

IDENTIFYN RESOLUTION STEPDOWN

H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human γ H2Ax (Serine 139) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555

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

7

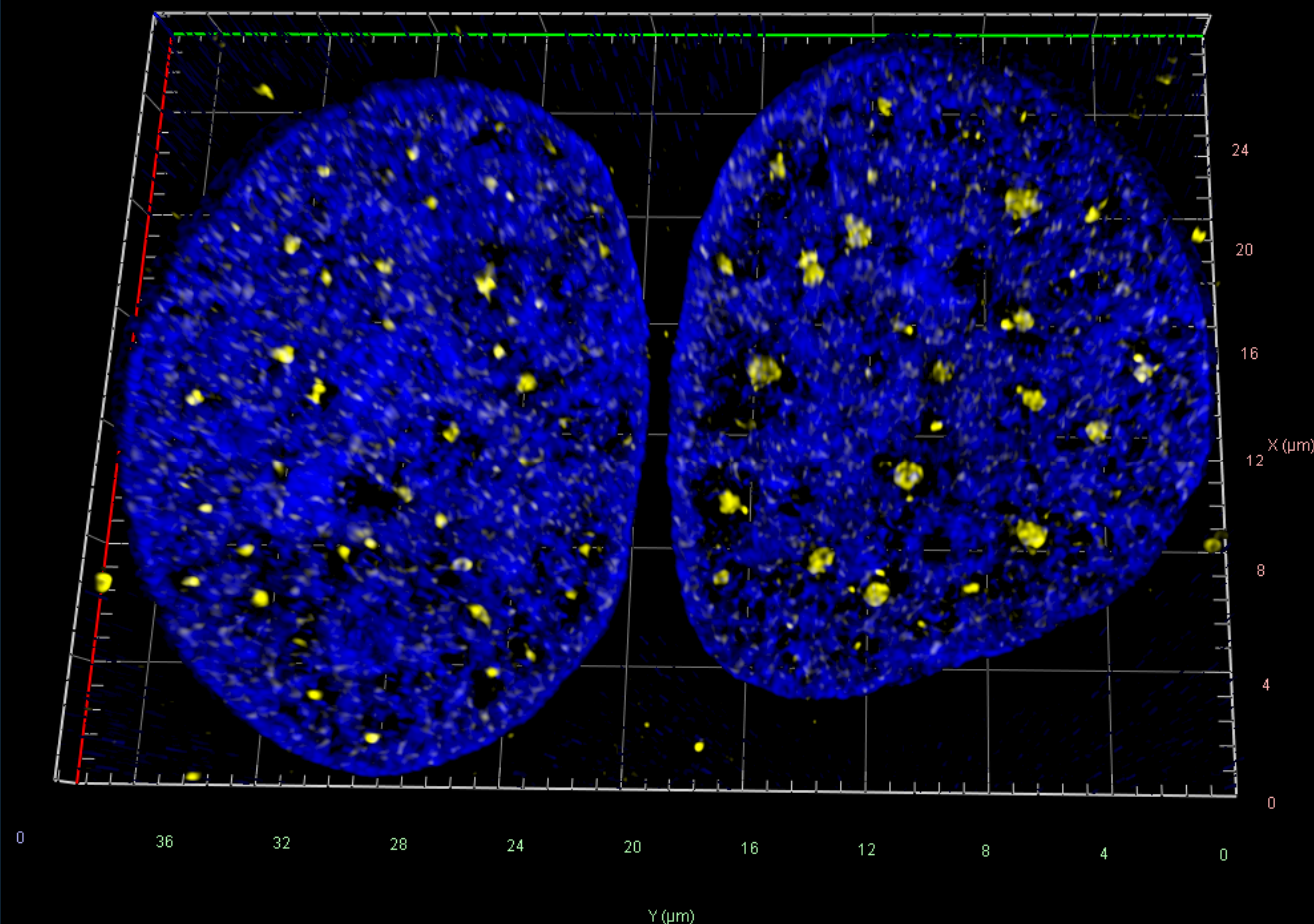
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000018 | 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-000018 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-000018 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	555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555	2; 45 Texas Red; 49 DAPI; 540-580; 335-383; 593-668; 420-470; 553
Widefield SIM — Apotome	405/DAPI; 555	2; AF532-49014; 49 DAPI; 515 - 545; 335-383; 555 - 595; 420-470; 553
Confocal	405/DAPI; 488; 555	3; None; 561nm @0.01%; 488nm @0.01%; 405nm @0.2%; 553; 493; 353
Airyscan	405/DAPI; 488; 555	3; None; 560nm @0.2%; 488nm @0.10%; 405nm @0.7%; 553; 493; 353
SIM	405/DAPI; 488; 532; 555; 647	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820 #2-SR; 561
SIM ²	405/DAPI; 488; 532; 555; 647	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T1-Axiocam 820 #2-SR; T2-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 555.

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 MR R3, and X-Cite Xylis Lamp, was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 864 X 571; Image Size (Pixels): 864 X 571; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 125ms; 26ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

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; 555.

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: 29 Slices (2.8µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 976 X 599; Image Size (Pixels): 976 X 599; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 50ms; 25ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%; X-Cite Xylis Lamp Intensity = @8%. 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: 38.

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; 555.

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: 159 Slices (4.74µm); Channels: 3; Scaling (Per Pixel): 0.030µm X 0.030µm X 0.030µm; Image size (Pixels): 976 X 976; Image Size (Pixels): 976 X 976; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT; GaAsP-Pmt. Timing: Pixel Time: 0.22µs; Frame Time: 1.02s. Illumination: Laser Wavelength: 561nm @0.01%; 488nm @0.01%. Optical/scan: Pinhole: 1.00AU / 55µm; 1.00AU / 49µm; Scan Zoom: 4.5; LSM Scan Speed: 15; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 7.2(3D, Auto). Structured source metadata values: 59.

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; 555.

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 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 146 Slices (3.71µm); Channels: 3; Scaling (Per Pixel): 30.00nm X 30.00nm X 30.00nm; Image size (Pixels): 1096 X 1096; Image Size (Pixels): 1096 X 1096; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.69µs; Frame Time: 8.15s. Illumination: Laser Wavelength: 560nm @0.2%; 488nm @0.10%. Optical/scan: Pinhole: 5.00 AU / 254µm; 5.00 AU / 216µm; Scan Zoom: 3.0; LSM Scan Speed: 8; 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; AiryScan Mode: Super Resolution 8.5 (3D, Auto); Super Resolution 8.0 (3D, Auto). Structured source metadata values: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

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, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 34 Slices (660nm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 20.00nm; Image size (Pixels): 1782 X 1265; Image Size (Pixels): 1782 X 1265; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @2.50%; 405nm @6.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 40°, Scale Z 114%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 555; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 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, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 47Slices (920nm); Channels: 2; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm; Image size (Pixels): 2057 X 2872; Image Size (Pixels): 2057 X 2872; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @2.50%; 405nm @6.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 168%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 555; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000018 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): 30.00nm X 30.00nm X 30.00nm -> 0.03000 μ m X 0.03000 μ m X 0.02559 μ m. 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)=1096 X 1096; Image Size (Scaled)=33.08 μ m X 33.08 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.60%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89nm X 16.89nm X 20.00nm -> 0.01689 μ m X 0.01689 μ m X 19.41176 μ m. 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)=1782 X 1265; Image Size (Scaled)=30.10 μ m X 21.37 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm -> 0.01320 μ m X 0.01320 μ m X 19.57447 μ m. 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)=2057 X 2872; Image Size (Scaled)=27.14 μ m X 37.90 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.05%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200uM; 2 μ M -> Etoposide = 2 μ M. The governing scientist identified every 200 μ M occurrence as a technician transcription error. The canonical historical concentration is 2 μ M. 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.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 139. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human γ H2Ax (Serine 139) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555

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 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

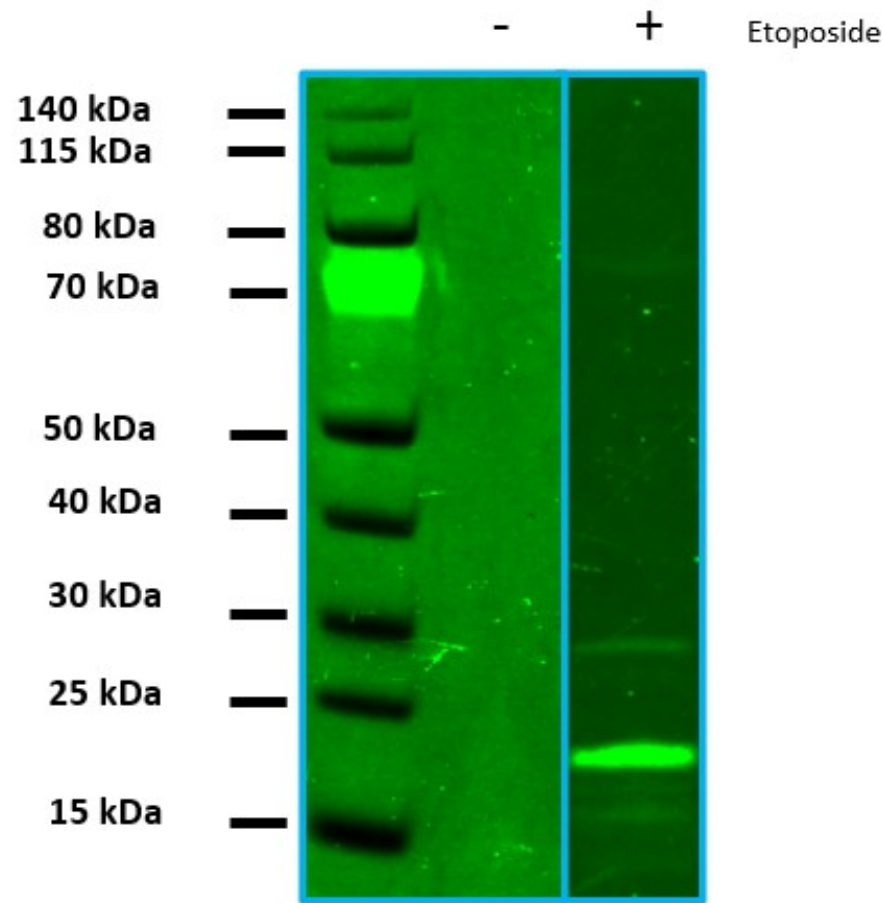
This dossier preserves the complete available evidence progression for DC-000018. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN	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 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	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	HEK293
DETECTED DYES	555
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	Kyle Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

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

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

Control Data - Primary Unconjugated Antibody

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

Scan Settings

Detection mode = Fluorescence

Laser = 532nm

Emission Filter = 572±28nm

Detector = PMT

Intensity = 2

Pixel size = 100 μ m

Scan Speed = High

Quality = High

Focus = Membrane (0 μ m in Z-axis)

Post Processing

None

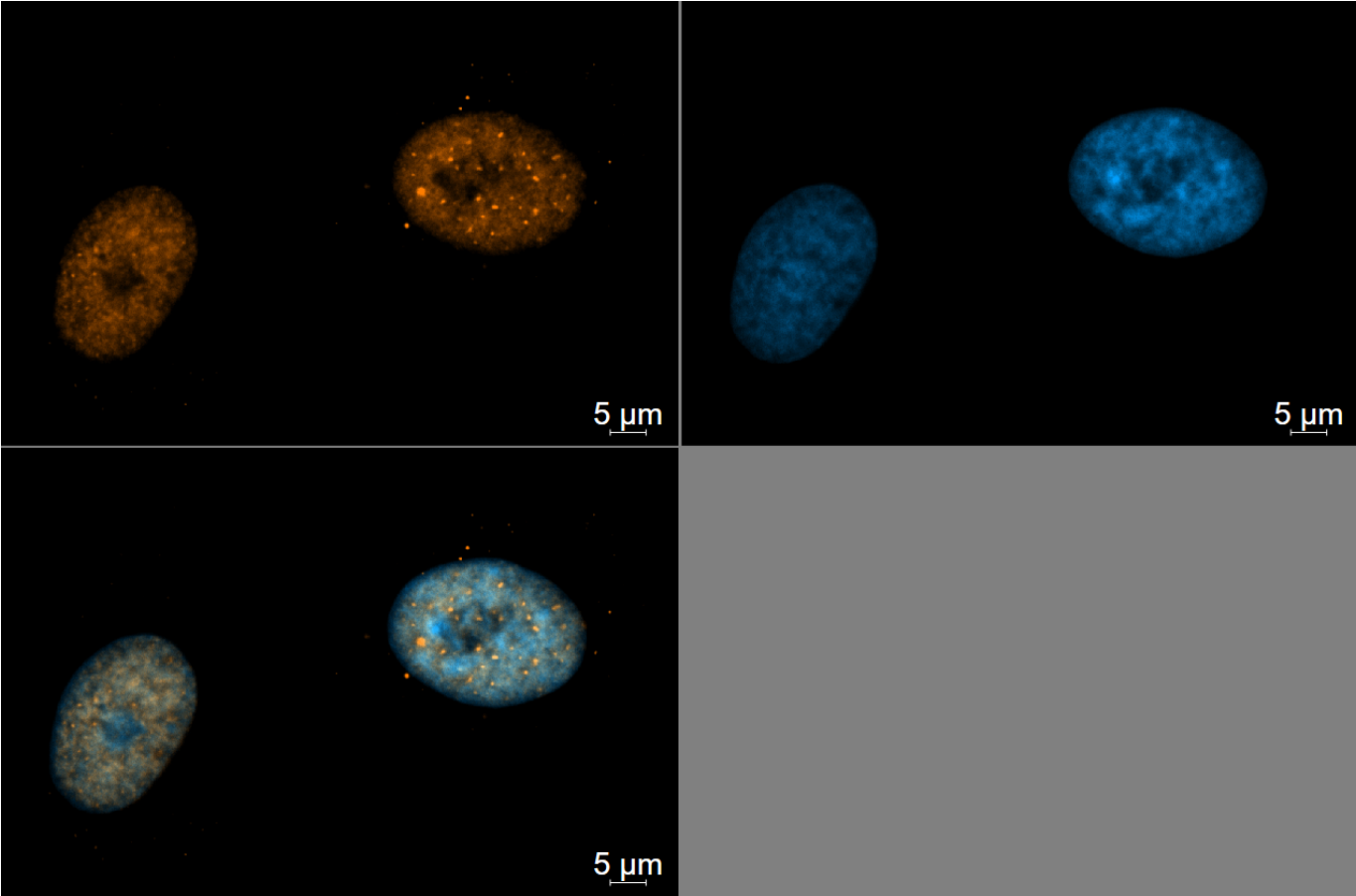
Image: Kyle Small

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SOURCE PROVENANCE

Catalog	DC-000018
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HEK293
Image SHA-256	53572510DF8D8909A64B63634B7CD1F9878944E7155254D0EC310CCFBF9D4952
Method PDF SHA-256	A6D825AEE794EA259E65AD827A54084D0C749770E93A9FFFBEB7945C720709F

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
METADATA VALUES	30

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 MR R3, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	864 X 571
Image Size (Scaled)	94.63µm X 62.54µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	45 Texas Red 49 DAPI
Beam Splitter	585 395
Filter Excitation Wavelength	540-580 335-383
Filter Emission Wavelength	593-668 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	125ms 26ms
Excitation Wavelength	553 353
Emission Wavelength	568 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.88µm 0.72µm
Binning Mode	1,1

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110 μ m X 0.110 μ m

Image size (Pixels) = 864 X 571

Image Size (Scaled) = 94.63 μ m X 62.54 μ m

Bit Depth = 12 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

555 -

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 @25%
 Exposure Time = 125ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Imaging Device = Axiocam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.88µm
 Binning Mode = 1,1

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%
 Exposure Time = 26ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 1,1

Post Processing

None

Split View

Split View, From right, Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 -
 Direct Conjugate, DAPI, and Merge

Image Correction

None

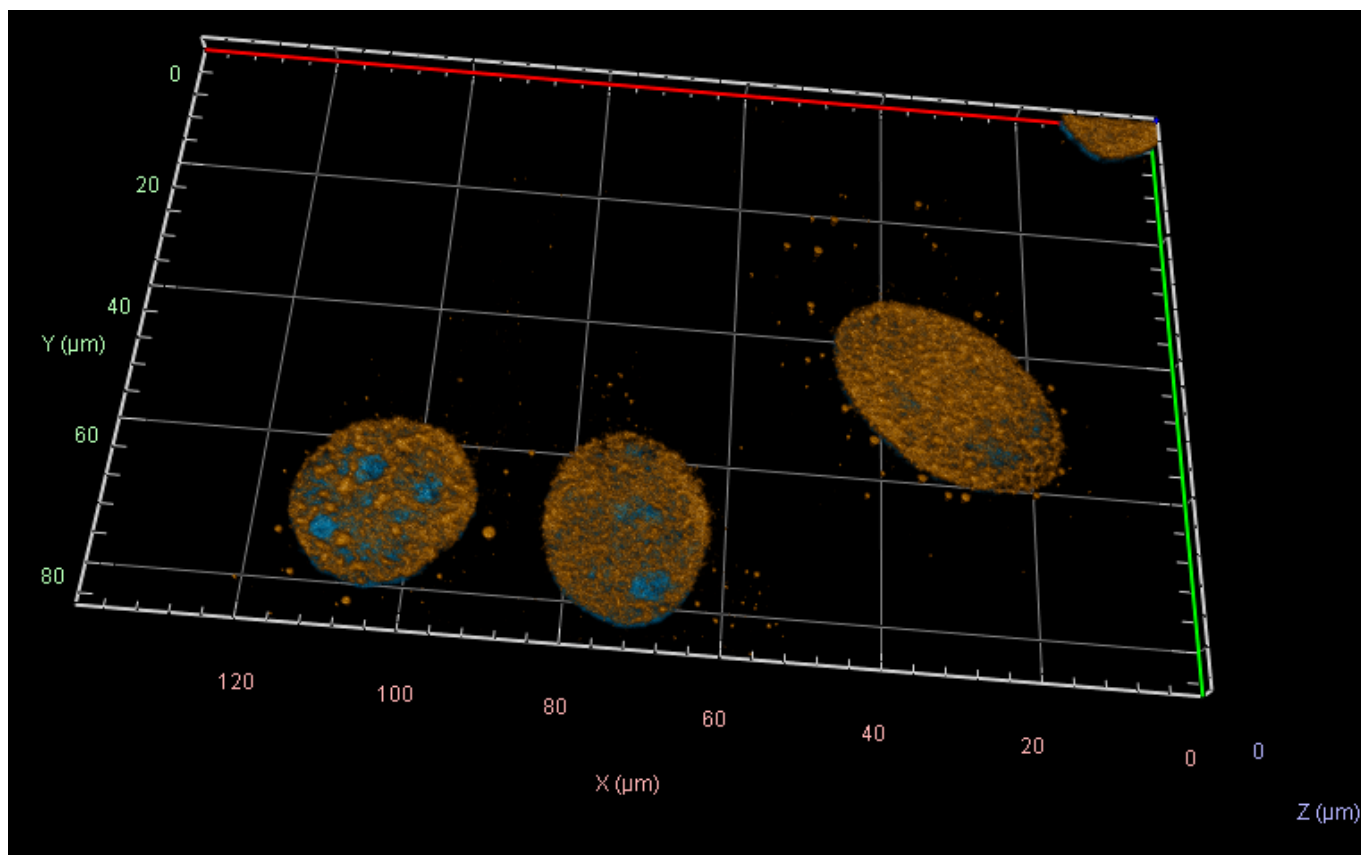
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	DC-000018
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B1739FAE47ACF7BB0B0E90A7BB8D7F5FF869D5AB0318F58F14EE607A3E4CD8F6
Method PDF SHA-256	30DD3501D550DE516E193883F0A44315A3F6AF64544C8AEEA2699BCEFF0B4504

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
METADATA VALUES	38

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	29 Slices (2.8µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	976 X 599
Image Size (Scaled)	139.43µm X 85.57µm
Bit Depth	14 Bit
Image Center Position	X: 85.58mm, Y: 47.89mm
Stage Position	X: 85.58mm, Y: 47.89mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	AF532-49014 49 DAPI
Beam Splitter	550 395
Filter Excitation Wavelength	515 - 545 335-383
Filter Emission Wavelength	555 - 595 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15% X-Cite Xylis Lamp Intensity = @8%
Exposure Time	50ms 25ms
Excitation Wavelength	553 353
Emission Wavelength	568 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.10µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% for all channels, ramp 50% for all channels, and maximum 100% for all channels.
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. 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 Xylys Lamp was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 29 Slices (2.8 μ m)

Channels = 2

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m

Image Size (Pixels) = 976 X 599

Image Size (Scaled) = 139.43 μ m X 85.57 μ m

Bit Depth = 14 Bit

Image Center Position = X: 85.58mm, Y: 47.89mm

Stage Position = X: 85.58mm, Y: 47.89mm

ROI Center Offset = X: 1.14 μ m, Y: 0.00 μ m

555 -

Reflector = AF532-49014
 Beam Splitter = 550
 Filter Excitation Wavelength = 515 - 545
 Filter Emission Wavelength = 555 - 595
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15%
 Exposure Time = 50ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 1.10µm
 Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335-383
 Filter Emission Wavelength = 420-470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity = @8%
 Exposure Time = 25ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 0.90µm
 Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

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

Image Corrections

None

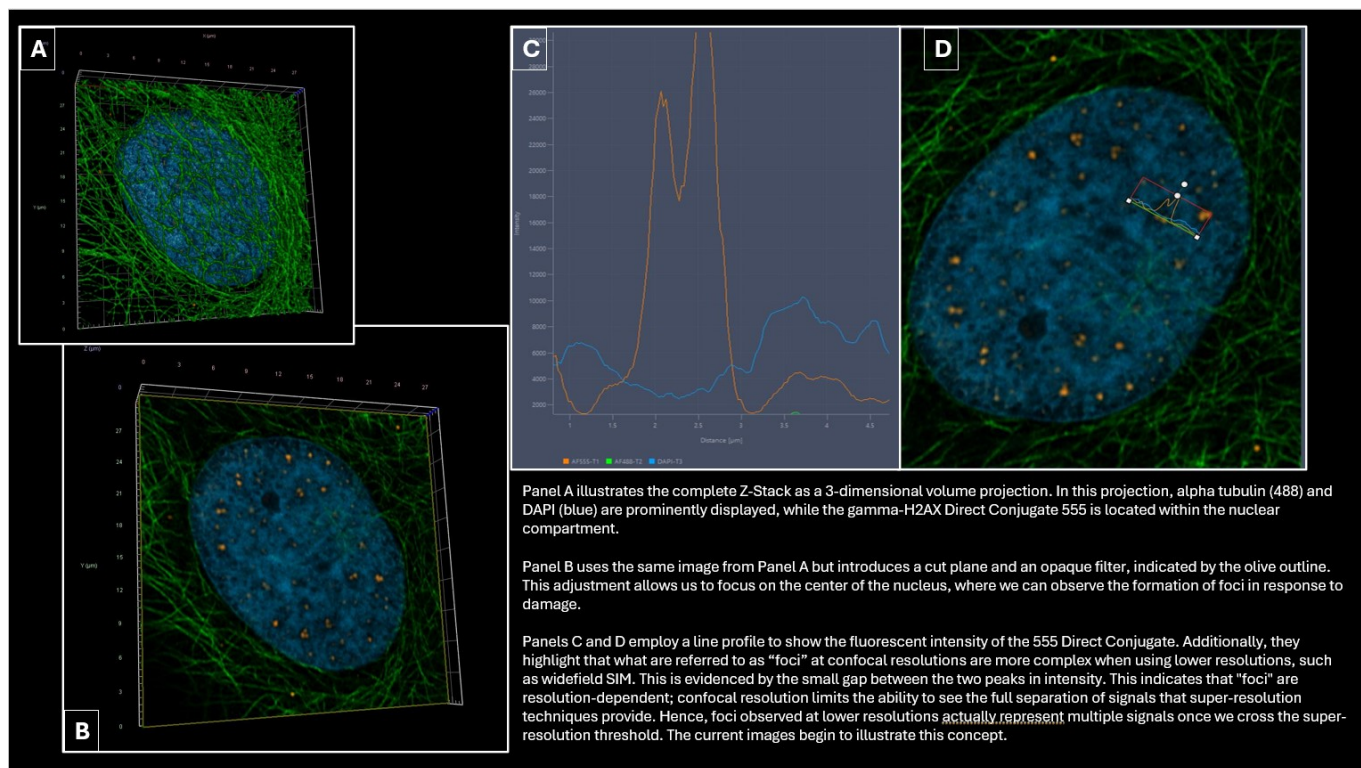
Image by E.F. Simon

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SOURCE PROVENANCE

Catalog	DC-000018
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	92CACFBAF09C1E6F37328171623A3DDF88B4082EA180810FA014128F6DBBB658
Method PDF SHA-256	EBC88D9B368E94F9CAEBA500A03AA2903944A06A63AE9B699006AEABD1DE714F

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	59

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	159 Slices (4.74µm)
Channels	3
Scaling (Per Pixel)	0.030µm X 0.030µm X 0.030µm
Image Size (Pixels)	976 X 976
Image Size (Scaled)	29.21µm X 29.21µm
Bit Depth	16 Bit
Image Center Position	X: 103.64mm, Y: 49.57mm
Stage Position	X: 103.64mm, Y: 49.57mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 55µm 1.00AU / 49µm 0.72AU / 30µm
LMS MBS	Plate MBS 488/561, 405/730
LMS SBS	Mirror
Laser Wavelength	561nm @0.01% 488nm @0.01% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	4.5
Rotation	0°
Sampling	2.36
Pixel Time	0.22µs
Frame Time	1.02s
LSM Scan Speed	15
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	550 - 600 480 - 549 410 - 470
Imaging Device	GaAsP-Pmt3
Detector Type	GaAsP-PMT GaAsP-Pmt

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	600 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) Filter: 7.2(3D, Auto) Filter: 6.3 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% (555), Threshold 2% (488), Threshold 1% (DAPI), Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. An olive outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.
Position of Clip	-0%
Clip Y	0°
Clip Z	0°
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 4.89, Y (Min 214 and Max 36892)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

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)

Z-Stack = 159 Slices (4.74µm)

Channels = 3

Scaling (Per Pixel) = 0.030µm X 0.030µm X 0.030µm

Image Size (Pixels) = 976 X 976

Image Size (Scaled) = 29.21µm X 29.21µm

Bit Depth = 16 Bit

Image Center Position = X: 103.64mm, Y: 49.57mm

Stage Position = X: 103.64mm, Y: 49.57mm

ROI Center Offset = X: 0.00µm, Y: 0.00µm

555 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 55µm

LMS MBS = Plate MBS 488/561, 405/730

LMS SBS = Mirror

Laser Wavelength = 561nm @0.01%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 4.5

Rotation = 0°

Sampling = 2.36

Pixel Time = 0.22µs

Frame Time = 1.02s

LSM Scan Speed = 15

Scan Direction = Bidirectional

Line Step = 1

Averaging - 4

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Detection Wavelength = 550 - 600

Imaging Device = GaAsP-Pmt3

Detector Type = GaAsP-PMT

Detector Gain = 600 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = Filter: 7.2 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 49µm
 LMS MBS = Plate MBS 488/561, 405/730
 LMS SBS = Mirror
 Laser Wavelength = 488nm @0.01%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 4.5
 Rotation = 0°
 Sampling = 2.36
 Pixel Time = 0.22µs
 Frame Time = 1.02s
 LSM Scan Speed = 15
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 480 - 549
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-Pmt
 Detector Gain = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.2(3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 0.72AU / 30µm
 LMS MBS = Plate MBS 488/561, 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 4.5
 Rotation = 0°
 Sampling = 2.36
 Pixel Time = 0.22µs
 Frame Time = 1.02s
 LSM Scan Speed = 15
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-Pmt
 Detector Gain = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.3 (3D, Auto)

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 2% (555), Threshold 2% (488), Threshold 1% (DAPI), Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process Panels C and D -

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human γH2AX Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

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

Position of Clip = -0%

Clip Y = 0°

Clip Z =0°

Profile View Display

The line profile was used to show the intensity of the fluorescent signal of the direct conjugate using the 561 laser at 0.01% and the separation between two foci or the gain in resolving foci at confocal resolution using LSMP processing in Zen.

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 4.89, Y (Min 214 and Max 36892)

Image Corrections

None

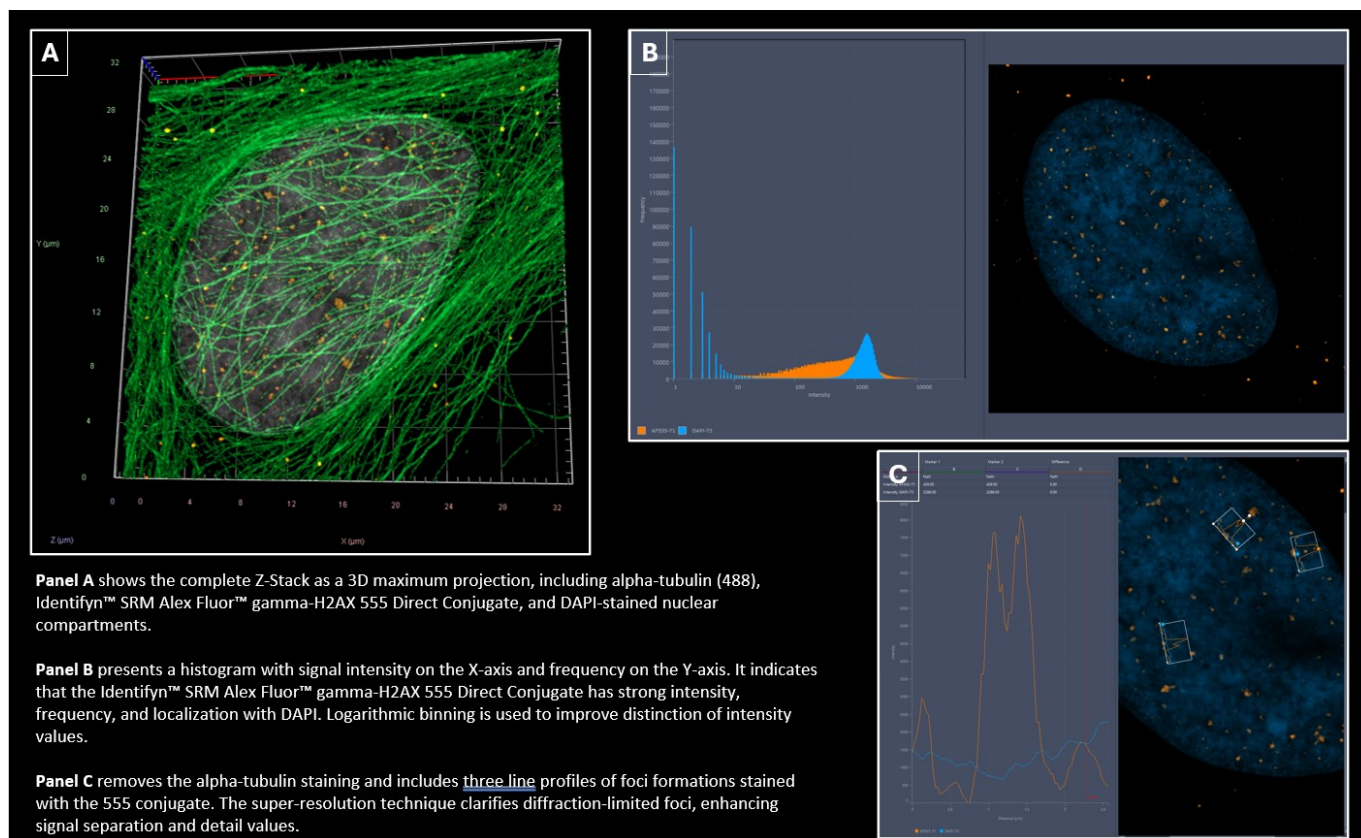
Image by P. Raut

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SOURCE PROVENANCE

Catalog	DC-000018
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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CDFC4A630E259F7FBF83030DDC25532B0E84F5878C664F3603BC9E9D05477C6E
Method PDF SHA-256	2B3560F64F31882F573276897C29CC07E009154CD9B100DBD075CF98EE57B29A

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	146 Slices (3.71µm)
Channels	3
Scaling (Per Pixel)	0.03000 µm X 0.03000 µm X 0.02559 µm
Image Size (Pixels)	1096 X 1096
Image Size (Scaled)	33.08µm X 33.08µm
Bit Depth	16 Bit
Image Center Position	X: 71.36mm, Y: 51.06mm
Stage Position	X: 71.36mm, Y: 51.06mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 254µm 5.00 AU / 216µm 5.00 AU / 183µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	560nm @0.2% 488nm @0.10% 405nm @0.7%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	-0°
Sampling	2.34
Pixel Time	0.69µs
Frame Time	8.15s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	565-650 480-545 400-467
Imaging Device	AiryScan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V 850 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.5 (3D, Auto) Super Resolution 8.0 (3D, Auto) Super Resolution 7.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (555), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 79%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 2.57), Y (Min 2 and Max 8514)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 146 Slices (3.71µm)

Channels = 3
 Scaling (Per Pixel) = 30.00nm X 30.00nm X 30.00nm
 Image Size (Pixels) = 1096 X 1096
 Image Size (Scaled) = 33.08µm X 33.08µm
 Bit Depth = 16 Bit
 Image Center Position = X: 71.36mm, Y: 51.06mm
 Stage Position = X: 71.36mm, Y: 51.06mm
 ROI Center Offset = X: 0.00µm, Y: 0.00µm

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 254µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 560nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = -0°
 Sampling = 2.34
 Pixel Time = 0.69µs
 Frame Time = 8.15s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 565-650
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.5 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 216µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.10%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = -0°
 Sampling = 2.34
 Pixel Time = 0.69µs
 Frame Time = 8.15s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 480-545
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.0 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 183µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.7%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = -0°
 Sampling = 2.34
 Pixel Time = 0.69µs
 Frame Time = 8.15s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400-467
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 7.3 (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 1% (555), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 79%, 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)

Histogram View - Panel B

Histogram Definition - Normal, Histo Table was Frequency, Bin Count 256, Bin Size 256, Lower Threshold 1, Upper Threshold 65534, Showing Threshold, and deploying Logarithmic Binning

Histogram View - X and Y axis auto, Channel Transparency 100%, Statistic Table, Frequency Table, and Internal Measurements were not selected.

Profile View Display - Panel C

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 2.57), Y (Min 2 and Max 8514)

Image Corrections -

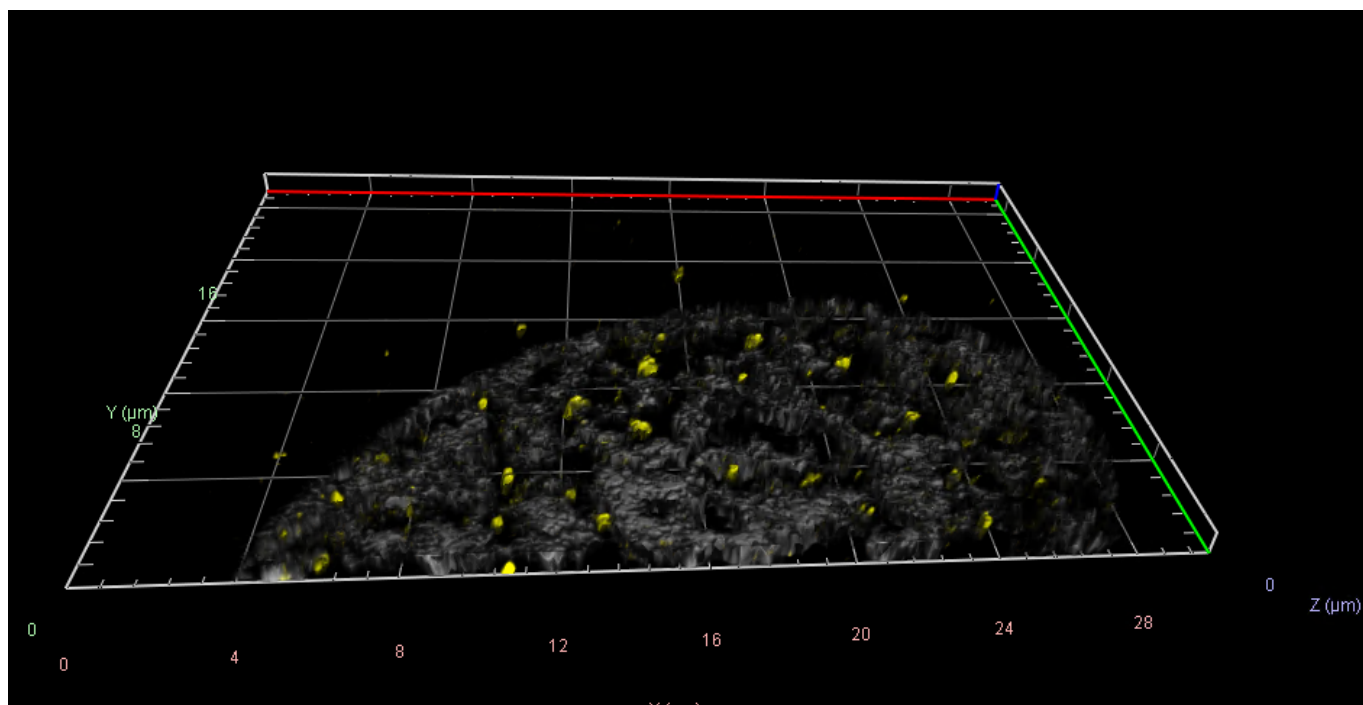
None

Image by E.F. Simon

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SOURCE PROVENANCE	
Catalog	DC-000018
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DF19A02DD880F3064C3854C738C968F8263AD74BCFBCBB6627D9008C5C548A4F
Method PDF SHA-256	9596319BA8CC56194AE80C6C13921DFB2502A2E2836EC574D422D6CD3F30D27F

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 555; 647
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	34 Slices (660nm)
Channels	2 1
Scaling (Per Pixel)	0.01689 μ m X 0.01689 μ m X 19.41176 μ m
Image Size (Pixels)	1782 X 1265
Image Size (Scaled)	30.10 μ m X 21.37 μ m
Bit Depth	16 Bit
Image Center Position	X: 46.37mm, Y: 43.33mm
Stage Position	X: 46.37mm, Y: 43.33mm
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms 50ms
Depth of Focus	0.92 μ m 0.69 μ m
Binning Mode	2,2
Laser Wavelength	561nm @2.50% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.8465
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Volume Projection	Precise
Appearance	Threshold 1% (532), Threshold 1% (DAPI), Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 56°, Elongation -39°, Enabled Tone Mapping
Projection	View Angle 40°, Scale Z 114%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 34 Slices (660nm)

Channels = 2

Scaling (Per Pixel) = 16.89nm X 16.89nm X 20.00nm

Image Size (Pixels) = 1782 X 1265

Image Size (Scaled) = 30.10 μ m X 21.37 μ m

Bit Depth = 16 Bit

Image Center Position = X: 46.37mm, Y: 43.33mm

Stage Position = X: 46.37mm, Y: 43.33mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

532 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 647 - 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 = 100ms
 Depth of Focus = 0.92µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @2.50%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 27.5µm grating

DAPI -

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

Post Processing - SIM Processing

Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 2
 Channels = 1
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 10.8465
 Input SNR = N/A
 Regularization Weight = N/A
 Iterations = N/A
 Filter = N/A
 Fitting = N/A
 Normalization = Automatic
 Experimental PSF = N/A

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 1% (532), Threshold 1% (DAPI), Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 56°, Elongation -39°, Enabled Tone Mapping
 Projection = View Angle 40°, Scale Z 114%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

Copyright 2026 GMD12 LLC.

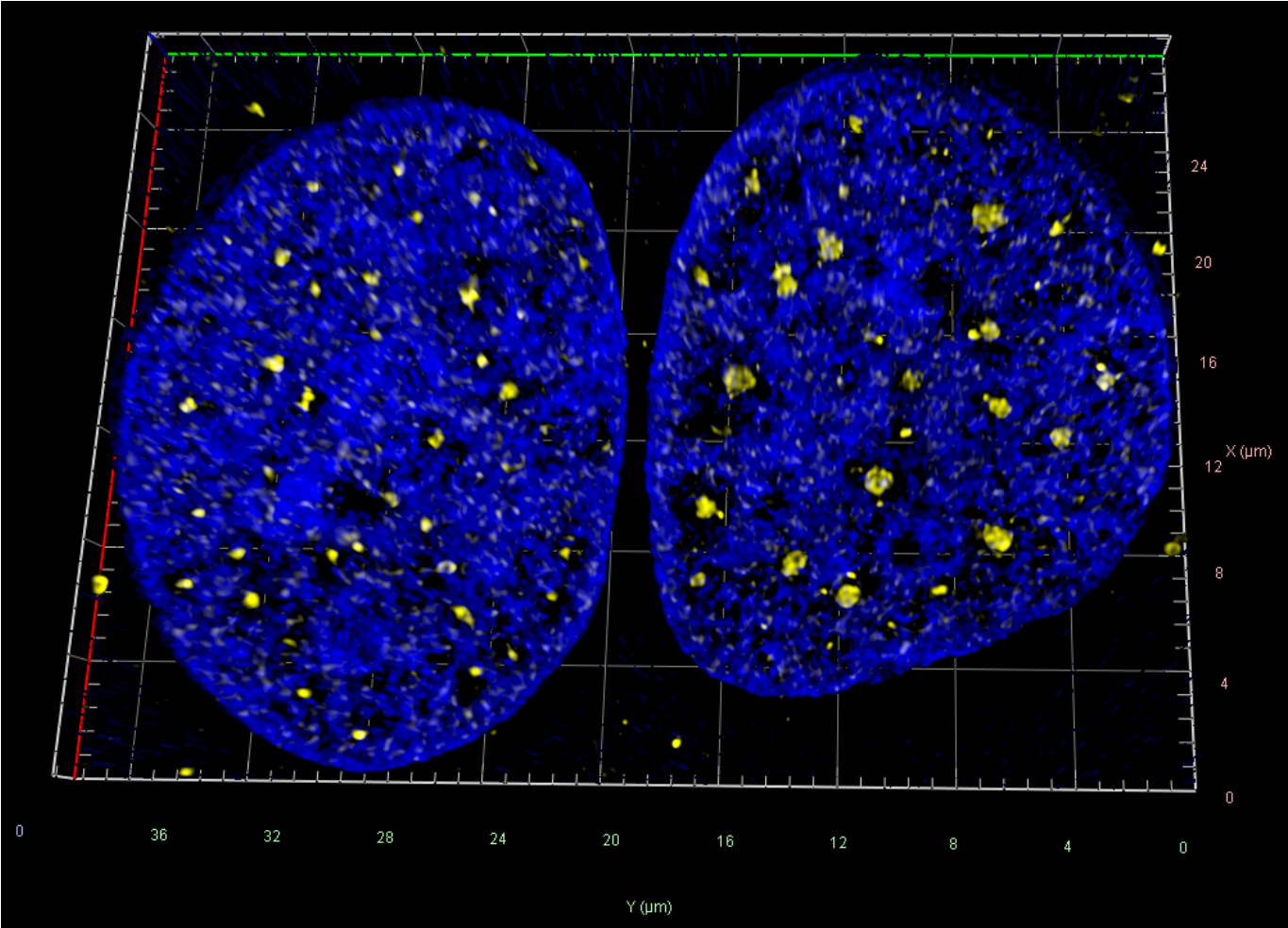
SOURCE PROVENANCE	
Catalog	DC-000018
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3A81E174966D9906771F8B4188F2DC417E0B1E94E092470259CC8A23C12A67D2

SOURCE PROVENANCE

Method PDF SHA-256

0BE54A1787AD56CCC5C61E54D1A292C0A4CE9523C054878C0B1D38507166801E

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 555; 647
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	47Slices (920nm)
Channels	2 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 19.57447 µm
Image Size (Pixels)	2057 X 2872
Image Size (Scaled)	27.14µm X 37.90µm
Bit Depth	16 Bit
Image Center Position	X: 44.78mm, Y: 42.85mm
Stage Position	X: 44.78mm, Y: 42.85mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms 50ms
Depth of Focus	0.92µm 0.69µm
Binning Mode	2,2
Laser Wavelength	561nm @2.50% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.1358
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Maximum Projection	Precise
Appearance	Threshold 2% (532), Threshold 3% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 168%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate
Cat ID# - DC 000018

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate was incubated for 1 hour at room temperature at a dilution of 1:250. Slides were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 47Slices (920nm)

Channels = 2
Scaling (Per Pixel) = 13.20nm X 13.20nm X 20.00nm
Image Size (Pixels) = 2057 X 2872
Image Size (Scaled) = 27.14 μ m X 37.90 μ m
Bit Depth = 16 Bit
Image Center Position = X: 44.78mm, Y: 42.85mm
Stage Position = X: 44.78mm, Y: 42.85mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

532 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 647 - 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 = 100ms
 Depth of Focus = 0.92µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @2.50%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 27.5µm grating

DAPI -

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

Post Processing - SIM2 Processing

Processing Dimension - 3D

Result Sampling = 4

Process Sampling = 4

Channels = 1

Algorithm = SIM2

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.1358

Input SNR = N/A

Regularization Weight = N/A

Iterations = N/A

Filter = N/A

Fitting = N/A

Normalization = Automatic

Experimental PSF = N/A

3D Image Processing

3D Maximum Projection = Precise

Appearance = Threshold 2% (532), Threshold 3% (DAPI), Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 168%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

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SOURCE PROVENANCE

Catalog	DC-000018
Stage	SIM ²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F6C27F33FC589A6048FF31425F6781687FB77E96647F4C1D2A187D9DE7F0CD31

SOURCE PROVENANCE

Method PDF SHA-256

93827B2B52A1DA56B34DE9E213FF9BF19D4C7A2FD5108AD775FB6760F6E2B71E