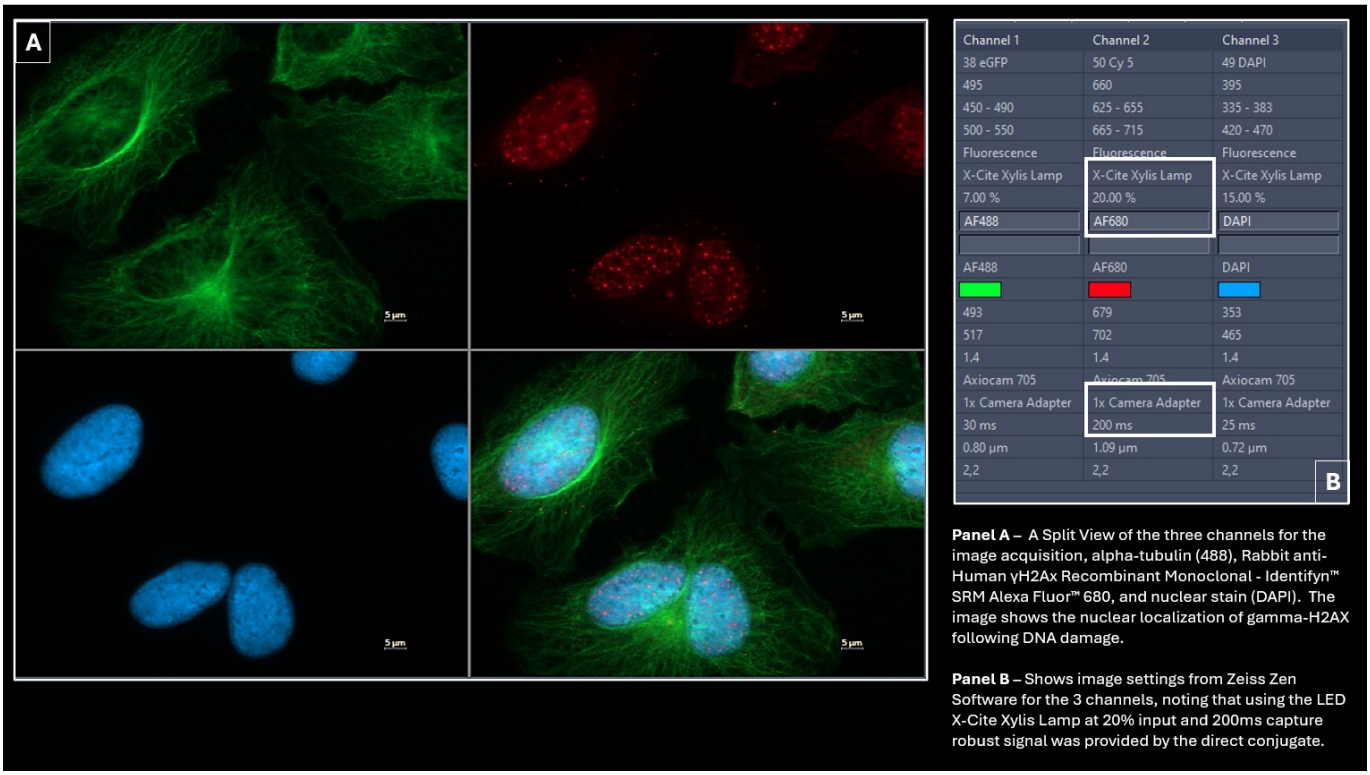
None

Image: Kyle Small

SOURCE PROVENANCE

Catalog	DC-000022
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	HEK293
Image SHA-256	66CFE5B7939549ECAACEE82753BC0EADF2E491A450241F0D235B0B46BC7E42F2
Method PDF SHA-256	500977D9115B3155A99F39A49D7228D2DADD29310F7355BF79371F80891D20A5

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Channel 1	Channel 2	Channel 3
38 eGFP	50 Cy 5	49 DAPI
495	660	395
450 - 490	625 - 655	335 - 383
500 - 550	665 - 715	420 - 470
Fluorescence	Fluorescence	Fluorescence
X-Cite Xylis Lamp	X-Cite Xylis Lamp	X-Cite Xylis Lamp
7.00 %	20.00 %	15.00 %
AF488	AF680	DAPI
AF488	AF680	DAPI
493	679	353
517	702	465
1.4	1.4	1.4
Axiocam 705	Axiocam 705	Axiocam 705
1x Camera Adapter	1x Camera Adapter	1x Camera Adapter
30 ms	200 ms	25 ms
0.80 µm	1.09 µm	0.72 µm
2,2	2,2	2,2

Panel A – A Split View of the three channels for the image acquisition, alpha-tubulin (488), Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, and nuclear stain (DAPI). The image shows the nuclear localization of gamma-H2AX following DNA damage.

Panel B – Shows image settings from Zeiss Zen Software for the 3 channels, noting that using the LED X-Cite Xylis Lamp at 20% input and 200ms capture robust signal was provided by the direct conjugate.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 sCMOS, 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)	108.87 X 77.98
Image Size (Scaled)	83.90µm X 109.63µm
Bit Depth	16 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	50 Cy5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @7.0% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	200ms | 30ms | 25ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4 | .5
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.09µm | 0.80µm | 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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 sCMOS, 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) = 108.87 X 77.98
Image Size (Scaled) = 83.90μm X 109.63μm
Bit Depth = 16 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

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 @20%
Exposure Time = 200ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @7.0%
Exposure Time = 30ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80µm
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 = 25ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA =.5
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Summary

Panel A - A Split View of the three channels for the image acquisition, alpha-tubulin (488), Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, and nuclear stain (DAPI). The image shows the nuclear localization of gamma-H2AX following DNA damage.

Panel B - Shows image settings from Zeiss Zen Software for the 3 channels, noting that using the LED X-Cite Xylis Lamp at 20% input and 200ms capture, a robust signal was provided by the direct conjugate.

Post Processing

None

Image Correction

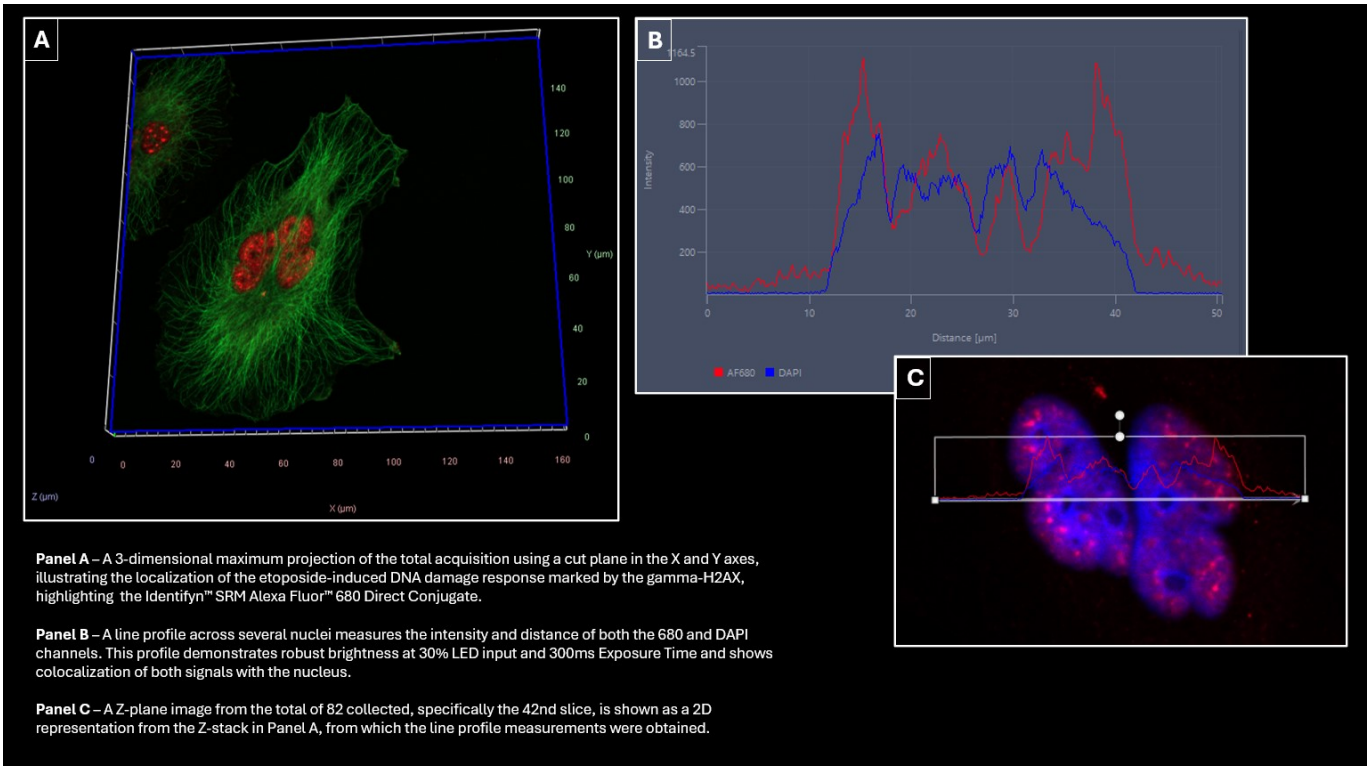
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	DC-000022
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 sCMOS, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4011C846328FB30129772712AD1BF60A2FC601EAD2E6B51F4C3729AB7A8B6BA3
Method PDF SHA-256	5EFAA6AB56E44A888CEC3531BE105F8C27E6F8AE08DC8C851B09656484E32CFD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A – A 3-dimensional maximum projection of the total acquisition using a cut plane in the X and Y axes, illustrating the localization of the etoposide-induced DNA damage response marked by the gamma-H2AX, highlighting the Identifyn™ SRM Alexa Fluor™ 680 Direct Conjugate.

Panel B – A line profile across several nuclei measures the intensity and distance of both the 680 and DAPI channels. This profile demonstrates robust brightness at 30% LED input and 300ms Exposure Time and shows colocalization of both signals with the nucleus.

Panel C – A Z-plane image from the total of 82 collected, specifically the 42nd slice, is shown as a 2D representation from the Z-stack in Panel A, from which the line profile measurements were obtained.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	53

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	82 Slices (8.81µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	1126 X 1068
Image Size (Scaled)	160.86µm X 152.57µm
Bit Depth	14 Bit
Image Center Position	X: 49.34mm, Y: 49.94mm
Stage Position	X: 49.34mm, Y: 49.94mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	50 Cy5 | 38 HE eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30% | X-Cite Xylis Lamp Intensity @15% | X-Cite Xylis Lamp Intensity @6%
Exposure Time	300ms | 100ms | 10ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.36µm | 1.00µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 125%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

FIELD	SOURCE-DOCUMENTED VALUE
X-Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A blue outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.
Position of Clip	9%
Clip Y	-0°
Clip Z	0°
Profile Definition	Stroke Thickness 3.25, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 50.5), Y (Min 3.0 and Max 1164)

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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 82 Slices (8.81µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.100µm
Image Size (Pixels) = 1126 X 1068
Image Size (Scaled) = 160.86µm X 152.57µm
Bit Depth = 14 Bit
Image Center Position = X: 49.34mm, Y: 49.94mm
Stage Position = X: 49.34mm, Y: 49.94mm
ROI Center Offset = X: 1.14, Y: 0.00µm

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 @30%
Exposure Time = 300ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.36µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 100ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.00µm
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%
Exposure Time = 10ms
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 Maximum Projection = Precise
Appearance = Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 125%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel A

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A blue outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = 9%
Clip Y = -0°
Clip Z =0°

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 3.25, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 50.5), Y (Min 3.0 and Max 1164)

Summary

Panel A - A 3-dimensional maximum projection of the total acquisition using a cut plane in the X and Y axes, illustrating the localization of the etoposide-induced DNA damage response marked by the gamma-H2AX, highlighting the Identifyn® SRM Alexa Fluor™ 680 Direct Conjugate.

Panel B - A line profile across several nuclei measures the intensity and distance of both the 680 and DAPI channels. This profile demonstrates robust brightness at 30% LED input and shows colocalization of both signals with the nucleus.

Panel C - A Z-plane image from the 82 collected, specifically the 42nd slice, is shown as a 2D representation from the Z-stack in Panel A, from which the line profile measurements were obtained.

Image Corrections

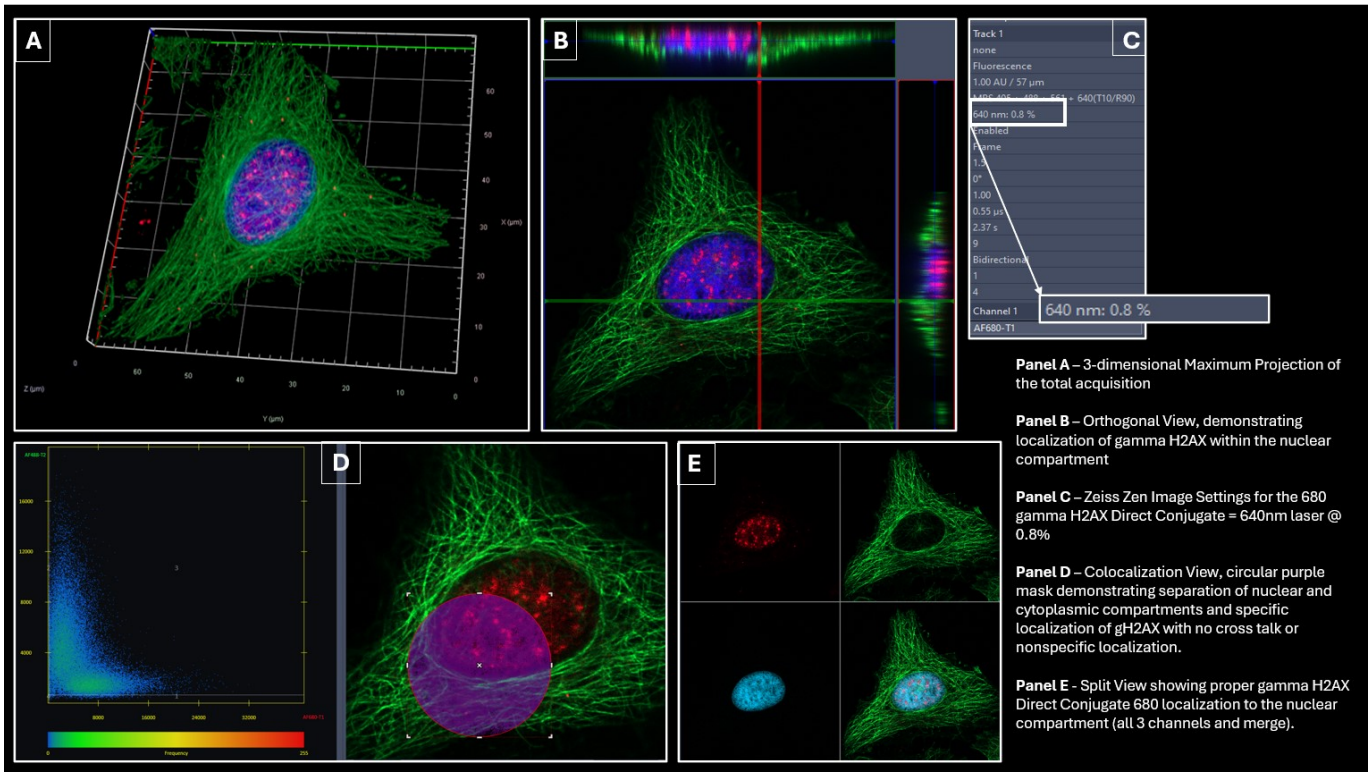
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	DC-000022
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	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2FC3CE28BAC72628922AD817F475E377A417D6B7495F485979D89AD89D1A2F16
Method PDF SHA-256	7C88EA489401ECEDFCF0C2A8ED097166EA1B2C239D66C4CB9F8EFCE8B2897C86

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	56

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 Confocal, 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	151 Slices (4.50µm)
Channels	3
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.030µm
Image Size (Pixels)	958 X 958
Image Size (Scaled)	67.61µm X 67.61µm
Bit Depth	16 Bit
Image Center Position	X: 72.91mm, Y: 50.86mm
Stage Position	X: 72.91mm, Y: 50.86mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 57µm | 1.00AU / 43µm | 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @0.8% | 488nm @0.5% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.5
Rotation	0°
Sampling	1.00
Pixel Time	0.55µs
Frame Time	2.37s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	656 - 700 | 480 - 550 | 410 - 470
Imaging Device	GaAsP-Pmt2 | Airyscan | GaAsP-Pmt1
Detector Type	GaAsP-PMT
Detector Gain	700 V | 650 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 3% (680), Threshold 2% (488), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 4% (680), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Cut Lines	X 588, Y 602, Z 53
Line Width	X 10, Y 10, Z 1
Cut Line Opacity	57
680 gamma H2AX Direct Conjugate	640nm laser @ 0.8%

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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 DSBs. When DSBs occur in chromatin, γH2AX is rapidly phosphorylated explicitly 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM 900 Confocal, 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 = 151 Slices (4.50µm)

Channels = 3
Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.030µm
Image Size (Pixels) = 958 X 958
Image Size (Scaled) = 67.61µm X 67.61µm
Bit Depth = 16 Bit
Image Center Position = X: 72.91mm, Y: 50.86mm
Stage Position = X: 72.91mm, Y: 50.86mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel E

Plane 47 of 151

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 57µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.8%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55µs
Frame Time = 2.37s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 700 V
Detector Offset = 0
Detector Digital Gain = 1.0

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55µs
Frame Time = 2.37s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480 - 550
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 = 1.00AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55µs
Frame Time = 2.37s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
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 3% (680), Threshold 2% (488), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Zoomed Inset

3D Maximum Projection = Precise
Appearance = Threshold 4% (680), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Orthogonal View - Panel B

Uses Image 53 of the Z-Stack 151 images.

Cut Lines = X 588, Y 602, Z 53
Line Width = X 10, Y 10, Z 1
Cut Line Opacity = 57
Maximum Intensity Projection (MIP) was NOT selected

Zeiss Zen Info Tab - Panel C

Zeiss Zen Image Settings for the
680 gamma H2AX Direct Conjugate = 640nm laser @ 0.8%

Colocalization View - Panel D

Horizontal Axis - 680 Threshold 263, Range Auto
Vertical Axis - 488 Threshold 676, Range Auto

Colocalization with the circular mask transversing a section of both the nuclear and cytoplasmic compartments (purple) is measured; as expected, no colocalization is seen, demonstrating the specificity of both targets and, importantly, the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate to the nucleus in response to DNA damage.

Split View - Panel E

Split View shows gamma H2AX in the upper right corner (680, Red), the alpha-tubulin in the right uppermost corner (488, Green), the DAPI or nuclear staining in the lower left (Blue), and the merge in the lower right panel. Demonstrating specificity.

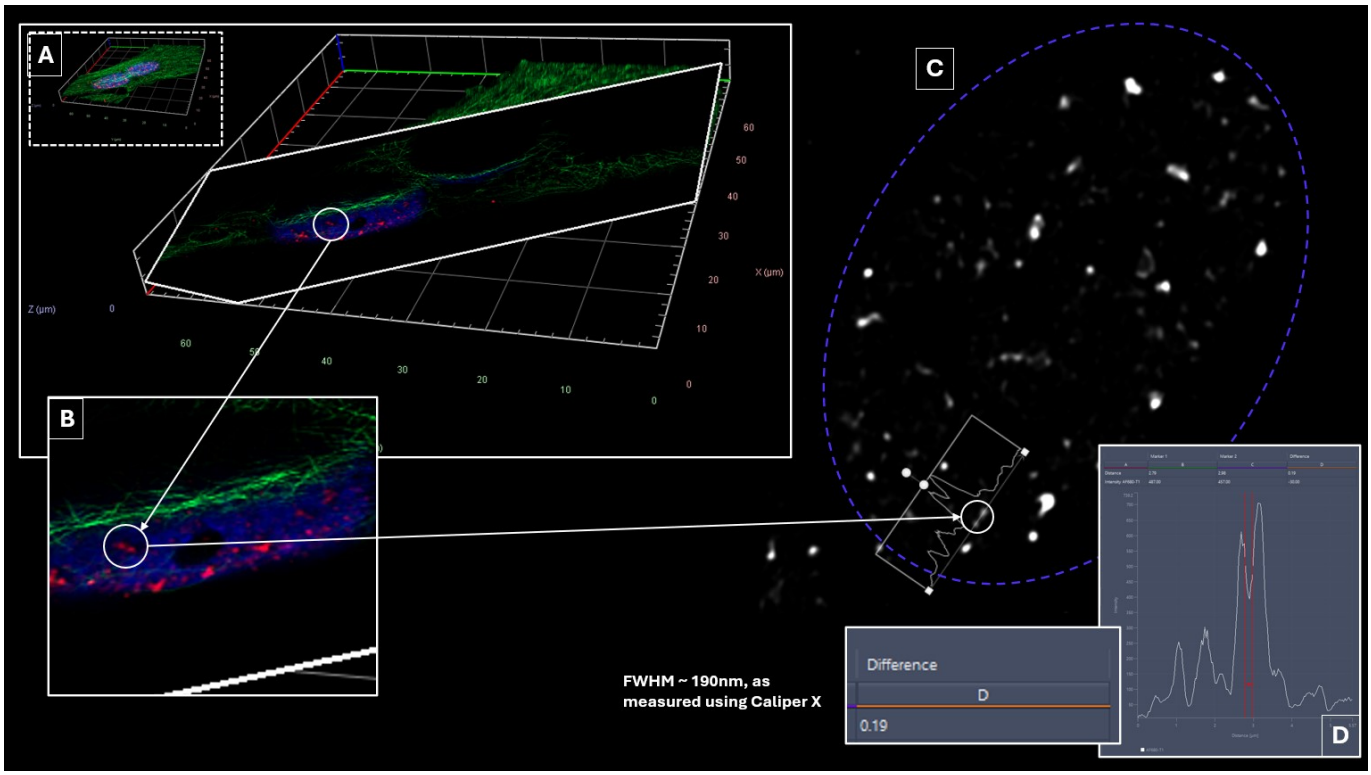
Image Corrections -

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	DC-000022
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, 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	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	798791BAF650F5D1E6FF7F8CB7CA79B1C96BCCAF504D975932B74322BE6F1A5E
Method PDF SHA-256	42C6DE3BA97DC5C93B77E620EDBBAA9B031DC0E1B2036840225D6075130C0ED8

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680; 750
METADATA VALUES	63

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	171 Slices (5.10µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1884 X 1884 | 715 X 627
Image Size (Scaled)	66.50µm X 66.50µm | 24.24µm X 22.13µm
Bit Depth	16 Bit
Image Center Position	X: 105.31mm, Y: 54.62mm
Stage Position	X: 105.31mm, Y: 54.62mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 328µm | 5.00AU / 247µm | 5.46AU / 235µm
LMS MBS	Plate MBS 488/639 MBS 405/730
LMS SBS	SBS LP 10° | SBS SP 550 | SBS SP 505
Laser Wavelength	639nm @0.08% | 488nm @0.01% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	2.00
Pixel Time	1.10µs
Frame Time	18.77s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	573 - 620, 655 - 735 | 380 - 548 | 350 - 499
Imaging Device	Airyscan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 10.0 (3D, Auto) | Super Resolution 9.4 (3D, Auto) | Super Resolution 8.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (680), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% (680), Ramp 1% (DAPI), Maximum 100% all channels
Background	Black
Light	Tone Mapping was NOT Enabled
Projection	View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Y-Z	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A white outline of the clipped perimeter was shown, the Clip Front was selected, and the Clip Back was NOT selected.
Position of Clip	-12%
Clip Y	-78°
Clip Z	30°
Profile Definition	Stroke Thickness 1.00, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 5.57), Y (Min 8.0 and Max 739)

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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 DSBs. When DSBs occur in chromatin, γH2AX is rapidly phosphorylated explicitly 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

Microscope

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

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

Z-Stack =171 Slices (5.10µm)

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm X 30.00nm
Image Size (Pixels) = 1884 X 1884
Image Size (Scaled) = 66.50µm X 66.50µm
Bit Depth = 16 Bit
Image Center Position = X: 105.31mm, Y: 54.62mm
Stage Position =X: 105.31mm, Y: 54.62mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

2D Single Plane - Panel C & D

Plane 65 of 171

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm X 30.00nm
Image Size (Pixels) = 715 X 627
Image Size (Scaled) = 24.24µm X 22.13µm
Bit Depth = 16 Bit
Image Center Position = X: 105.31mm, Y: 54.62mm
Stage Position =X: 105.31mm, Y: 54.62mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = Plate MBS 488/639 MBS 405/730
LMS SBS = SBS LP 10°
Laser Wavelength = 639nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10µs
Frame Time = 18.77s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 573 - 620, 655 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 247µm
LMS MBS = Plate MBS 488/639 MBS 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.01%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10µs
Frame Time = 18.77s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 380 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.4 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.46AU / 235µm
LMS MBS = Plate MBS 488/639 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10µs
Frame Time = 18.77s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.0 (3D, Auto)

3D Image Processing - Panels A & B

3D Maximum Projection = Precise
Appearance = Threshold 1% (680), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% (680), Ramp 1% (DAPI), Maximum 100% all channels
Background = Black
Light = Tone Mapping was NOT Enabled
Projection = View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panels A & B

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

Y-Z = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A white outline of the clipped perimeter was shown, the Clip Front was selected, and the Clip Back was NOT selected.

Position of Clip = -12%
Clip Y = -78°
Clip Z =30°

Profile View Display - Panels C & D

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

Caliper X measured the FWHM of the two "foci" signals for which the lines cross in panel C, measuring at ~190nm between the two distinct signals.

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 5.57), Y (Min 8.0 and Max 739)

Summary

Panel A features a three-dimensional maximum projection view of the entire acquisition, showcasing Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red), alpha-tubulin (green, 488), and nuclear staining using DAPI (blue).
In addition, the primary image in Panel A is the same as that in the upper left inset of the panel. Still, it utilizes a Y-Z cut plane to illuminate the interior of the nuclear compartment. This reveals the localization of the gamma H2AX 680 conjugate within the nucleus following etoposide-induced DNA damage. The panel also highlights a region of interest (ROI) that concentrates on two foci, limited here to Airyscan super-resolution of approximately 120 nm in both the X and Y dimensions.

Panel B, located in the lower left, shows a 3.5X zoom of the ROI area and its surrounding features, providing enhanced visualization of the ROI outlined in Panel A.

Panel C presents a previously mentioned ROI (refer to the data labeled "2D single plane in settings"), which uses a single plane from the z-stack. This eliminates the 488 and DAPI channels to emphasize the gamma H2AX 680 direct conjugate in the nucleus. The image includes a dotted blue line to indicate the perimeter of the nuclear compartment. Furthermore, the image has been modified in Zeiss Zen software from the default dark red color to white for improved visualization of the ROI displayed in Panels A and B. A line was drawn across the signals, measured in Panel D.

Panel D illustrates the line profile for the drawn line across the two signals shown in Panel C. It demonstrates that at 0.08% of the 640 nm laser, the gamma H2AX direct conjugate exhibited extreme brightness, correlating with the peak signals in the adjacent graph. The Caliper X tool was used to measure the Full Width at Half Maximum (FWHM). Although placement is user-dependent, the data shows the signal below the diffraction limit, measured at approximately 190 nm separation. While Airyscan can achieve resolutions of up to 120 nm, this separation distance may be limited by the actual localization of the protein.

Image Corrections

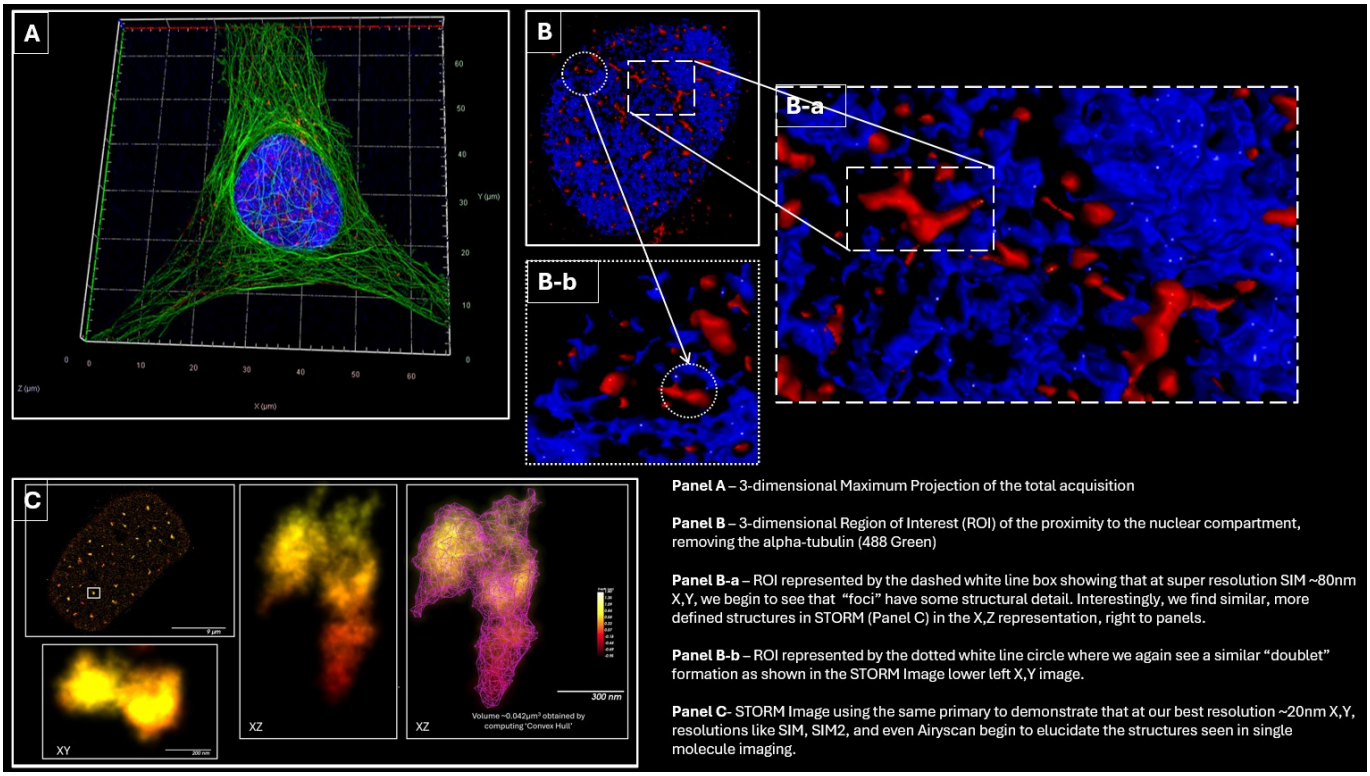
Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate was pseudo-colored in Zen Software from dark red, the default color, to white, in panel C, to provide visualization of the signal shown in dark red in panels A and B.

Image by J. K. Bennett

SOURCE PROVENANCE

Catalog	DC-000022
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DF53F0BBC2DDB41F96ADB4BC166EA0556843480D07BE0099197E90B21AB5EC22
Method PDF SHA-256	36A13D75840DB983E914540B3FD23A197DCE5FD15E93AD6A471A37D1C6EA9631

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	63

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using 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	121 Slices (3.60µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056
Image Size (Scaled)	66.72µm X 66.72µm
Bit Depth	16 Bit
Image Center Position	X: 64.17mm, Y: 39.11mm
Stage Position	X: 64.17mm, Y: 39.11mm
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, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 100ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @3.0% | 488nm @1.50% | 405nm @6.0%
Frame Time	1.59s | 1.33s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.4257 (680), 11.3010 (488), 9.0027 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Maximum Projection	Precise
Appearance	Threshold 4% (680), Threshold 4% (488), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 30% of all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Azimuth 80°, Elongation -130° , Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 DSBs. When DSBs occur in chromatin, γH2AX is rapidly phosphorylated explicitly 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

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 = 121 Slices (3.60µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 64.17mm, Y: 39.11mm
Stage Position = X: 64.17mm, Y: 39.11mm
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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @3.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.50%
Frame Time = 1.33s
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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 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 = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.4257 (680), 11.3010 (488), 9.0027 (DAPI)
Fitting = Fast Fit
Histogram = Scale to Min/Max
Baseline = Cut

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 4% (680), Threshold 4% (488), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panels B (B-a, B-b)

3D Surface Projection = Precise
Appearance = Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 30% of all channels
Background = Black
Light = Azimuth 80°, Elongation -130° , Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - Shows a 3-dimensional maximum projection of the total acquisition.

Panel B -Illustrates a 3-dimensional region of interest (ROI) focused on the proximity to the nuclear compartment, excluding alpha-tubulin (488 nm, green).

Panel B-a - The ROI is highlighted by a dashed white line box, indicating that at super-resolution SIM (~80 nm in X, Y), we begin to observe that the "foci" exhibit some structural detail. Notably, we find similar, more defined structures in the STORM representation (Panel C), particularly in the X, Z view on the right side of the panels.

Panel B-b - The ROI is outlined by a dotted white circle, where we again observe a similar "doublet" formation, as depicted in the STORM analysis's lower left X, Y image.

Panel C - The STORM image employs the same primary antibodies, showing that at our optimal resolution of ~20 nm in X, Y, methods such as SIM, SIM2, and even Airyscan begin to clarify the structures observed in single-molecule imaging.

The comparative imaging from Widefield to Single Molecule microscopy clearly shows that "foci" are non-structural. The level of structural detail is fundamentally limited by the resolution used. As we move beyond the diffraction barrier

into super-resolution techniques-starting with Airyscan, advancing to SIM, and culminating in Single Molecule imaging-it becomes undeniable, supported by thousands of data points, that our interpretations must correspond to the resolution achieved. We should stress that until we obtain resolutions capable of revealing a single event or structure, our scientific claims must accurately reflect the limitations inherent in the resolution applied.

Image Corrections

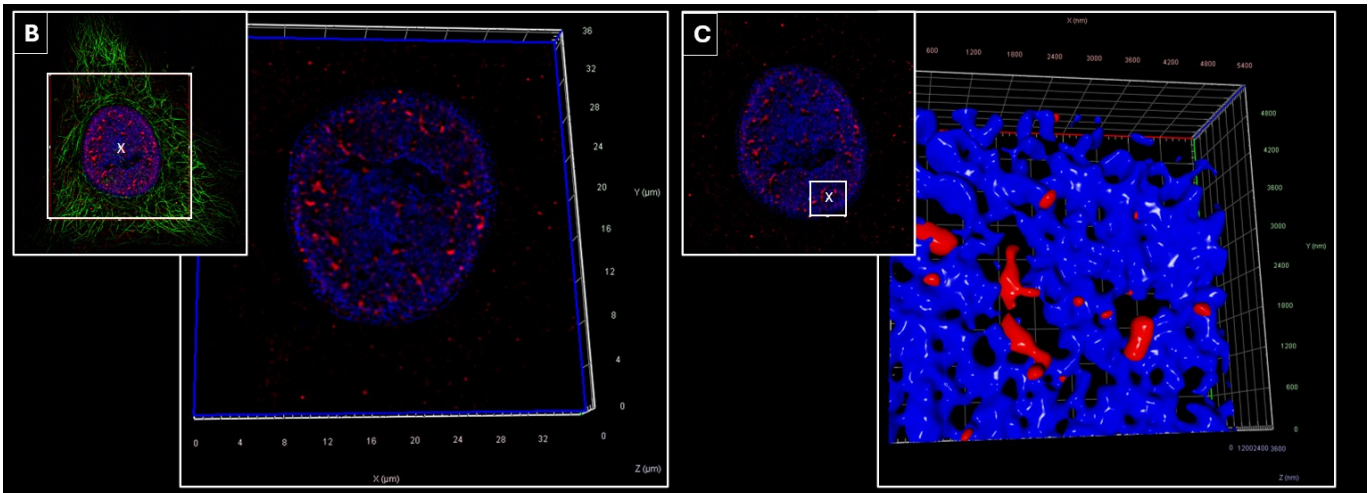
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	DC-000022
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	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5A82E9D9D2C90E62E7C16CF09B84CBE648D8C52B8F94F7F2DF1918990588A00B
Method PDF SHA-256	67C5154CC199A3358BB79842CC46551095ABD8802AA8CFE01A3AAE206F2F86EF

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A – This panel presents a three-dimensional representation of the entire image acquired across the three channels, utilizing a Maximum Projection.

Panel B – A Region of Interest (ROI) has been selected from the original image shown in Panel A, highlighted the by white box with an X. This ROI is displayed as a three-dimensional Maximum Projection using a X,Y Cut-Plane, highlighting the gamma H2AX 680 Direct Conjugate within the nuclear compartment.

Panel C – An additional ROI was taken from the Maximum Projection Cut Plane image in Panel B, indicated by the white box with an X. This ROI is displayed on the far right as a three-dimensional Surface Model. This comparison illustrates that as we improve resolution towards single-molecule detection (via STORM), we observe seemingly enhanced structural details compared to the SIM image. The resolution improves from approximately 80 nm in the X and Y dimensions with SIM to about ~ 60 nm in the X and Y dimensions when applying the SIM² processing.

It is important to note that both SIM and SIM² are algorithm-based image processing techniques and the difference in resolution between the two may be in some cases difficult to distinguish when the structural details fall below these resolutions.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	70

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	153 Slices (4.56µm)
Channels	3 | 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 413 X 401
Image Size (Scaled)	66.72µm X 66.72µm | 5.45µm X 5.29µm
Bit Depth	16 Bit
Image Center Position	X: 65.22mm, Y: 38.05mm
Stage Position	X: 65.22mm, Y: 38.05mm
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, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 100ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @3.0% | 488nm @1.50% | 405nm @6.0%
Frame Time	1.59s | 1.33s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.5440 (680), 10.3380 (488), 7.9518 (DAPI)
Order Combination	Wiener Filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Maximum Projection	Precise
Appearance	Threshold 1% (680), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 30% of all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Azimuth 80°, Elongation -130° , Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000022

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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.

Control Data - Primary Unconjugated Antibody

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) - Panels A & B

Z-Stack = 153 Slices (4.56µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 65.22mm, Y: 38.05mm
Stage Position = X: 65.22mm, Y: 38.05mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel C

Z-Stack = 153 Slices (4.56µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 413 X 401
Image Size (Scaled) = 5.45µm X 5.29µm
Bit Depth = 16 Bit
Image Center Position = X: 65.22mm, Y: 38.05mm
Stage Position = X: 65.22mm, Y: 38.05mm
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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @3.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.50%
Frame Time = 1.33s
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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 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 - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 1
Algorithm = SIM2
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.5440 (680), 10.3380 (488), 7.9518 (DAPI)
Order Combination = Wiener Filter
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

3D Image Processing - Panels A & B

3D Maximum Projection = Precise
Appearance = Threshold 1% (680), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 30% of all channels
Background = Black
Light = Azimuth 80°, Elongation -130° , Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 181%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel presents a three-dimensional representation of the entire image acquired across the three channels, utilizing a Maximum Projection.

Panel B - A Region of Interest (ROI) has been selected from the original image shown in Panel A, highlighted by the white box with an X. This ROI is displayed as a three-dimensional Maximum Projection using an X, Y Cut-Plane, highlighting the gamma H2AX 680 Direct Conjugate within the nuclear compartment.

Panel C - An additional ROI was taken from the Maximum Projection Cut Plane image in Panel B, indicated by the white box with an X. This ROI is displayed on the far right as a three-dimensional Surface Model. This comparison illustrates that as we improve resolution towards single-molecule detection (via STORM), we observe seemingly enhanced structural details compared to the SIM image. The resolution improves from approximately 80 nm in the X and Y dimensions with SIM to about ~ 60 nm in the X and Y dimensions when applying the SIM2 processing.

It is important to note that both SIM and SIM2 are algorithm-based image processing techniques, and the difference in resolution between the two may be, in some cases, difficult to distinguish when the structural details fall below these resolutions.

Image Corrections

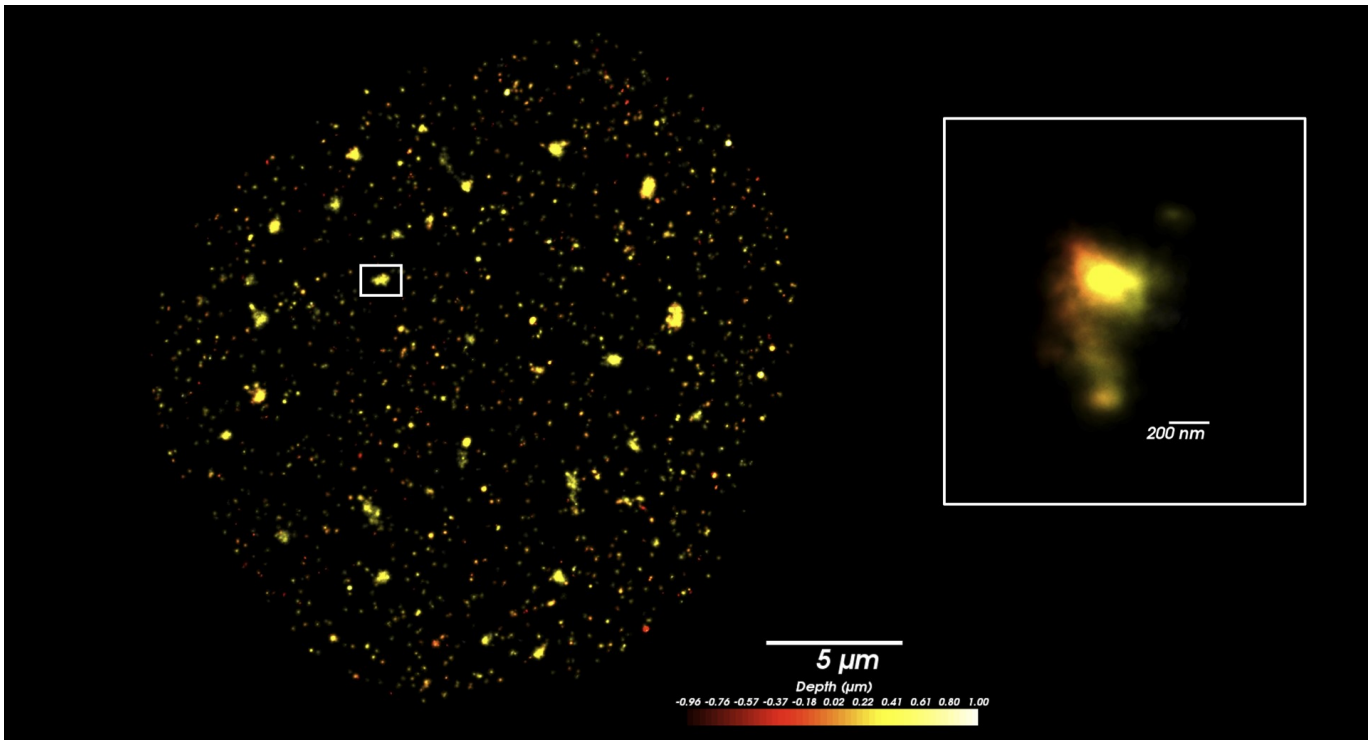
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	DC-000022
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	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	861B58254F26A7718CEC285F8E41BC206A6B0C75CB32129FBED31249A181EE8A
Method PDF SHA-256	FFAA9E2C0FB99BCECF4785D7140D40440067C4793FD2A6A1567A52DC854A036F

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	680
METADATA VALUES	79

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	160.5 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @1% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	200ms
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: 162.3; End: 162.3
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 20000
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	35%
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Radial
Particle size	4nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.05
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	7 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	30
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.07um (700nm)
Maximum Particle Count to Form Cluster	20
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate
Cat ID# - DC 000030

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 DSBs. When DSBs occur in chromatin, γH2AX is rapidly explicitly phosphorylated 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 - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer, followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Control Data - Primary Unconjugated Antibody

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope configuration

Biplane Module: 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: 160.5 µm

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

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

Advanced Tools Option

Widefield camera Exposure Time: 200ms
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: 162.3; End: 162.3

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

SuperRes 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

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 20000
Z Positions: 1 using Steps: 0.2µm (200nm)
Cycles: 1
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 35%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 15

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Radial
Particle size: 4nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.05
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 7 Intervals
Pixel Size: 50nm
Smoothing: 8.00

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

PSF Z offset: -600 to +600
Radial precision: 30
Axial precision: 70

The other parameters were not applied.

Statistical analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.07um (700nm)
Maximum Particle Count to Form Cluster: 20
Computer Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

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Image by S. Thakur

SOURCE PROVENANCE

Catalog	DC-000022
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	79
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D0E6C7A329CA29AE3384C689CB842AEAB8BE2DEACD239C0A4A9CEAE710AA67FD
Method PDF SHA-256	8DF1B237E82128F1360CB29A10D1153E53004DB5716A66B23FB2B405FA331312