BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

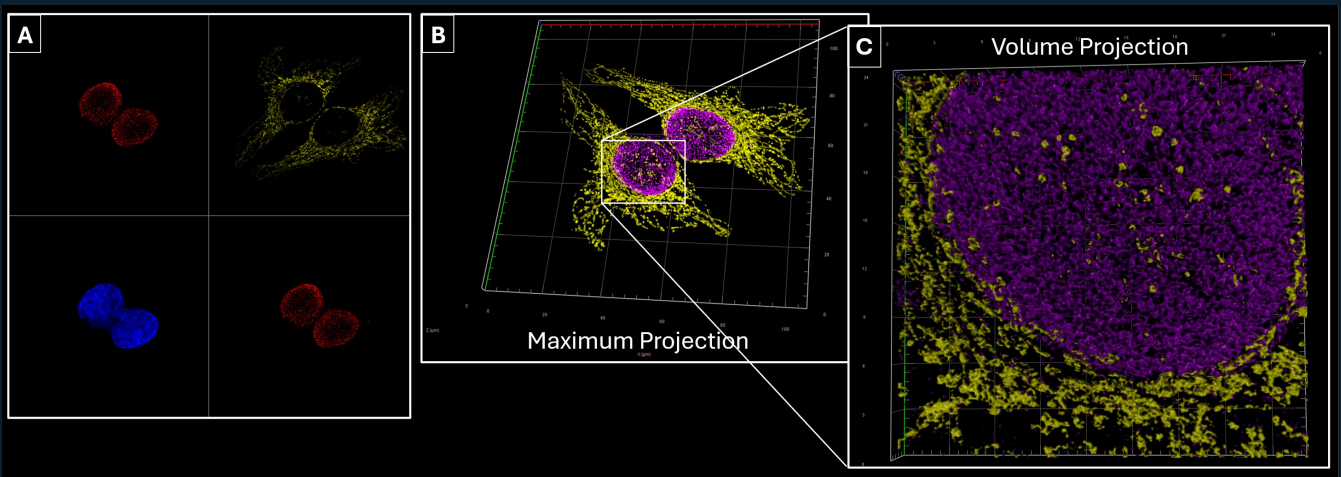
H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680

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

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A - This panel illustrates a split view demonstrating the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 532 (yellow). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels.

Panel B - This panel presents a three-dimensional maximum projection of the same region as shown in Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the maximum view in Zeiss Zen software. A Region of Interest is indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional Volume projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the volume view in Zeiss Zen software.

Image Edit - For visual contrast, the H2AX/680 direct conjugate was changed from its default color, dark red, to purple in Zeiss Zen software.

It is noteworthy that our observations with the H2AX mouse monoclonal primary antibody, counterstained with a secondary fluorescent antibody, as well as the other direct conjugates across all samples and images employing super-resolution techniques such as Airyscan, SIM, SIM Squared, and STORM, show consistent structural details. This consistency validates the three-dimensional localization and structural integrity of the images.

While this image may suggest chromatin organization due to the ubiquitous nature of H2AX in the nucleus, this interpretation is hypothetical. All images demonstrate this pattern, which appears to be biologically relevant, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets using super resolution.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000038 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for 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

BENNETT ASSESSMENT

The preserved DC-000038 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / NIR-BOUNDED PARTIAL STEPDOWN

The preserved DC-000038 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

The record preserves 7 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 680	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 680	3; 50 Cy 5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 680	3; None; 639 nm @1.50%; 488 nm @0.05%; 405 nm @0.2%; 679; 493; 353
Airyscan	405/DAPI; 488; 680; 750	3; None; 639 nm @2.50%; 488 nm @0.05%; 405 nm @0.3%; 679; 493; 353
SIM	405/DAPI; 488; 532; 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 #2-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 532; 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 #2-SR; T3-Axiocam 820 #2-SR; 640

MODALITY ASSESSMENT / PART 1 OF 7

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 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 7

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 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 7

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 86 Slices (8.5 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 676 X 435; Image Size (Pixels): 676 X 435; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 300 ms; 50 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @12.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

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 7

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.4 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 930 X 930; Image Size (Pixels): 930 X 930; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.36 µs; Frame Time: 3.08 s. Illumination: Laser Wavelength: 639 nm @1.50%; 488 nm @0.05%. Optical/scan: Pinhole: 1.00 AU / 68 µm; 1.00 AU / 49µm; Scan Zoom: 2.4; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.2 (3D, Auto); 8.0 (3D, Auto). Structured source metadata values: 64.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 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 7

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 113 Slices (3.36 µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 838 X 912; 271 X 273; Image Size (Pixels): 838 X 912; 271 X 273; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.99 µs; Frame Time: 20.84 s. Illumination: Laser Wavelength: 639 nm @2.50%; 488 nm @0.05%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 247 µm; Scan Zoom: 1.8; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution 9.6 (3D, Auto); SuperResolution 10.0 (3D, Auto). Structured source metadata values: 59.

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 7

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 92 Slices (2.73 µm); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 4278 X 3499; 860 X 822; Image Size (Pixels): 4278 X 3499; 860 X 822; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @2.50%; 488 nm @8.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 60.

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; 532; 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 7

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 94 Slices (2.79 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1238 X 1173; Image Size (Pixels): 5056 X 5056; 1238 X 1173; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @2.50%; 488 nm @8.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 65.

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; 532; 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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000038 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=838 X 912; Image Size (Scaled)=29.58 µm X 32.19 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 µm X 0.01689 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=4278 X 3499; Image Size (Scaled)=72.26 µm X 59.10 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=106.75 µm X 106.75 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM -> 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.

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

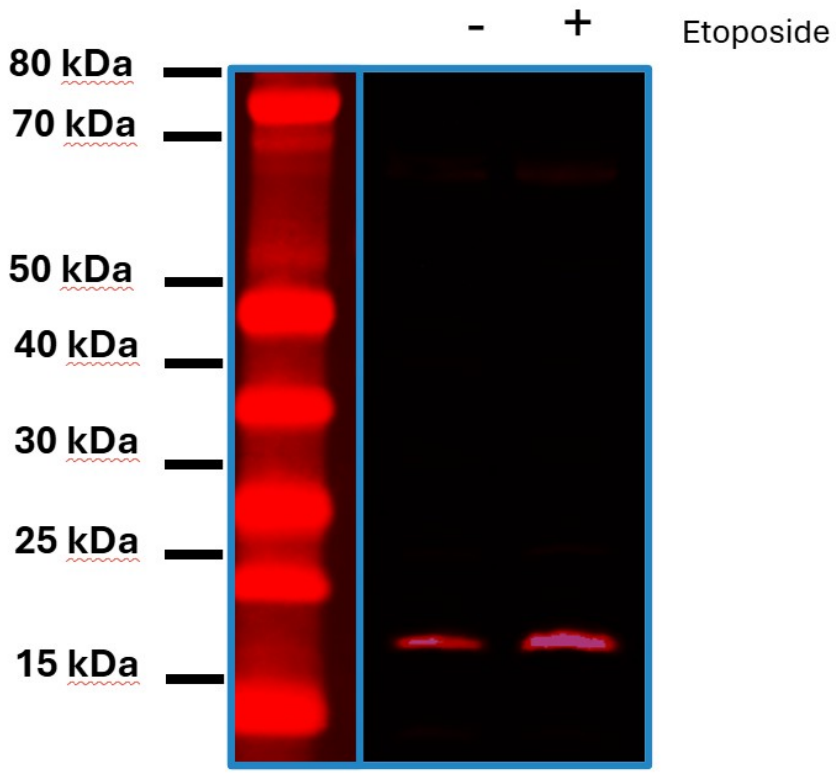
This dossier preserves the complete available evidence progression for DC-000038. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	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	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Expected Molecular Weight: 15 kDa

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

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 - Direct Conjugate for 2 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

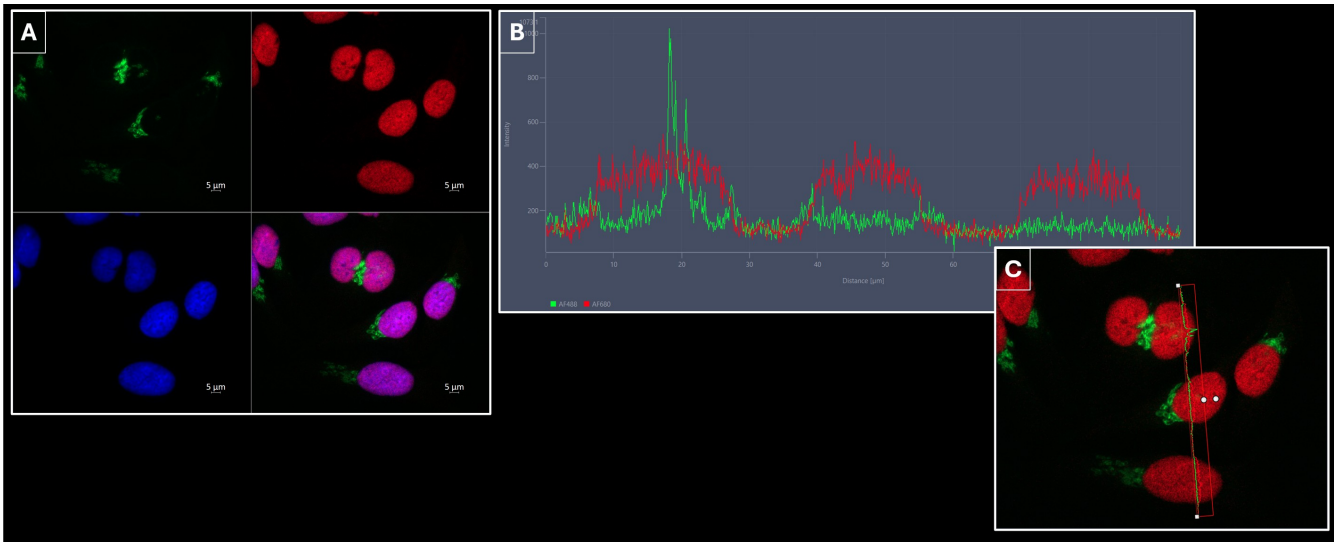
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000038
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6A50384347139045DC3FE8719B3EC966D6FE8C1E7E357869CC847BD59CCA27C2
Method PDF SHA-256	7C9C54F775CE33F1FD8AB83F95833AC79A129909B2923F0B6F9B67021DB8355A

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A – A split view illustrates the signal and localization of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 IgG1, direct conjugate. In the upper left, the image shows the Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). The upper right displays the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 IgG1 direct conjugate (red). The lower left features DAPI nuclear staining (blue). Finally, the lower right presents a merged image combining all three channels.

Note – The localization of the H2AX/680 direct conjugate is found to be nuclear with a "pan" distribution, similar to what is observed with the unconjugated primary antibody. This pan-nuclear staining is expected at diffraction-limited widefield resolution; however, as we transition toward super-resolution imaging, the sample will begin to reveal structural details.

Panels B and C – These panels present a profile view, with the image in Panel C positioned next to the histogram in Panel B. A line has been drawn through several cells, specifically through the nuclei, measuring both the distance and fluorescent intensity. This measurement displays the intensity values of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 IgG1 direct conjugate (red) at an LED excitation power of 15% and an exposure time of 300 ms, thereby confirming the localization and high brightness of the direct conjugate. Additionally, the Golgi primary antibody, counterstained with Identifyn™ SRM Alexa Fluor™ 488, is included in the measurement.

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, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @8.00%
Exposure Time	300 ms | 100 ms | 20 ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.09 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 93.6), Y (Min 11 and Max 1073)

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

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage. It plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat

Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAP, I cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18 µm X 112.59 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 300 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09 µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel features Golgi visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the top-right panel shows Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Profile View Display - Panel B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 93.6), Y (Min 11 and Max 1073)

Summary

Panel A - A split view illustrates the signal and localization of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680 IgG1, direct conjugate. In the upper left, the image shows the Golgi primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The upper right displays the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680 IgG1 direct conjugate (red. The lower left features DAPI nuclear staining (blue).

Finally, the lower right presents a merged image combining all three channels.

Note - The localization of the H2AX/680 direct conjugate is found to be nuclear with a "pan" distribution, similar to what is observed with the unconjugated primary antibody. This pan-nuclear staining is expected at diffraction-limited widefield resolution; however, as we transition toward super-resolution imaging, the sample will begin to reveal structural details.

Panels B and C - These panels present a profile view, with the image in Panel C positioned next to the histogram in Panel B. A line has been drawn through several cells, specifically through the nuclei, measuring both the distance and fluorescent intensity This measurement displays the intensity values of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680 IgG1 direct conjugate (red) at an LED excitation power of 15% and an exposure time of 300 ms, thereby confirming the localization and high brightness of the direct conjugate. Additionally, the Golgi primary antibody, counterstained with Identifyn® SRM Alexa Fluor™ 488, is included in the measurement.

Image Correction

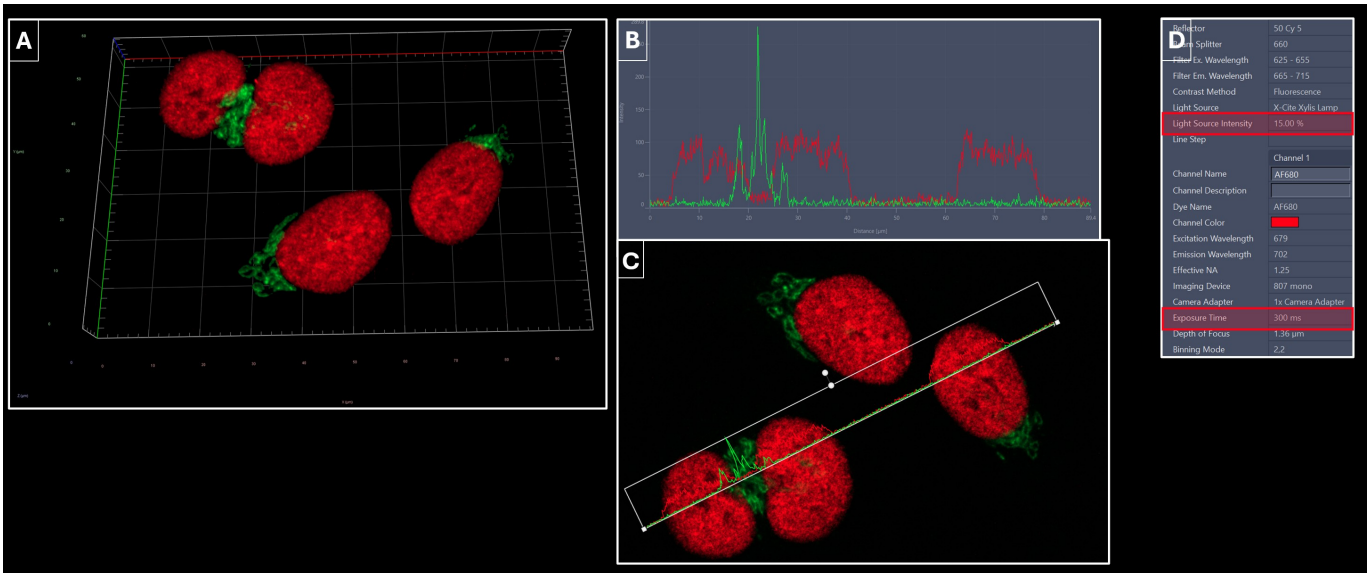
None

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

SOURCE PROVENANCE

Catalog	DC-000038
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7EC85E5DC3CCC5193DF7F54A7D8CA93E38DF1A0F9586679F0F5EAC9906CE9618
Method PDF SHA-256	56723BA23EBCCDD9D3C0279B59F2005F03577608D17EEA8D7AF73A2693354926

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A – This panel presents a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) conjugate and a Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), utilizing widefield structured illumination microscopy (SIM) resolution.

Note: Tone Mapping is NOT enabled, removing manipulation of the coloring to demonstrate the high brightness of the H2AX/680 direct conjugate.

Panels B and C – These panels present a profile view, where the image in Panel C is below the histogram in Panel B. A line has been drawn through several cells, specifically through the nuclei. This line measures both the distance and fluorescent intensity. We use this measurement to display the intensity values of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 IgG1 direct conjugate (red) at an LED excitation power of 15% and an exposure time of 300ms, thus confirming the localization and high brightness of the direct conjugate. Additionally, we include the Golgi primary antibody, counterstained with Identifyn™ SRM Alexa Fluor™ 488 (green) in the line profile measurement.

Panel D - A clipped image from Zeiss Zen software, showing the settings for the image, demonstrating the use of 15% LED excitation and 300ms exposure time.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	50

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	86 Slices (8.5 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	676 X 435
Image Size (Scaled)	96.57 µm X 62.14 µm
Bit Depth	14 Bit
Image Center Position	X: 42.58 mm, Y: 47.75 mm
Stage Position	X: 42.58 mm, Y: 47.75 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 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 @15.00% | X-Cite Xylis Lamp Intensity @12.00% | X-Cite Xylis Lamp Intensity @6.00%
Exposure Time	300 ms | 50 ms | 10 ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.36 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping was NOT enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Z-Position	49 of 86

FIELD	SOURCE-DOCUMENTED VALUE
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 89.4), Y (Min 0 and Max 290)
Note	Tone Mapping is NOT enabled, removing manipulation of the coloring to demonstrate the high brightness of the H2AX/680 direct conjugate.

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

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage and plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat

Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:100 . 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)- Panel A

Z-Stack = 86 Slices (8.5 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 676 X 435
Image Size (Scaled) = 96.57 µm X 62.14 µm
Bit Depth = 14 Bit
Image Center Position = X: 42.58 mm, Y: 47.75 mm
Stage Position = X: 42.58 mm, Y: 47.75 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 300 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.36 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @12.00%
Exposure Time = 50 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.00%
Exposure Time = 10 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

3D Image Processing - Panel A

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

Profile View Display - Panel B & C

Z-Position = 49 of 86
Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 89.4), Y (Min 0 and Max 290)

Summary

Panel A - This panel presents a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red) conjugate and a Golgi primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), utilizing widefield structured illumination microscopy (SIM) resolution.

Note: Tone Mapping is NOT enabled, removing manipulation of the coloring to demonstrate the high brightness of the H2AX/680 direct conjugate.

Panels B and C - These panels present a profile view, where the image in Panel C is below the histogram in Panel B. A line has been drawn through several cells, specifically through the nuclei. This line measures both the distance and fluorescent intensity. We use this measurement to display the intensity values of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680 IgG1 direct conjugate (red) at an LED excitation power of 15% and an exposure time of 300ms, thus confirming the localization and high brightness of the direct conjugate Additionally, we include the Golgi primary antibody, counterstained with Identifyn® SRM Alexa Fluor™ 488 (green) in the line profile measurement.

Panel D - A clipped image from Zeiss Zen software, showing the settings for the image, demonstrating the use of 15% LED excitation and 300ms exposure time.

Image Correction

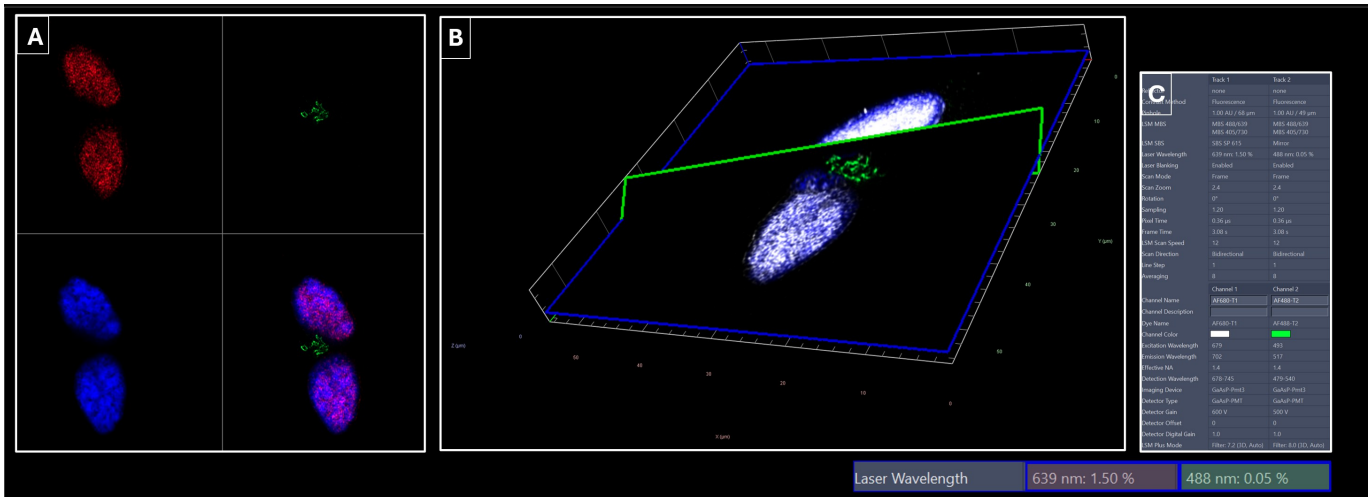
None

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

SOURCE PROVENANCE

Catalog	DC-000038
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0D7CD3384BAA0EC96E2061405EE1DFD966BA62F22F6EFB48EBBCD82B3E7D00F2
Method PDF SHA-256	B848543AAFACCA4E99EE3A3D2F9349F083A9AD7AF63D42CB08832378B50CF062

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - Split View illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate. The Upper left displays Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 (red), upper right, is Golgi primary antibody with an Identifyn™ Alexa Fluor™ 488 secondary (green), lower left DAPI nuclear staining (blue), and in the lower right presents a merged image of the three channels.

Panel B – This panel presents a 3-dimensional Maximum Projection of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate (red), Golgi primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Y (blue outline) and X-Z planes (green outline). In the X-Y cut plane, the position is set at -45% and the angles X and Y are set to 0 degrees (flat), and in the X-Z cut plane, the position is set at 3% and the X is at 45 degrees and the Z at 15 degrees, to provide the cross-cut angle.

Panel C - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 181 planes. **We note that the 639nm laser power is set at % to obtain maximal imaging of the direct conjugate. We also note the Iderntifyn™ Alexa Fluor™ 488 uses 0.05% of the 488nm laser.**

Indicates that the H2AX 680 direct conjugate is localized to the nucleus, and at a 1.50% laser input from a 639nm source, it displays high brightness.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	181 Slices (5.4 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	930 X 930
Image Size (Scaled)	54.71 µm X 54.71 µm
Bit Depth	16 Bit
Image Center Position	X: 82.73 mm, Y: 54.51 mm
Stage Position	X: 82.73 mm, Y: 54.51 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 68 µm | 1.00 AU / 49µm | 1.00 AU / 43 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 615 | Mirror
Laser Wavelength	639 nm @1.50% | 488 nm @0.05% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	1.20
Pixel Time	0.36 µs
Frame Time	3.08 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	678-745 | 497-540 | 435-474
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.2 (3D, Auto) | 8.0 (3D, Auto) | 6.2 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Z	Active was selected, and Textured Opaque was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment. A green outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.
Position of Clip	3% | -45%
Clip X	45° | 0°
Clip Z	15°
X-Y	Active was selected, and Textured Opaque was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment. A blue outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.
Clip Y	0°

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage. It plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat

Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 51 of 181 total - Panel A

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

Z-Stack = 181 Slices (5.4 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 930 X 930
Image Size (Scaled) = 54.71 µm X 54.71 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.73 mm, Y: 54.51 mm
Stage Position = X: 82.73 mm, Y: 54.51 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 68 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @1.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 678-745
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.2 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 49µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 497-540
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.36 µs
Frame Time = 3.08 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435-474
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.2 (3D, Auto)

Split View - Panel A

The top-left panel features Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), the top-right panel shows Golgi visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This image was taken at a Z-Position 51 of 181.

3D Image Processing - Panel B

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

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

X-Z = Active was selected, and Textured Opaque was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment. A green outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.

Position of Clip = 3%
Clip X = 45°
Clip Z = 15°

X-Y = Active was selected, and Textured Opaque was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment. A blue outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.

Position of Clip = -45%
Clip X = 0°
Clip Y = 0°

Summary

Panel A - Split View illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal -Identifyn® SRM Alexa Fluor™ 680, IgG1 direct conjugate The Upper left displays Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 (red), upper right, is Golgi primary antibody with an Identifyn® Alexa Fluor™ 488 secondary (green), lower left DAPI nuclear staining (blue), and in the lower right presents a merged image of the three channels.

Panel B - This panel presents a 3-dimensional Maximum Projection of Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 direct conjugate (red), Golgi primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue) at confocal resolution. The image employs a double "wedge" or a double cut plane on the X-Y (blue outline) and X-Z planes (green outline). In the X-Y cut plane, the position is set at -45%, and the angles X and Y are set to 0 degrees (flat), and in the X-Z cut plane, the position is set at 3%, and the X is at 45 degrees and the Z at 15 degrees, to provide the cross-cut angle.

Panel C - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 181planes. We note that the 639 nm laser power is set at 1.50% to obtain maximal imaging of the direct conjugate. We also note the Iderntifyn™ Alexa Fluor™ 488 uses 0.05% of the 488 nm laser.

Indicates that the H2AX 680 direct conjugate is localized to the nucleus, and at a 1.50% laser input from a 639 nm source, it displays high brightness.

Image Corrections

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7) was pseudo-colored in Zen Software from red, the default color, to white.

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

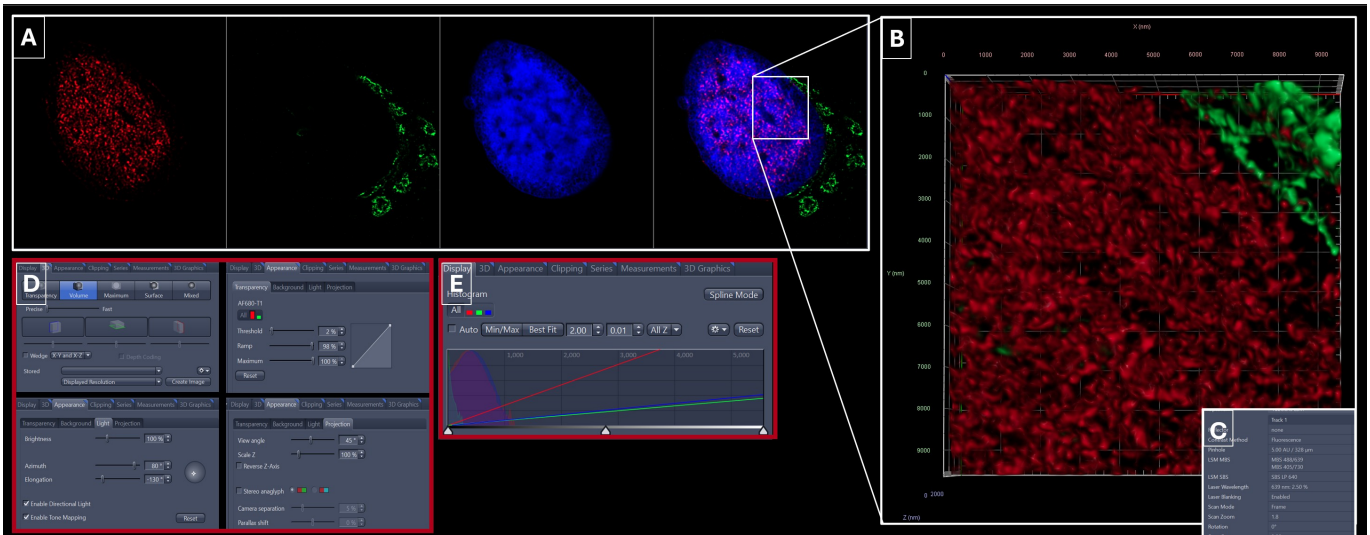
SOURCE PROVENANCE

Catalog	DC-000038
Stage	Confocal

SOURCE PROVENANCE

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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C6E9EBA33DF0E996C1A87B0D7233D81786FF7B9BE850ABCC378C7BAC43F0CE74
Method PDF SHA-256	30773C86620C61EFFD3E70A5340906BF0A206FEF91A52363DA4075A5F047B845

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A - Split View illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate. The left panel displays Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 (red), next panel to the right is Golgi primary antibody with an Identifyn™ Alexa Fluor™ 488 secondary (green), to the right, DAPI nuclear staining (blue), and in the far right we presents a merged image of the three channels. The white box denotes the Region of Interest we chose to investigate further in panel B.

Panel B – Taken from the ROI in Panel A, this panel presents a 3-dimensional Volume Projection of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate (red), and Golgi primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green) at super resolution Airyscan ~ 100nm in X, Y.

Panel C - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 181 planes. **We note that the 639 nm laser power is set at 2.50% to obtain maximum imaging of the direct conjugate.**

Panel D - Provides transparency in our presentation of the three-dimensional settings. All displayed settings are default configurations. In the upper left panel, we see that Volume Projection is set to "Precise." The upper right panel indicates that no thresholding has been performed, as the default settings are a 2% threshold, a 98% ramp, and a maximum of 100%. In the lower right panel, the settings for Brightness, Azimuth, and Elongation remain at their default values. The lower left panel indicates that the View Angle and Scale Z settings are also set to default.

Panel E - The histogram illustrates that no changes were made to the acquired images. This panel uses Auto Spline mode, with no gamma correction applied. The H2AX 680 direct conjugate is localized to the nucleus and displays high brightness.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680; 750
METADATA VALUES	59

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	113 Slices (3.36 µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	838 X 912 | 271 X 273
Image Size (Scaled)	29.58 µm X 32.19 µm | 9.57 µm X 9.64 µm
Bit Depth	16 Bit
Image Center Position	X: 83.07 mm, Y: 55.68 mm
Stage Position	X: 83.07 mm, Y: 55.68 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 247 µm | 5.46 AU / 235 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 640 | SBS SP 550 | SBS SP 505
Laser Wavelength	639 nm @2.50% | 488 nm @0.05% | 405 nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	2.00
Pixel Time	0.99 µs
Frame Time	20.84 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	643-735 | 380-548 | 350-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 9.6 (3D, Auto) | SuperResolution 10.0 (3D, Auto) | SuperResolution 8.5 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage and plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Golgi GOLPH2 Polyclonal Antibody, was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat

Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 54 of 113 total - Panel A

Z Stack - (3D) - Panel A

Z-Stack = 113 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 838 X 912
Image Size (Scaled) = 29.58 µm X 32.19 µm
Bit Depth = 16 Bit
Image Center Position = X: 83.07 mm, Y: 55.68 mm
Stage Position = X: 83.07 mm, Y: 55.68 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 113 Slices (3.36 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 271 X 273
Image Size (Scaled) = 9.57 µm X 9.64 µm
Bit Depth = 16 Bit
Image Center Position = X: 83.07 mm, Y: 55.68 mm
Stage Position = X: 83.07 mm, Y: 55.68 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @2.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.99 µs
Frame Time = 20.84 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.6 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 247 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 550
Laser Wavelength = 488 nm @0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.99 µs
Frame Time = 20.84 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 380-548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.46 AU / 235 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.99 µs
Frame Time = 20.84 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.5 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

Split View - Panel A

The first panel on the left shows Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), the second panel features Golgi visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the third panel shows DAPI nuclear staining, and the last panel on the right is the merged image of all channels This split view image was taken at Z-Position 54 of 113.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - Split View illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 direct conjugate. The left panel displays Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 (red), next panel to the right is Golgi primary antibody with an Identifyn® Alexa Fluor™ 488 secondary (green), to the right, DAPI nuclear staining (blue), and in the far right we present a merged image of the three channels The white box denotes the Region of Interest we chose to investigate further in panel B.

Panel B - Taken from the ROI in Panel A, this panel presents a 3-dimensional Volume Projection of Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 direct conjugate (red), and Golgi primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green) at super resolution Airyscan ~ 100nm in X, Y.

Panel C - A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 181 planes. We note that the 639 nm laser power is set at 2.50% to obtain maximum imaging of the direct conjugate.

Panel D - Provides transparency in our presentation of the three-dimensional settings. All displayed settings are default configurations. In the upper left panel, we see that Volume Projection is set to "Precise." The upper right panel indicates that no thresholding has been performed, as the default settings are at 2% threshold, a 98% ramp, and a maximum of 100%. In the lower right panel, the settings for Brightness, Azimuth, and Elongation remain at their default values. The lower left panel indicates that the View Angle and Scale Z settings are also set to default.

Panel E - The histogram illustrates that no changes were made to the acquired images. This panel uses Auto Spline mode, with no gamma correction applied. The H2AX 680 direct conjugate is localized to the nucleus and displays high brightness.

Image Corrections

None

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

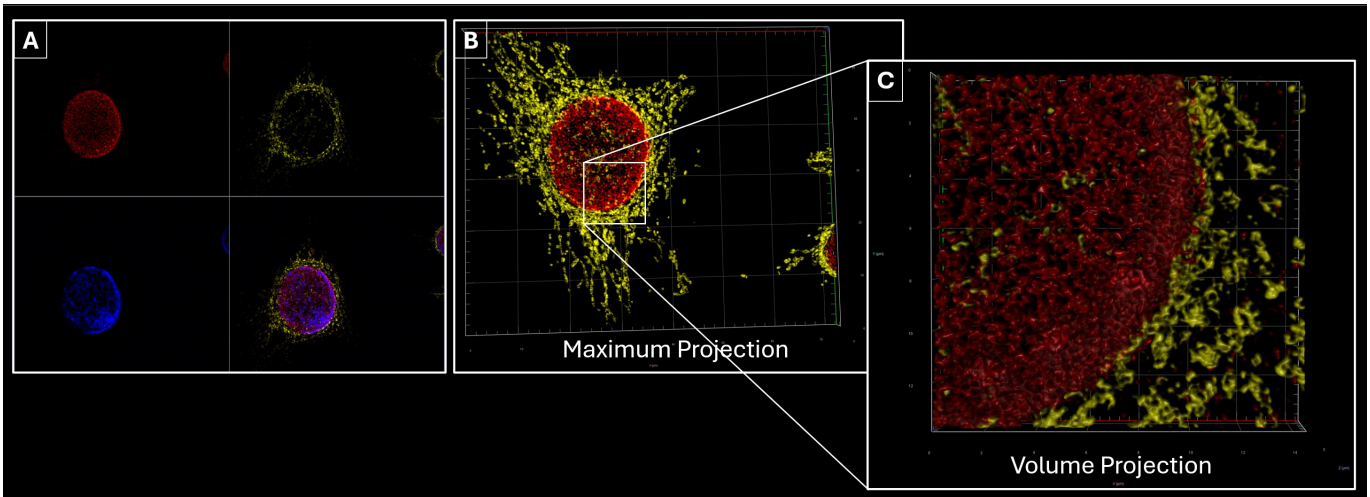
SOURCE PROVENANCE

Catalog	DC-000038
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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6D624B352A9D0D404E6776C84243E8FC6C7CCDDF5C12954B7CFFBD11FC0709A4

SOURCE PROVENANCE

Method PDF SHA-256	0739818C6CCAE2C3A50DF1DBE337A34DB841276C9B487474DA74F5A590E2FF37

ORIGINAL IMAGE EVIDENCE / SIM



Panel A - This panel illustrates a split view demonstrating the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, an IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 532 (yellow). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels.

Panel B - This panel presents a three-dimensional maximum projection of the same region as shown in Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM, with no signal manipulation beyond selecting the maximum view in Zeiss Zen software. A Region of Interest is indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional Volume projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM, with no signal manipulation beyond selecting the volume view in Zeiss Zen software.

It is noteworthy that our observations with the H2AX mouse monoclonal primary antibody, counterstained with a secondary fluorescent antibody, as well as the other direct conjugates across all samples and images employing super-resolution techniques such as Airyscan, SIM, SIM Squared, and STORM, show consistent structural details. This consistency validates the three-dimensional localization and structural integrity of the images.

While this image may suggest chromatin organization due to the ubiquitous nature of H2AX in the nucleus, this interpretation is hypothetical. All images demonstrate this pattern, which appears to be biologically relevant, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets using super resolution.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 680
METADATA VALUES	60

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	92 Slices (2.73 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	4278 X 3499 | 860 X 822
Image Size (Scaled)	72.26 µm X 59.10 µm | 14.53 µm X 13.88 µm
Bit Depth	16 Bit
Image Center Position	X: 58.67 mm, Y: 33.88 mm
Stage Position	X: 58.67 mm, Y: 33.88 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820 #2-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	120 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @2.50% | 488 nm @8.00% | 405 nm @5.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.5818 (680), 10.0886 (532), 8.9033 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage and plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532

Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAP, I cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 47 of 92 total - Panel A

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

Z-Stack = 92 Slices (2.73 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 4278 X 3499
Image Size (Scaled) = 72.26 µm X 59.10 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.67 mm, Y: 33.88 mm
Stage Position = X: 58.67 mm, Y: 33.88 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 92 Slices (2.73 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 860 X 822
Image Size (Scaled) = 14.53 µm X 13.88 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.67 mm, Y: 33.88 mm
Stage Position = X: 58.67 mm, Y: 33.88 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

532 -

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 #2-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 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @8.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.5818 (680), 10.0886 (532), 8.9033 (DAPI)
Fitting = Fast Fit
Normalization = Auto

Split View - Panel A

The top-left panel features Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), the top-right panel shows Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This image was taken at Z-Position 47 of 92.

3D Image Processing - Panel C

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

Summary

Panel A - This panel illustrates a split view demonstrating the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 532 (yellow). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels.

Panel B - This panel presents a three-dimensional maximum projection of the same region as shown in Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM, with no signal manipulation beyond selecting the maximum view in Zeiss Zen software. A Region of Interest is indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional Volume projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM, with no signal manipulation beyond selecting the volume view in Zeiss Zen software.

It is noteworthy that our observations with the H2AX mouse monoclonal primary antibody, counterstained with a secondary fluorescent antibody, as well as the other direct conjugates across all samples and images employing super-resolution techniques such as Airyscan, SIM, SIM Squared, and STORM, show consistent structural details. This consistency validates the three-dimensional localization and structural integrity of the images.

While this image may suggest chromatin organization due to the ubiquitous nature of H2AX in the nucleus, this interpretation is hypothetical. All images demonstrate this pattern, which appears to be biologically relevant, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets using super resolution.

Image Corrections

None

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

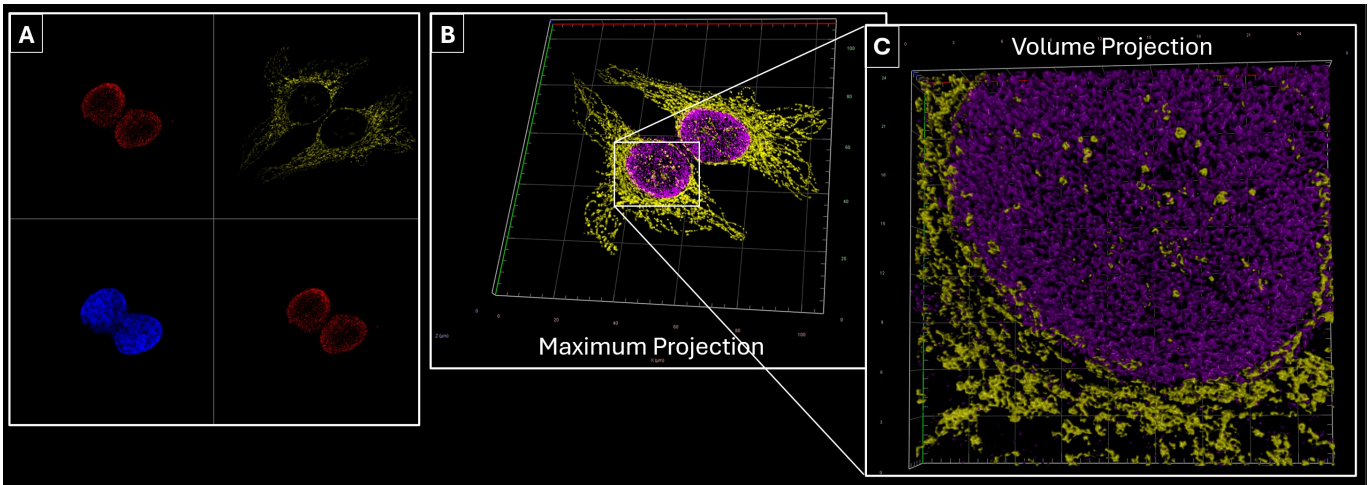
SOURCE PROVENANCE

Catalog	DC-000038
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1459552F3D1ABBF6AA3752BF66B2FD9A1A34BBAD1E39600067B5C767448CB0A7
Method PDF SHA-256	82E9C3B1729D5A9273D6D663A5DA97BA4BFC2BEC601318B288EB417EBDEBF5B8

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - This panel illustrates a split view demonstrating the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 532 (yellow). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels.

Panel B - This panel presents a three-dimensional maximum projection of the same region as shown in Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the maximum view in Zeiss Zen software. A Region of Interest is indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional Volume projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the volume view in Zeiss Zen software.

Image Edit - For visual contrast, the H2AX/680 direct conjugate was changed from its default color, dark red, to purple in Zeiss Zen software.

It is noteworthy that our observations with the H2AX mouse monoclonal primary antibody, counterstained with a secondary fluorescent antibody, as well as the other direct conjugates across all samples and images employing super-resolution techniques such as Airyscan, SIM, SIM Squared, and STORM, show consistent structural details. This consistency validates the three-dimensional localization and structural integrity of the images.

While this image may suggest chromatin organization due to the ubiquitous nature of H2AX in the nucleus, this interpretation is hypothetical. All images demonstrate this pattern, which appears to be biologically relevant, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets using super resolution.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 680
METADATA VALUES	65

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	94 Slices (2.79 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1238 X 1173
Image Size (Scaled)	106.75 µm X 106.75 µm | 26.14 µm X 24.77 µm
Bit Depth	16 Bit
Image Center Position	X: 57.29 mm, Y: 37.13 mm
Stage Position	X: 57.29 mm, Y: 37.13 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820 #2-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	120 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @2.50% | 488 nm @8.00% | 405 nm @5.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM²
SIM² Settings	Standard
Input SNR	Low
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7)
Cat ID# - DC 000038

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000024

H2AX, a variant of the histone protein H2A, and its primary function relates to the organization and structure of chromatin, as well as the cellular response to DNA damage. Here are the essential functions and roles of H2AX:

Nucleosome Formation: H2AX, like other histone proteins, contributes to the formation of nucleosomes. A nucleosome is the fundamental unit of chromatin, consisting of DNA wrapped around a core of histone proteins (including two molecules each of H2A, H2B, H3, and H4). This organization plays a crucial role in compacting DNA within the cell nucleus and regulating gene expression by controlling access to DNA.

DNA Damage Response (DDR): One of H2AX's key functions, distinct from those of other histone proteins, is its function in the DNA damage response. When DNA suffers double-strand breaks (DSBs), H2AX is phosphorylated at a specific serine residue (serine 139), leading to the formation of γ-H2AX (gamma H2AX). This phosphorylation serves as an early signal of DNA damage.

Recruitment of DNA Repair Proteins: Phosphorylation of H2AX to form γ-H2AX creates a binding site for various proteins involved in DNA repair. It helps recruit proteins involved in the recognition, signaling, and repair of DNA damage, such as MDC1 (Mediator of DNA Damage Checkpoint 1), 53BP1 (p53-binding protein 1), BRCA1 (Breast Cancer 1), and others. These proteins work together to repair the damage and maintain genomic stability.

Chromatin Remodeling: Through its role in signaling and repair, H2AX plays a role in chromatin remodeling during the DNA damage response. The formation of γ-H2AX marks regions where chromatin structure is altered to allow access for repair proteins and facilitate the repair process.

H2AX's functions are fascinating studies in molecular biology. They involve a complex interplay of structural and signaling roles. Its structural role in chromatin and its specialized role in signaling and facilitating the DNA damage response, particularly in double-strand breaks, are of immense interest. Through its phosphorylation to form γ-H2AX, it acts as a key marker for DNA damage and plays an essential role in the recruitment and organization of proteins implicated in the DNA repair process.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated for 1 hour at room temperature at a dilution of

1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 45 of 94 total - Panel A

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

Z-Stack = 94 Slices (2.79 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 106.75 µm X 106.75 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.29 mm, Y: 37.13 mm
Stage Position = X: 57.29 mm, Y: 37.13 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 94 Slices (2.79 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1238 X 1173
Image Size (Scaled) = 26.14 µm X 24.77 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.29 mm, Y: 37.13 mm
Stage Position = X: 57.29 mm, Y: 37.13 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

532 -

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 #2-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 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @8.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM² Processing

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

Split View - Panel A

The top-left panel features Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7), the top-right panel shows Mitochondria visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels. This image was taken at Z-Position 45 of 94.

3D Image Processing - Panel A

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

3D Image Processing - Panel B

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

Summary

Panel A - This panel illustrates a split view demonstrating the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red). In contrast, the upper right shows the Mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 532 (yellow). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels.

Panel B - This panel presents a three-dimensional maximum projection of the same region as shown in Panel A. It includes the Mouse anti-HumanH2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the maximum view in Zeiss Zen software. A Region of Interest is indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional Volume projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 680 (red) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 532 (yellow). This was achieved using super-resolution SIM Squared, with no signal manipulation beyond selecting the volume view in Zeiss Zen software.

Image Edit - For visual contrast, the H2AX/680 direct conjugate was changed from its default color, dark red, to purple in Zeiss Zen software.

It is noteworthy that our observations with the H2AX mouse monoclonal primary antibody, counterstained with a secondary fluorescent antibody, as well as the other direct conjugates across all samples and images employing super-resolution techniques such as Airyscan, SIM, SIM Squared, and STORM, show consistent structural details. This consistency validates the three-dimensional localization and structural integrity of the images.

While this image may suggest chromatin organization due to the ubiquitous nature of H2AX in the nucleus, this interpretation is hypothetical. All images demonstrate this pattern, which appears to be biologically relevant, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets using super resolution.

Image Corrections

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG1 - (1H1D9/B7) was pseudo-colored in Zen Software from dark-red, the default color, to purple.

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

SOURCE PROVENANCE

Catalog	DC-000038
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9B10B5EAFFCA4FB02A46DC0D936C8BBE4546C108C3A509A6F68D52DF78C4DE90
Method PDF SHA-256	CDCFE314BCA6DE19704AE6CEFD0F06AC31153F41CC59073BCE29B3E18BDD2362