BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

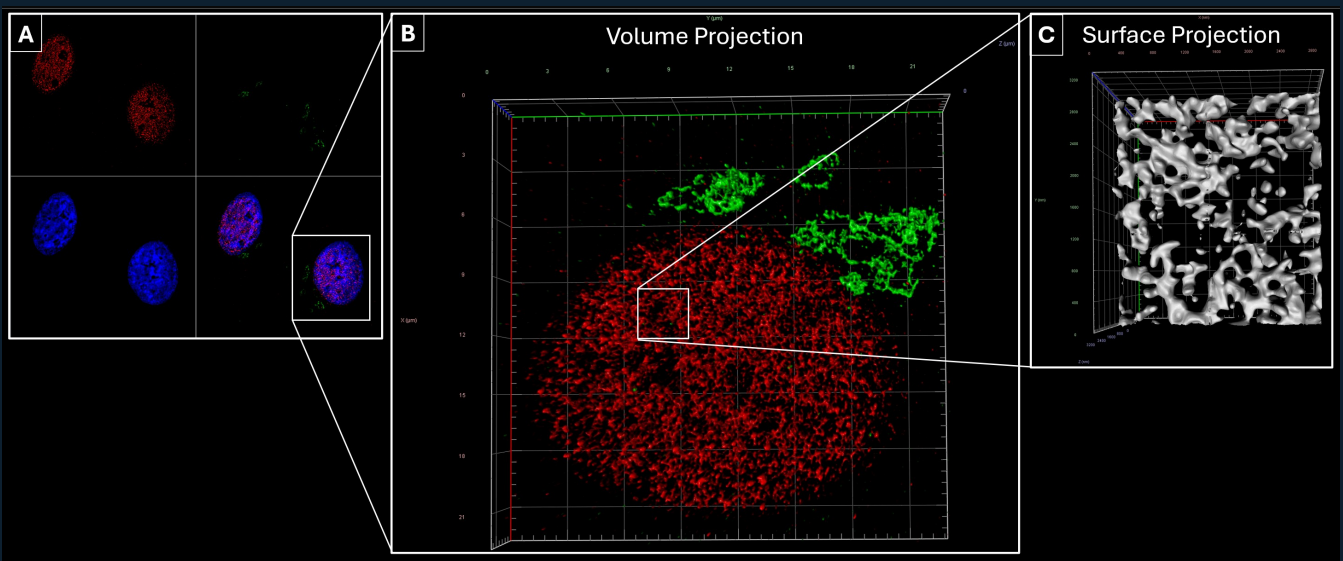
H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594

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™ 594, an IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels. A Region of Interest is indicated by the white box, which will be explored further in Panel B.

Panel B - This panel presents a three-dimensional volume projection of the Region of Interest from Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 (red) and the Golgi primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green). This was achieved using super-resolution SIM Squared, with no manipulation of the signal beyond selecting the volume view in Zeiss Zen software. A Region of Interest is again indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional surface projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, which has been pseudo-colored to white in Zeiss Zen software to enhance visualization of the structural details against a black background, utilizing super-resolution SIM Squared.

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 makes biological sense, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets at this level of resolution, approximately 40 nm in the X and Y dimensions.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000037 | 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-000037 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000037 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 45 Texas Red; 38 eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561 nm @0.5%; 488 nm @0.06%; 405 nm @0.2%; 590; 493; 353
Airyscan	405/DAPI; 488; 594	3; None; 561 nm @3.40%; 488 nm @0.05%; 405 nm @0.2%; 590; 493; 353
SIM	405/DAPI; 488; 594	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 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561
SIM²	405/DAPI; 488; 594	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 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561

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 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 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: 80 ms; 60 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @8.00%; X-Cite Xylis Lamp Intensity @6.00%. 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; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 65 Slices (6.4 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 683 X 703; Image Size (Pixels): 683 X 703; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 75 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @8.00%; X-Cite Xylis Lamp Intensity @10.00%. 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: 49.

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; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 121 Slices (3.6 µm); Channels: 3; Scaling (Per Pixel): 0.054 µm X 0.054 µm X 0.030 µm; Image size (Pixels): 1101 X 1101; Image Size (Pixels): 1101 X 1101; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.31 µs; Frame Time: 3.63 s. Illumination: Laser Wavelength: 561 nm @0.5%; 488 nm @0.06%. Optical/scan: Pinhole: 1.00 AU / 59 µm; 1.00 AU / 49 µm; Scan Zoom: 2.2; 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); 7.4 (3D, Auto). Structured source metadata values: 54.

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; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 121 Slices (3.6 µm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 677 X 561; Image Size (Pixels): 1566 X 1566; 677 X 561; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66 µs; Frame Time: 7.82 s. Illumination: Laser Wavelength: 561 nm @3.40%; 488 nm @0.05%. Optical/scan: Pinhole: 5.00 AU / 286 µm; 5.00 AU / 250 µm; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; 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; AiryScan Mode: SuperResolution 8.5 (3D, Auto); SuperResolution 8.9 (3D, Auto). Structured source metadata values: 60.

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; 594.

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: 100 Slices (2.97 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1236 X 1064; 464 X 365; Image Size (Pixels): 1236 X 1064; 464 X 365; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @8.00%; 488 nm @1.00%; Illumination Wavelength: 561; 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); View Angle 35°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 73.

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; 594.

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: 132 Slices (3.93 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 2663 X 2318; 1079 X 1081; Image Size (Pixels): 2663 X 2318; 1079 X 1081; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2-SR; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @8.00%; 488 nm @1.00%; Illumination Wavelength: 561; 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 77%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 91%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 72.

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; 594.

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-000037 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1566 X 1566; Image Size (Scaled)=55.27 µm X 55.27 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1236 X 1064; Image Size (Scaled)=26.10 µm X 22.46 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 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)=2663 X 2318; Image Size (Scaled)=56.22 µm X 48.94 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

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

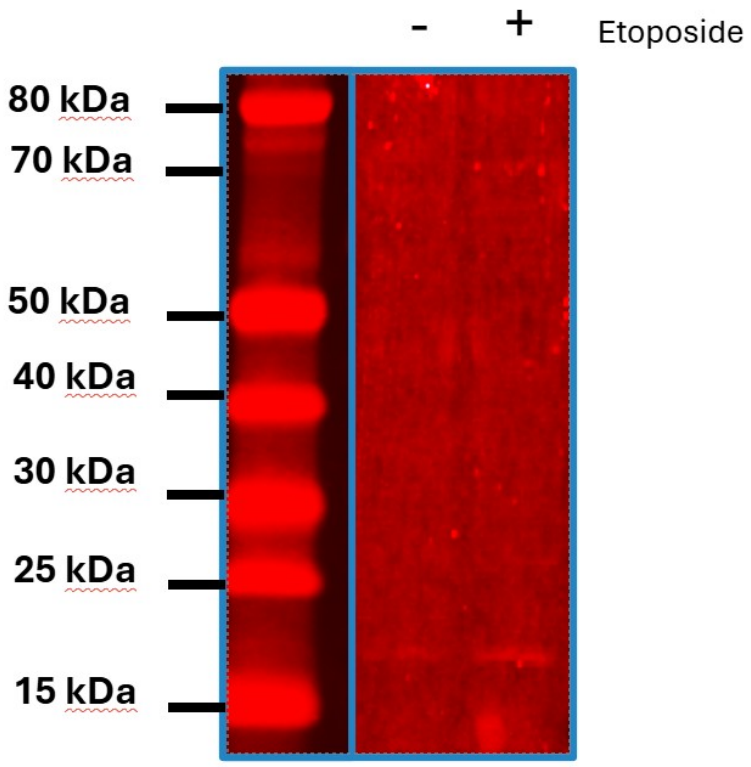
This dossier preserves the complete available evidence progression for DC-000037. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	594
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	572±28nm
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™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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 Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 594 - 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 = 532nm
Emission Filter = 572±28nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

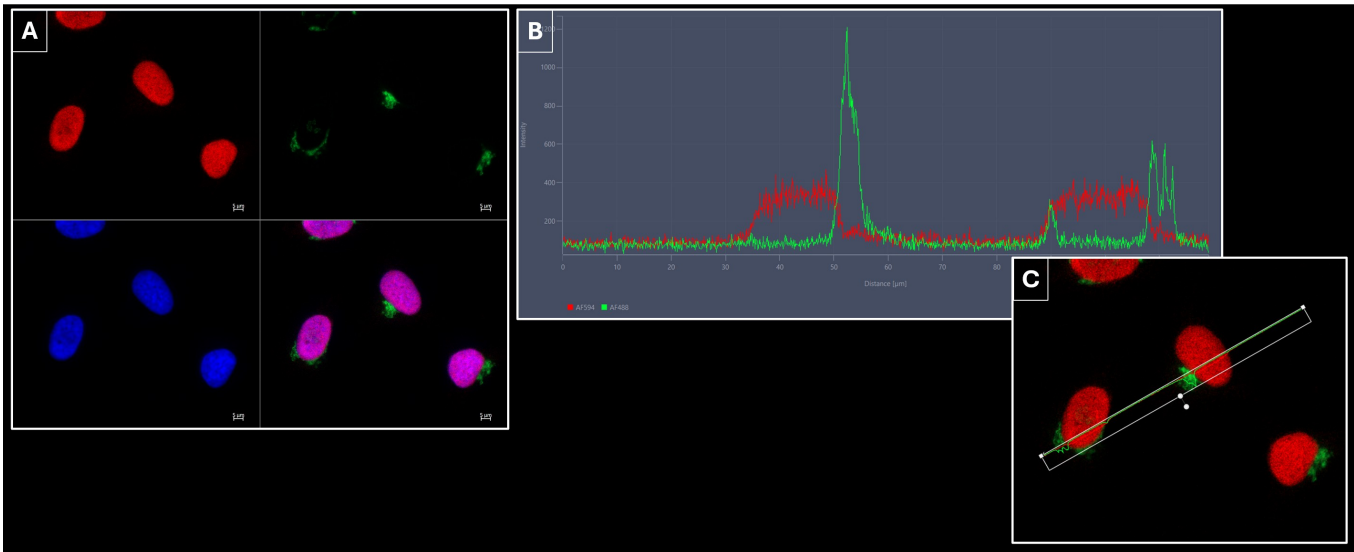
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000037
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	4D1E5C2FCA4C8B86EEA860E9FD465DBA8D42F56F36304B19806265638BFE5EF5
Method PDF SHA-256	9417B9B947A0ECC210139A40338851B602EE5CA63301C05BBC7C6EEA54A96F1C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A– A split view illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 IgG1, direct conjugate. In the upper left, the image shows the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 IgG1, direct conjugate (red). The upper right displays the Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). The lower left features DAPI nuclear staining (blue). Finally, the lower right presents a merged image combining all three channels.

Note – We observe that the localization of the H2AX/594 direct conjugate is nuclear with a "pan" distribution, similar to what is seen with the unconjugated primary antibody. This pan-nuclear staining is expected at diffraction-limited widefield resolution; however, as we shift towards super-resolution imaging, the sample will begin to reveal structural details.

Panels B and C – These panels present a profile view, where the image in Panel C is next to 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™ 594 IgG1 direct conjugate (red) at an LED excitation power of 8% and an exposure time of 80ms, 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 in the measurement.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
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	45 Texas Red | 38 eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @8.00% | X-Cite Xylis Lamp Intensity @6.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	80 ms | 60 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96 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 119.1), Y (Min 25 and Max 1267)

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™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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™ 594, 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, at#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

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 80 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.96 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 @6.00%
Exposure Time = 60 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 @10.00%
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 Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, 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.

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 119.1), Y (Min 25 and Max 1267)

Summary

Panel A - A split view illustrates the signal and localization of Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594 IgG1, direct conjugate. In the upper left, the image shows the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594 IgG1, direct conjugate (red). The upper right displays the Golgi primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left features DAPI nuclear staining (blue). Finally, the lower right presents a merged image combining all three channels.

Note - We observe that the localization of the H2AX/594 direct conjugate is nuclear with a "pan" distribution, similar to what is seen with the unconjugated primary antibody. This pan-nuclear staining is expected at diffraction-limited widefield resolution; however, as we shift towards super-resolution imaging, the sample will begin to reveal structural details.

Panels B and C - These panels present a profile view, where the image in Panel C is next to 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 H2AxMonoclonal - Identifyn® SRM Alexa Fluor™ 594 IgG1 direct conjugate (red) at an LED excitation power of 8% and an exposure time of 80ms, 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 in the measurement.

Image Correction

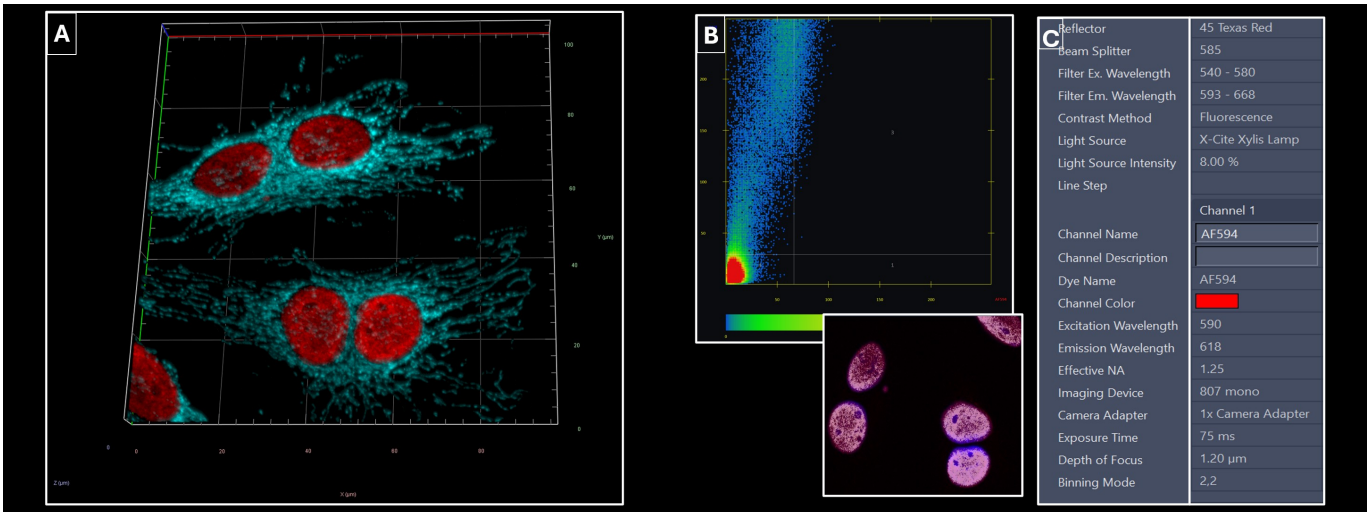
None

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

SOURCE PROVENANCE

Catalog	DC-000037
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	C1A6C496A2B5F3E79E242D80FD644A893E62FEA7E595FFECFF3B0F581CEDC6B8
Method PDF SHA-256	7582687E09166BCCF2BFFEEC26BCF5ACA4D7CE5FA0CC1F8160ABDE18B39A79EB

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A – This panel presents a 3-dimensional maximum projection of Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate (red) and a mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (pseudo colored teal, rather than default green), utilizing widefield SIM resolution. The maximum projection 3-dimensional image, unlike widefield, shows some additional volume and minor advancements as this application allows for 3-dimensional z-stacks. We note that the signal is robust and nuclear.

Note: Tone mapping was NOT enabled, meaning the robust signal is not artificially enhanced or balanced by Zeiss Zen. This supports the extreme brightness of the direct conjugate as demonstrated in the power settings shown in Panel C.

Panel B – This panel displays a colocalization view of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate, and DAPI. The upper panel illustrates the graphical representation of colocalization events for the nuclei represented in the panel below. The colocalization event was measured at z-plane 36 of the total 64 planes taken. We have also masked areas of colocalization in a white overlay, using the 3Col tab in Zeiss Zen. Both the graph and the image, along with the mask, support the localization of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate to the nucleus.

Panel C – This panel features a clipped image from the Zeiss Zen image acquisition settings for this z-stack, which comprises 64 planes. The LED input power is set to 8.0%, and the exposure time is set to 75ms to achieve maximum imaging of the direct conjugate. This configuration supports the extreme brightness for the H2AX/594 direct conjugate.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	49

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	65 Slices (6.4 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	683 X 703
Image Size (Scaled)	97.57 µm X 100.43 µm
Bit Depth	14 Bit
Image Center Position	X: 49.55 mm, Y: 52.25 mm
Stage Position	X: 49.55 mm, Y: 52.25 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	45 Texas Red | 38 HE eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @8.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	75 ms | 100 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.20 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	36 of 65

FIELD	SOURCE-DOCUMENTED VALUE
3Col Mask	White
Note	Tone mapping was NOT enabled, meaning the robust signal is not artificially enhanced or balanced by Zeiss Zen. This supports the extreme brightness of the direct conjugate as demonstrated in the power settings shown in Panel C.

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™ 594 , IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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™ 594, 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™ 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 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 = 65 Slices (6.4 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 683 X 703
Image Size (Scaled) = 97.57 µm X 100.43 µm
Bit Depth = 14 Bit
Image Center Position = X: 49.55 mm, Y: 52.25 mm
Stage Position = X: 49.55 mm, Y: 52.25 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @8.00%
Exposure Time = 75 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20 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 @10.00%
Exposure Time = 100 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 @8.00%
Exposure Time = 20 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)

Colocalization View - Panel B

Z-Position = 36 of 65
Horizontal Axis - AF594, Threshold 190, Range 256
Vertical Axis - DAPI, Threshold 86, Range 256
3Col Mask = White

Summary

Panel A - This panel presents a 3-dimensional maximum projection of Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate (red) and a mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 488 (pseudo colored teal, rather than default green), utilizing widefield SIM resolution. The maximum projection 3-dimensional image, unlike widefield, shows some additional volume and minor advancements, as this application allows for 3-dimensional z-stacks. We note that the signal is robust and nuclear.

Note: Tone mapping was NOT enabled, meaning the robust signal is not artificially enhanced or balanced by Zeiss Zen. This supports the extreme brightness of the direct conjugate as demonstrated in the power settings shown in Panel C.

Panel B - This panel displays a colocalization view of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate, and DAPI. The upper panel illustrates the graphical representation of colocalization events for the nuclei represented in the panel below. The colocalization event was measured at z-plane 36 of the total 64 planes taken. We have also masked areas of colocalization in a white overlay, using the 3Col tab in Zeiss Zen. Both the graph and the image, along with the mask, support the localization of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate to the nucleus.

Panel C - This panel features a clipped image from the Zeiss Zen image acquisition settings for this z-stack, which comprises 64 planes. The LED input power is set to 8.0%, and the exposure time is set to 75 ms to achieve maximum imaging of the direct conjugate. This configuration supports the extreme brightness for the H2AX/594 direct conjugate.

Image Correction

Identifyn® SRM Alexa Fluor™ 488 was pseudo-colored in Zen Software from green, the default color, to light blue to provide visual contrast between the 594/488 channels.

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

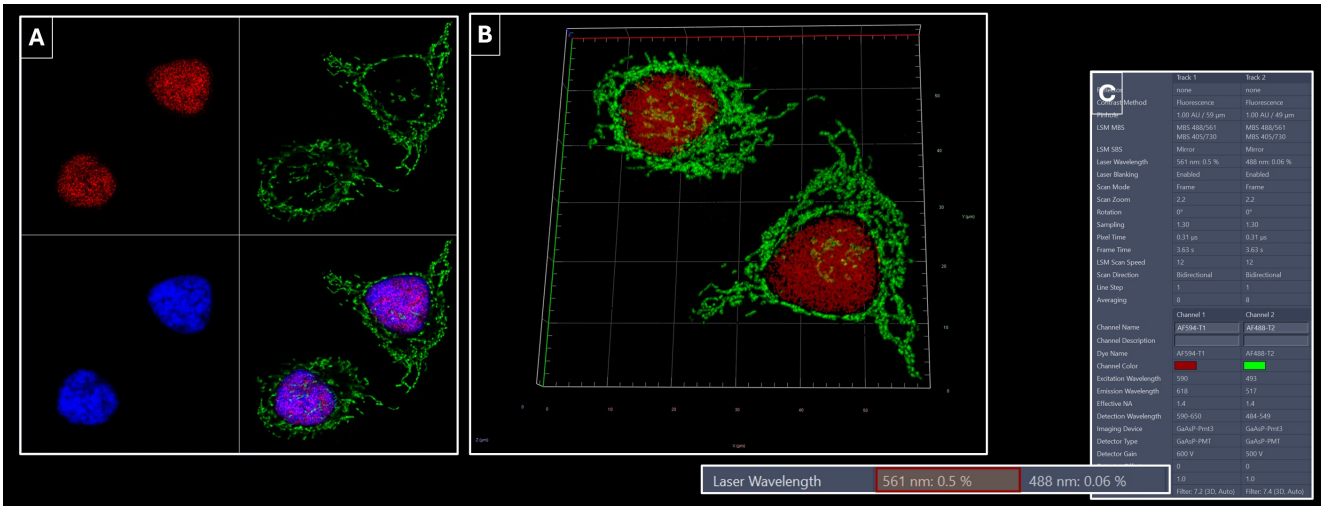
SOURCE PROVENANCE

Catalog	DC-000037
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:

SOURCE PROVENANCE

Structured source metadata values	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A7F1F39C37B0A35108F50AFF1BC694B8245DCAB945904BDD861F9459949CBF49
Method PDF SHA-256	376E5B19A68C2C88027321EBA440B9550FEB3923A11540057EEB41A724C8E581

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - Split view illustrates the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, 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™ 488 (green). 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 Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate (red) and the Mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green) at confocal resolution. The Image employs LMS Plus (LSMP processing); however, no adjustments were made post-acquisition. 3-dimensional modeling is set at default settings, and image quality and H2AX/594 brightness are not adjusted.

Panel C - This panel features a clipped image from the Zeiss Zen image acquisition settings for a z-stack comprising 121 planes. **The 561 nm laser power is set at 0.5% to achieve optimal imaging of the direct conjugate over a z-stack distance of 3.6 microns. We also note the Identifyn™ SRM Alexa Fluor™ 488 required 0.06%, indicating high brightness for all Identifyn™ products via our patent-pending conjugation chemistry.**

Both images demonstrate the alignment of the nucleus with the H2AX 594 direct conjugate and the DAPI staining.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	54

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	121 Slices (3.6 µm)
Channels	3
Scaling (Per Pixel)	0.054 µm X 0.054 µm X 0.030 µm
Image Size (Pixels)	1101 X 1101
Image Size (Scaled)	59.92 µm X 59.92 µm
Bit Depth	16 Bit
Image Center Position	X: 84.19 mm, Y: 56.05 mm
Stage Position	X: 84.19 mm, Y: 56.05 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 59 µm | 1.00 AU / 49 µm | 1.00 AU / 43 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @0.5% | 488 nm @0.06% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.30
Pixel Time	0.31 µs
Frame Time	3.63 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	590-650 | 484-549 | 430-480
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) | 7.4 (3D, Auto) | 6.5 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping 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)

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™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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 other. 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™ 594, 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™ 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 61 of 121 total - Panel A

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

Z-Stack = 121 Slices (3.6 µm)

Channels = 3
Scaling (Per Pixel) = 0.054 µm X 0.054 µm X 0.030 µm
Image Size (Pixels) = 1101 X 1101
Image Size (Scaled) = 59.92 µm X 59.92 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.19 mm, Y: 56.05 mm
Stage Position = X: 84.19 mm, Y: 56.05 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590-650
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 / 49 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 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 = 484-549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.30
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-480
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.5 (3D, Auto)

Split View - Panel A

The top-left panel shows Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, 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 is the merged image of all channels. This split-view image was taken at Z-Position 61 of 121.

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%, Tone Mapping 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)

Summary

Panel A - Split view illustrates the signal and localization of the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 594, 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™ 488 (green). 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 Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate (red) and the Mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green) at confocal resolution. The Image employs LMS Plus (LSMP processing); however, no adjustments were made post-acquisition. 3-dimensional modeling is set at default settings, and image quality and H2AX/594 brightness are not adjusted.

Panel C - This panel features a clipped image from the Zeiss Zen image acquisition settings for a z-stack comprising 121 planes. The 561 nm laser power is set at 0.5% to achieve optimal imaging of the direct conjugate over a z-stack distance of 3.6 microns. We also note the Identifyn® SRM Alexa Fluor™ 488 required 0.06%, indicating high brightness for all Identifyn® products via our patent-pending conjugation chemistry.

Both images demonstrate the alignment of the nucleus with the H2AX 594 direct conjugate and the DAPI staining.

Image Corrections

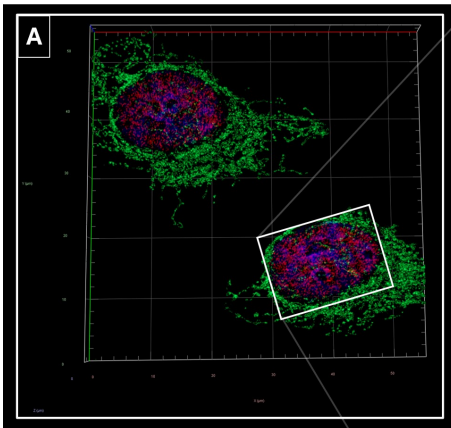
None

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

SOURCE PROVENANCE

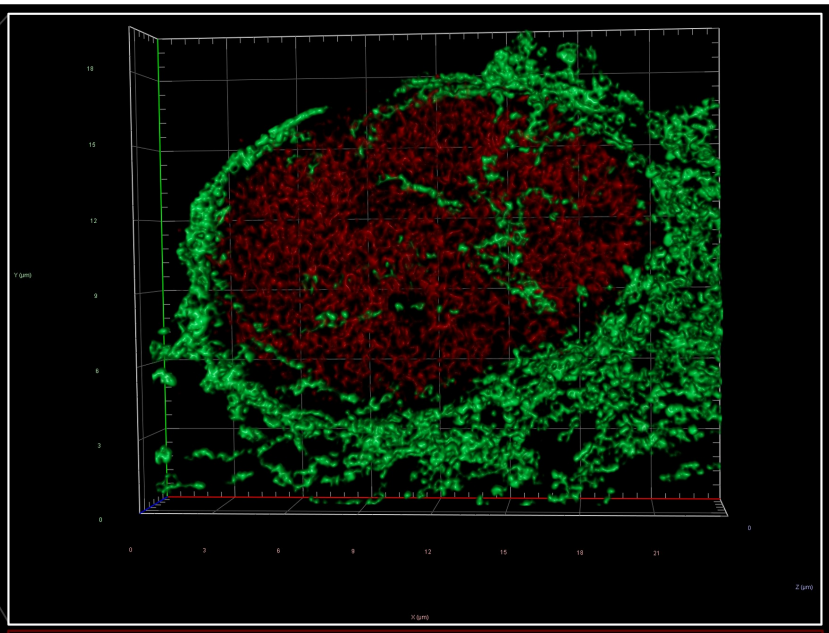
Catalog	DC-000037
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2D658A1ADE4471096364288678DE1C954A5B29C46E31DC14D2FFF840E8E6FFCA
Method PDF SHA-256	63F31D1AD59F46AFAD06B48D7F99873CF9FCBF984FA206116978FD32BEA7D634

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A - This panel displays a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate (red), combined with the mitochondrial primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), along with DAPI nuclear staining, utilizing super-resolution Airyscan imaging. A Region of Interest (ROI) is indicated in Panel A, which we will explore further to demonstrate the advantages of super-resolution Airyscan compared to diffraction-limited images in the H2AX 594 direct conjugate dataset.

Panel B - This panel presents a three-dimensional volume projection from the ROI identified in Panel A, again showing the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 (red) and the mitochondrial primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green). Noting importantly the Zeiss Zen default settings for the volume projection were used, no editing of the original acquisition occurred.



For the first time in the H2AX/594 direct conjugate dataset we transition from diffraction-limited techniques to Identifyn's™ patent-pending "step down" method, observing independent signals rather than the pan-nuclear (diffuse) staining typically seen with diffraction-limited applications such as widefield, widefield SIM, and to some extent, confocal microscopy. This transition reveals further structural details within the nuclear H2AX 594 direct conjugate staining. The findings are robustly supported by the use of a known marker for mitochondria, as Airyscan has consistently demonstrated the ability to begin resolving the organelle's center, as evidenced here. Given the control of resolution by mitochondria, the organization of the H2AX direct conjugate staining invites intriguing questions, though we make no claims regarding the underlying biology.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	60

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	121 Slices (3.6 µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1566 X 1566 | 677 X 561
Image Size (Scaled)	55.27 µm X 55.27 µm | 23.89 µm X 19.80 µm
Bit Depth	16 Bit
Image Center Position	X: 84.37 mm, Y: 52.39 mm
Stage Position	X: 84.37 mm, Y: 52.39 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 286 µm | 5.00 AU / 250 µm | 5.37 AU / 235 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 525 | SBS SP 550 | SBS SP 505
Laser Wavelength	561 nm @3.40% | 488 nm @0.05% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66 µs
Frame Time	7.82 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	573-627 | 499-548 | 420-480, 495-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 8.5 (3D, Auto) | SuperResolution 8.9 (3D, Auto) | SuperResolution 7.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels. | Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 145%, 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™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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 other. 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™ 594, 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™ 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:

Z Stack - (3D)- Panel A

Z-Stack = 121 Slices (3.6 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1566 X 1566
Image Size (Scaled) = 55.27 µm X 55.27 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.37 mm, Y: 52.39 mm
Stage Position = X: 84.37 mm, Y: 52.39 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D)- Panel B

Z-Stack = 121 Slices (3.6 µm)

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 677 X 561
Image Size (Scaled) = 23.89 µm X 19.80 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.37 mm, Y: 52.39 mm
Stage Position = X: 84.37 mm, Y: 52.39 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 286 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @3.40%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573-627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.5 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS SP 550
Laser Wavelength = 488 nm @0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499-548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.37 AU / 235 µm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420-480, 495-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 7.6 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

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 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 145%, 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 displays a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate (red), combined with the mitochondrial primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), along with DAPI nuclear staining, utilizing super-resolution Airyscan imaging. A Region of Interest (ROI) is indicated in Panel A, which we will explore further to demonstrate the advantages of super-resolution Airyscan compared to diffraction-limited images in the H2AX 594 direct conjugate dataset.

Panel B - This panel presents a three-dimensional volume projection from the ROI identified in Panel A, again showing the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594 (red) and the mitochondrial primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green). Notably, the Zeiss Zen default settings for the volume projection were used; no editing of the original acquisition occurred.

For the first time in the H2AX/594 direct conjugate dataset, we transition from diffraction-limited techniques to Identifyn's™ patent-pending "step down" method, observing independent signals rather than the pan-nuclear (diffuse) staining typically seen with diffraction-limited applications such as widefield, widefield SIM, and to some extent, confocal microscopy This transition reveals further structural details within the nuclear H2AX 594 direct conjugate staining. The findings are robustly supported using a known marker for mitochondria, as Airyscan has consistently demonstrated the ability to begin resolving the organelle's center, as evidenced here. Given the control of resolution by mitochondria, the organization of the H2AX direct conjugate staining invites intriguing questions, though we make no claims regarding the underlying biology.

Image Corrections

None

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

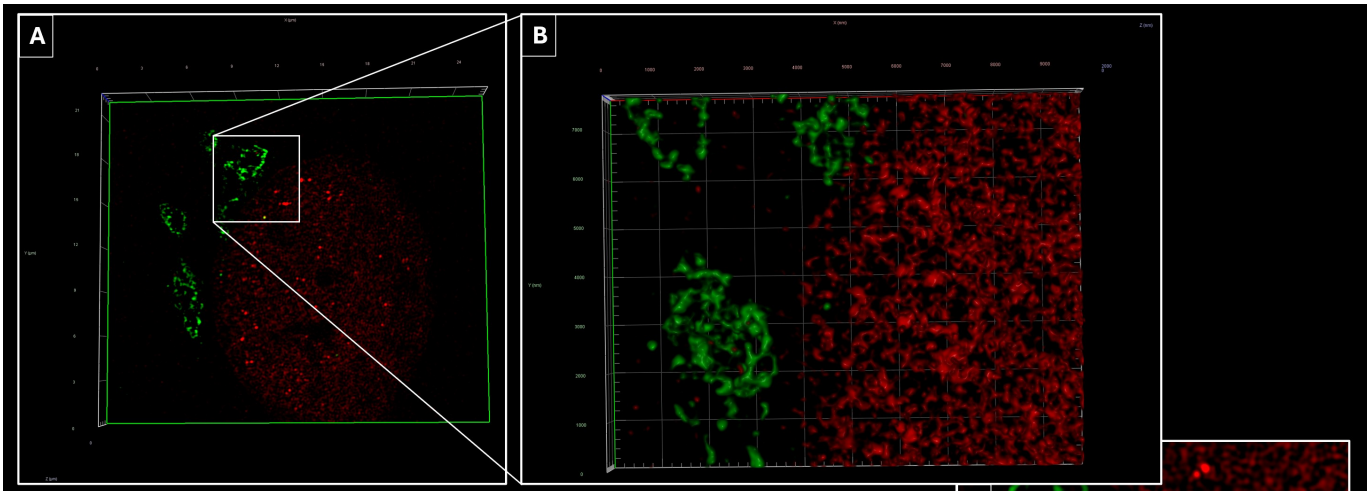
SOURCE PROVENANCE

Catalog	DC-000037
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:

SOURCE PROVENANCE

Structured source metadata values	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9A72A80B4AE452B97B24985A9CEA398B8620BEF34C0ADF9720EE2DC71AC13BB2
Method PDF SHA-256	BF1A900398C7CF9C6A8080183161B290B1366FD64D5F13E8285BAE7CC6E4983D

ORIGINAL IMAGE EVIDENCE / SIM



Panel A - This panel displays a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, IgG1 direct conjugate (red), combined with the Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), utilizing super-resolution SIM imaging. An X-Y cut plane is employed at position 36%, with X and Y at zero-degree angles; the plane is outlined in green. This cut plane was used to show the nuclear distribution of the H2AX/594 direct conjugate and the apparent nucleolar space devoid of signal, as expected. The image includes a Region of Interest (ROI) denoted by a white box.

Panel B - This panel presents a three-dimensional volume projection from the ROI identified in Panel A, again showing the Mouse anti-Human H2Ax Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 (red) and the Golgi primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green). It is essential to note that the Zeiss Zen default settings for volume projection were used, with no edits made to the original acquisition. Tone mapping was disabled to ensure the image was not artificially color-mapped or manipulated in any way.

Panel C - This is a two-dimensional image from z-plane 40 of the 100 total images taken to further investigate in a maximum projection view, or unmodeled structure, that we consistently observe when transitioning from super-resolution Airyscan to SIM, decreasing our axial resolution from 100-120 nm to 60-80 nm in the X and Y dimensions.

This transition reveals additional structural details within the nuclear compartment as they pertain to the localization of H2AX 594 direct conjugate staining. The findings are strongly supported by the use of a known marker for the Golgi, as SIM has consistently demonstrated the ability to resolve its structure, as evidenced here. Given the control for resolution employing the Golgi, the organization of the H2AX direct conjugate staining raises intriguing questions about the possible hypothesis of chromatin structural details. However, we make no claims regarding the underlying biology.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	73

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	100 Slices (2.97 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1236 X 1064 | 464 X 365
Image Size (Scaled)	26.10 µm X 22.46 µm | 9.80 µm X 7.71 µm
Bit Depth	16 Bit
Image Center Position	X: 61.35 mm, Y: 36.64 mm | X: 61.35 mm, Y: 36.64mm
Stage Position	X: 61.35 mm, Y: 36.64 mm | X: 61.35 mm, Y: 36.64mm
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	561 | 488 | 405
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	597 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503-546, 657-800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	150 ms | 120 ms | 50 ms
Depth of Focus	0.92 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @8.00% | 488 nm @1.00% | 405 nm @2.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating | 32 µm grating
Result Sampling	4
Process Sampling	N/A

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.1569 (594), 11.2316 (488), 9.9968 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% 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%, Tone Mapping NOT Enabled | 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) | View Angle 35°, Scale Z 20%, 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 define regions of special interest.
X-Y	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-36%
Clip X	0°
Clip Y	0°
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™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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 other. 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™ 594, 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 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:

Z Stack - (3D) - Panel A

Z-Stack = 100 Slices (2.97 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1236 X 1064
Image Size (Scaled) = 26.10 µm X 22.46 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.35 mm, Y: 36.64 mm
Stage Position = X: 61.35 mm, Y: 36.64 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 100 Slices (2.97 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 464 X 365
Image Size (Scaled) = 9.80 µm X 7.71 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.35 mm, Y: 36.64mm
Stage Position = X: 61.35 mm, Y: 36.64mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 40 of 100 total - Panel C

594 -

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 = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @8.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503-546, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32 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 @2.00%
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 = 11.1569 (594), 11.2316 (488), 9.9968 (DAPI)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping 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)

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

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

Position of Clip = -36%
Clip X = 0°
Clip Y = 0°

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 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel displays a three-dimensional maximum projection of the Mouse anti-Human H2Ax Monoclonal-Identifyn® SRM Alexa Fluor™ 594, IgG1 direct conjugate (red), combined with the Golgi primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), utilizing super-resolution SIM imaging. An X-Y cut plane is employed at position 36%, with X and Y at zero-degree angles; the plane is outlined in green. This cut plane was used to show the nuclear distribution of the H2AX/594 direct conjugate and the apparent nucleolar space devoid of signal, as expected. The image includes a Region of Interest (ROI) denoted by a white box.

Panel B - This panel presents a three-dimensional volume projection from the ROI identified in Panel A, again showing the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594 (red) and the Golgi primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green) It is essential to note that the Zeiss Zen default settings for volume projection were used, with no edits made to the original acquisition. Tone mapping was disabled to ensure the image was not artificially color-mapped or manipulated in any way.

Panel C - This is a two-dimensional image from z-plane 40 of the 100 total photos taken to further investigate, in a maximum projection view, or unmodeled structure, that we consistently observe when transitioning from super-resolution Airyscan to SIM, decreasing our axial resolution from 100-120 nm to 60-80 nm in the X and Y dimensions.

This transition reveals additional structural details within the nuclear compartment as they pertain to the localization of H2AX 594 direct conjugate staining. The findings are strongly supported by the use of a known marker for the Golgi, as SIM has consistently demonstrated the ability to resolve its structure, as evidenced here. Given the control for resolution employing the Golgi, the organization of the H2AX direct conjugate staining raises intriguing questions about the possible hypothesis of chromatin structural details. However, we make no claims regarding the underlying biology.

Image Corrections

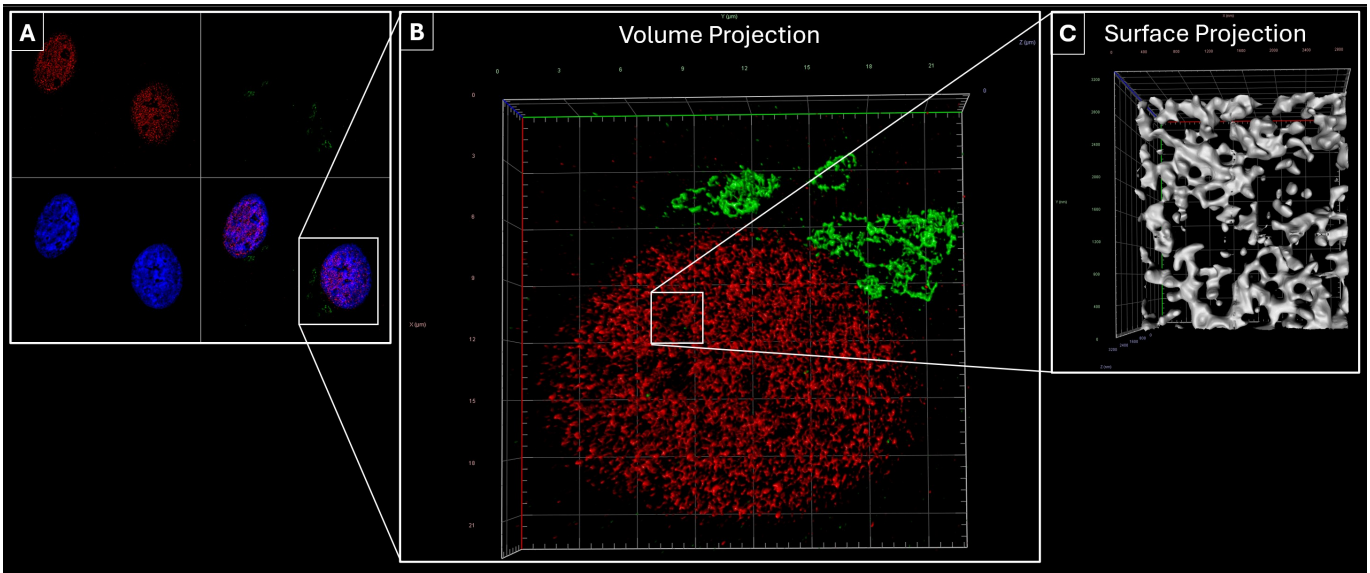
None

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

SOURCE PROVENANCE

Catalog	DC-000037
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:
Structured source metadata values	73
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	00F40C2FA7826277FEDC53825A3EA20FDEB460C9528C5AB1884AC1E2870103C7
Method PDF SHA-256	1FA0445BE67E8A0583B58DA72A4FA8CAE7471BA5D327017956653E20F71FB207

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™ 594, an IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Golgi primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels. A Region of Interest is indicated by the white box, which will be explored further in Panel B.

Panel B - This panel presents a three-dimensional volume projection of the Region of Interest from Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 (red) and the Golgi primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green). This was achieved using super-resolution SIM Squared, with no manipulation of the signal beyond selecting the volume view in Zeiss Zen software. A Region of Interest is again indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional surface projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn™ SRM Alexa Fluor™ 594, which has been pseudo-colored to white in Zeiss Zen software to enhance visualization of the structural details against a black background, utilizing super-resolution SIM Squared.

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 makes biological sense, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets at this level of resolution, approximately 40 nm in the X and Y dimensions.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	72

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	132 Slices (3.93 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	2663 X 2318 | 1079 X 1081 | 140 X 158
Image Size (Scaled)	56.22 µm X 48.94 µm | 22.78 µm X 22.95 µm | 2.96 µm X 3.34 µm
Bit Depth	16 Bit
Image Center Position	X: 61.35 mm, Y: 36.64 mm
Stage Position	X: 61.35 mm, Y: 36.64 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	561 | 488 | 405
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	597 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503-546, 657-800
Imaging Device	Axiocam 820 #2-SR | Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150 ms | 120 ms | 50 ms
Depth of Focus	0.92 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @8.00% | 488 nm @1.00% | 405 nm @2.00%
Frame Time	1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating | 32 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 Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels | Threshold 22%, Ambient Light 0%, Specular Light 18%, Shininess 36%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping | Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 77%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 35°, Scale Z 91%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 - (1H1D9/B7)
Cat ID# - DC 000037

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 other. 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™ 594, 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 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 61 of 132 total - Panel A

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 2663 X 2318
Image Size (Scaled) = 56.22 µm X 48.94 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.35 mm, Y: 36.64 mm
Stage Position = X: 61.35 mm, Y: 36.64 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1079 X 1081
Image Size (Scaled) = 22.78 µm X 22.95 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.35 mm, Y: 36.64 mm
Stage Position = X: 61.35 mm, Y: 36.64 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 140 X 158
Image Size (Scaled) = 2.96 µm X 3.34 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.35 mm, Y: 36.64 mm
Stage Position = X: 61.35 mm, Y: 36.64 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2-SR
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @8.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503-546, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 32 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 @2.00%
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™ 594, 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 Z-Position 61 of 132.

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 77%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 22%, Ambient Light 0%, Specular Light 18%, Shininess 36%
Background = Black
Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 91%, 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™ 594, an IgG1 direct conjugate. The upper left section displays the H2AX direct conjugate (red), while the upper right shows the Golgi primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The lower left features DAPI nuclear staining (blue), and the lower right presents a merged image of all three channels. A Region of Interest is indicated by the white box, which will be explored further in Panel B.

Panel B - This panel presents a three-dimensional volume projection of the Region of Interest from Panel A. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 594 (red) and the Golgi primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green). This was achieved using super-resolution SIM Squared, with no manipulation of the signal beyond selecting the volume view in Zeiss Zen software. A Region of Interest is again indicated by the white box, which will be examined further in Panel C.

Panel C - This panel presents a three-dimensional surface projection of the Region of Interest from Panel B. It includes the Mouse anti-Human H2AX Monoclonal - Identifyn® SRM Alexa Fluor™ 594, which has been pseudo-colored to white in Zeiss Zen software to enhance visualization of the structural details against a black background, utilizing super-resolution SIM Squared.

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 detail. 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 makes biological sense, but it is not conclusive. Nonetheless, it could serve as a valuable tool in colocalization studies with known protein targets at this level of resolution, approximately 40 nm in the X and Y dimensions.

Image Corrections

Panel C - Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG1 - (1H1D9/B7) was pseudo-colored in Zen Software from red, the default color, to white.

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

SOURCE PROVENANCE

Catalog	DC-000037
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	72
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	967163C847B016B12D9B77CF87F3A239CBA78FACED5BF855FF235E18A9BB37C7
Method PDF SHA-256	A4895878121498747B56930902BA954D113B02B2B7A6475F6823C364044590ED