BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

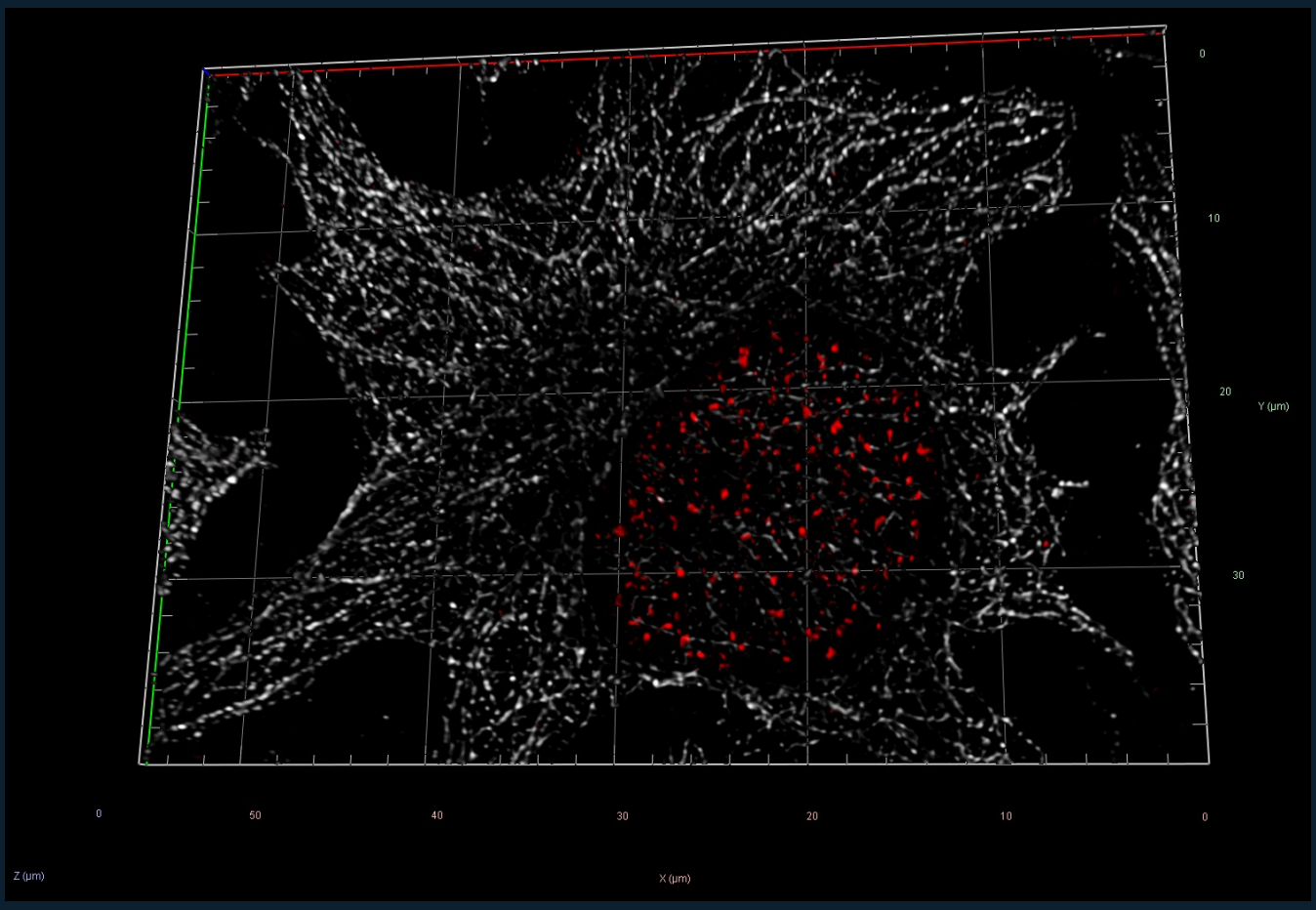
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human, IgG (H + L)

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

7	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000047 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

UNPUBLISHED / PENDING SCIENTIST REVIEW / NO PUBLICATION AUTHORIZED

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
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human, IgG (H + L), 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 -> Control

BENNETT ASSESSMENT

The preserved SA-000047 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / NIR-BOUNDED PARTIAL STEPDOWN

The preserved SA-000047 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540- 580; 335 - 383; 593 - 668; 420 - 470; 590
Widefield SIM — Apotome	405/DAPI; 488; 594	2; 45 Texas Red; 38HE eGFP; 540 - 580; 450 - 490; 593 - 668; 500 - 550; 590
Confocal	405/DAPI; 488; 594; 647; 750	2; None; 561nm @0.2%; 640nm @0.2%; 590; 653; 618; 668
Airyscan	405/DAPI; 488; 594; 647	2; None; 561nm @1.0%; 640nm @0.2%; 590; 653; 618; 668
SIM	405/DAPI; 488; 594; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820 #2-SR; T1-Axiocam 820 -SR; 561; 640
SIM²	405/DAPI; 488; 594; 647	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820 #2-SR; T1-Axiocam 820 -SR; 561
Control	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 590

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 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 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 Mono, 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): 691 X 556; Image Size (Pixels): 691 X 556; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 100ms; 40ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @15%. 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; 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 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 5 Slices (0.8µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 1018 X 1028; Image Size (Pixels): 1018 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 75ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%; X-Cite Xylis Lamp Intensity @20%. 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; 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 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 56 Slices (3.85µm); Channels: 2; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.070µm; Image size (Pixels): 1105 X 1105; Image Size (Pixels): 1105 X 1105; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-PMT; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.70µs; Frame Time: 4.02s. Illumination: Laser Wavelength: 561nm @0.2%; 640nm @0.2%. Optical/scan: Pinhole: 1.00AU / 51µm; 1.00AU / 57µm; Scan Zoom: 1.3; LSM Scan Speed: 8; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 594; 647; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 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: 21 Slices (1.00µm) - Subset of 63 Slices taken in Acquisition; Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 50.00nm; Image size (Pixels): 2849 X 2849; Image Size (Pixels): 2849 X 2849; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.73µs; Frame Time: 14.22s. Illumination: Laser Wavelength: 561nm @1.0%; 640nm @0.2%. Optical/scan: Pinhole: 5.00 AU / 252µm; 5.00 AU / 281µm; Scan Zoom: 1.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; 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 9.3 (3D, Auto); Super Resolution 8.9 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 594; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 60 Slices (2.95nµm); Channels: 2; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 2515 X 3066; Image Size (Pixels): 2515 X 3066; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 100ms; 120ms; Frame Time: 1.33s; 1.59s. Illumination: Laser Wavelength: 561nm @1.50%; 640nm @2.0%; Illumination Wavelength: 561; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 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; 594; 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 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 18 Slices (850nm) - Subset of 53 Slices taken in Acquisition; Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 3265 X 2365; Image Size (Pixels): 3265 X 2365; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 100ms; 120ms; Frame Time: 1.33s; 1.59s. Illumination: Laser Wavelength: 561nm @1.50%; 640nm @2.0%; Illumination Wavelength: 561; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 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; 594; 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.

MODALITY ASSESSMENT / PART 8 OF 8

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1393 X 829; Image Size (Pixels): 1393 X 829; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 594.

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000047 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30nm X 35.30nm X 50.00nm -> 0.03530 µm X 0.03530 µm X 0.05000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2849 X 2849; Image Size (Scaled)=100.57µm X 100.57µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689 µm X 0.01689 µm X 0.05000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2515 X 3066; Image Size (Scaled)=42.48µm X 51.79µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689 µm X 0.01689 µm X 47.22222 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)=3265 X 2365; Image Size (Scaled)=55.15µm X 39.95µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

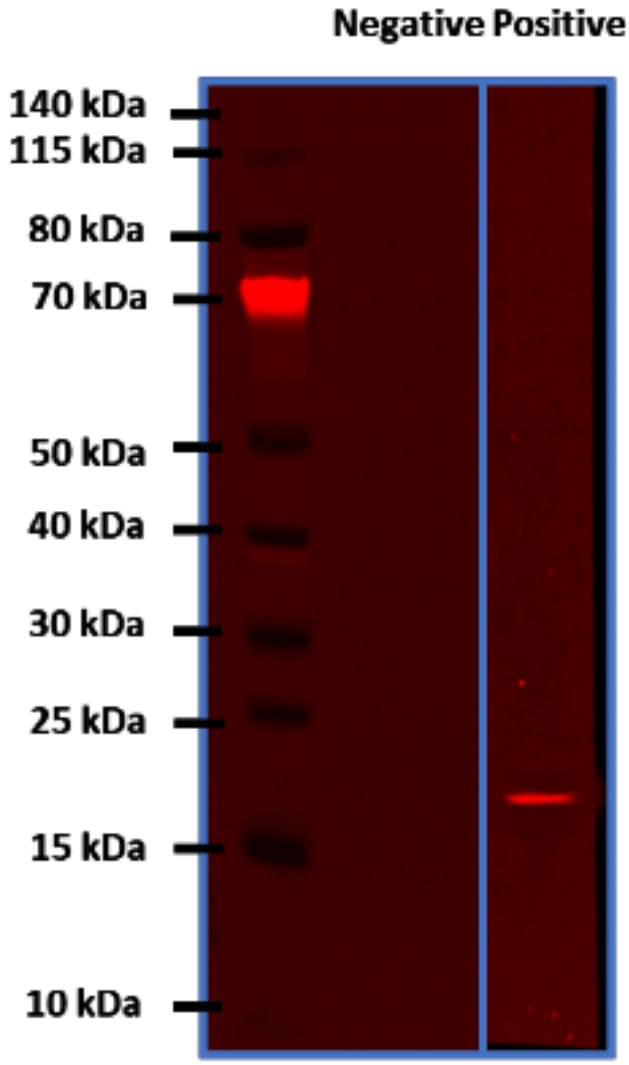
This dossier preserves the complete available evidence progression for SA-000047. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	594
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

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

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® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

Etoposide-treated (2 µM) HeLa cells were homogenized using an ultrasonication probe and centrifuged to pellet down the cell debris. The lysate supernatant was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80oC 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 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 Identifyn® Rabbit Anti-Human γH2AX chimeric antibody (Human Fc) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat anti-Human IgG was incubated for 1 hour, followed by washing 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

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

Post Processing

None

Image: Identifyn® LLC
Copyright 2026 GMD12 LLC.

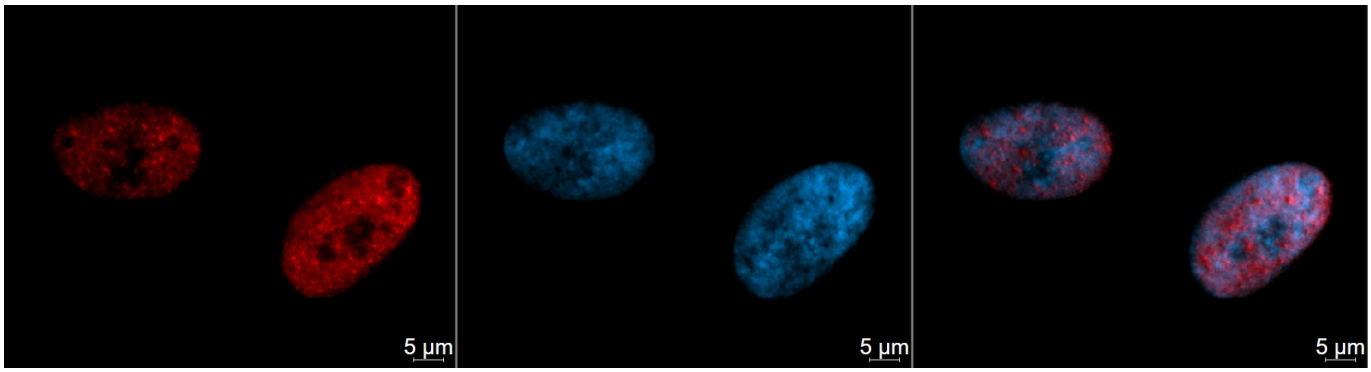
SOURCE PROVENANCE

Catalog	SA-000047
Stage	Western blot

SOURCE PROVENANCE

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	AB5B244A09427E16B5D8DE243156EC041BE1634D954DE26D5447DDDE521E2C71
Method PDF SHA-256	E89419BCDA13AA185D58BD60F6576A281A6C22DAB8566A2575899E547662178D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
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 705 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	691 X 556
Image Size (Scaled)	75.68µm X 60.90µm
Bit Depth	14 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 @20% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	100ms | 40ms
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96µm | 0.72µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies
Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110μm X 0.110μm
Image size (Pixels) = 691 X 556
Image Size (Scaled) = 75.68μm X 60.90μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

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 @20%
Exposure Time = 100ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.96µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 40ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Post Processing

None

Image Correction

None

Image by E.F. Simon

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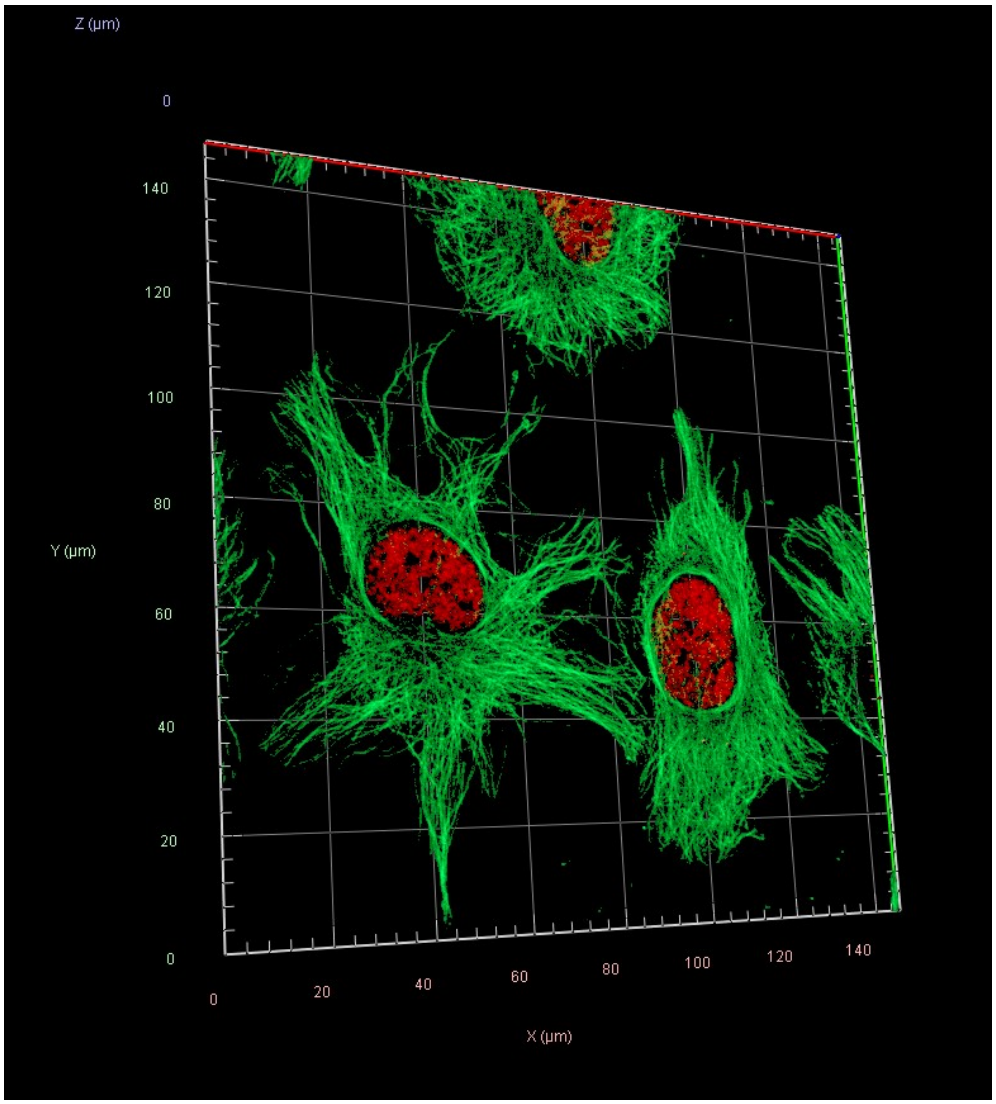
SOURCE PROVENANCE

Catalog	SA-000047

SOURCE PROVENANCE

Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3CA94215BD6DD7F0B1A3E40931340D5B217ADFFD5617D8BB108056FFD8AB4412
Method PDF SHA-256	F3888D589938B636ADF198BB125ADA084B4D6EA22708BE1ADB2C16AE9E834538

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; 488; 594
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	5 Slices (0.8µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	1018 X 1028
Image Size (Scaled)	145.43µm X 146.86µm
Bit Depth	14 Bit
Image Center Position	X: 80.28mm, Y: 48.46mm
Stage Position	X: 80.28mm, Y: 48.46mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	45 Texas Red | 38HE eGFP
Beam Splitter	585 | 495
Filter Excitation Wavelength	540 - 580 | 450 - 490
Filter Emission Wavelength	593 - 668 | 500 - 550
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15% | X-Cite Xylis Lamp Intensity @20%
Exposure Time	75ms | 100ms
Excitation Wavelength	590 | 493
Emission Wavelength	618 | 517
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.20µm | 1.00µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% (594), Threshold 13% (488), 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)

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

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

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

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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 for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 5 Slices (0.8µm)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.200µm
Image Size (Pixels) = 1018 X 1028
Image Size (Scaled) = 145.43µm X 146.86µm
Bit Depth = 14 Bit
Image Center Position = X: 80.28mm, Y: 48.46mm
Stage Position = X: 80.28mm, Y: 48.46mm
ROI Center Offset = X: 1.14, Y: 0.00µm

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 @15%
Exposure Time = 75ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.20µm
Binning Mode = 2,2

488 -

Reflector = 38HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 100ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 13% (594), Threshold 13% (488), 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)

Image Corrections

None

Image by E.F. Simon

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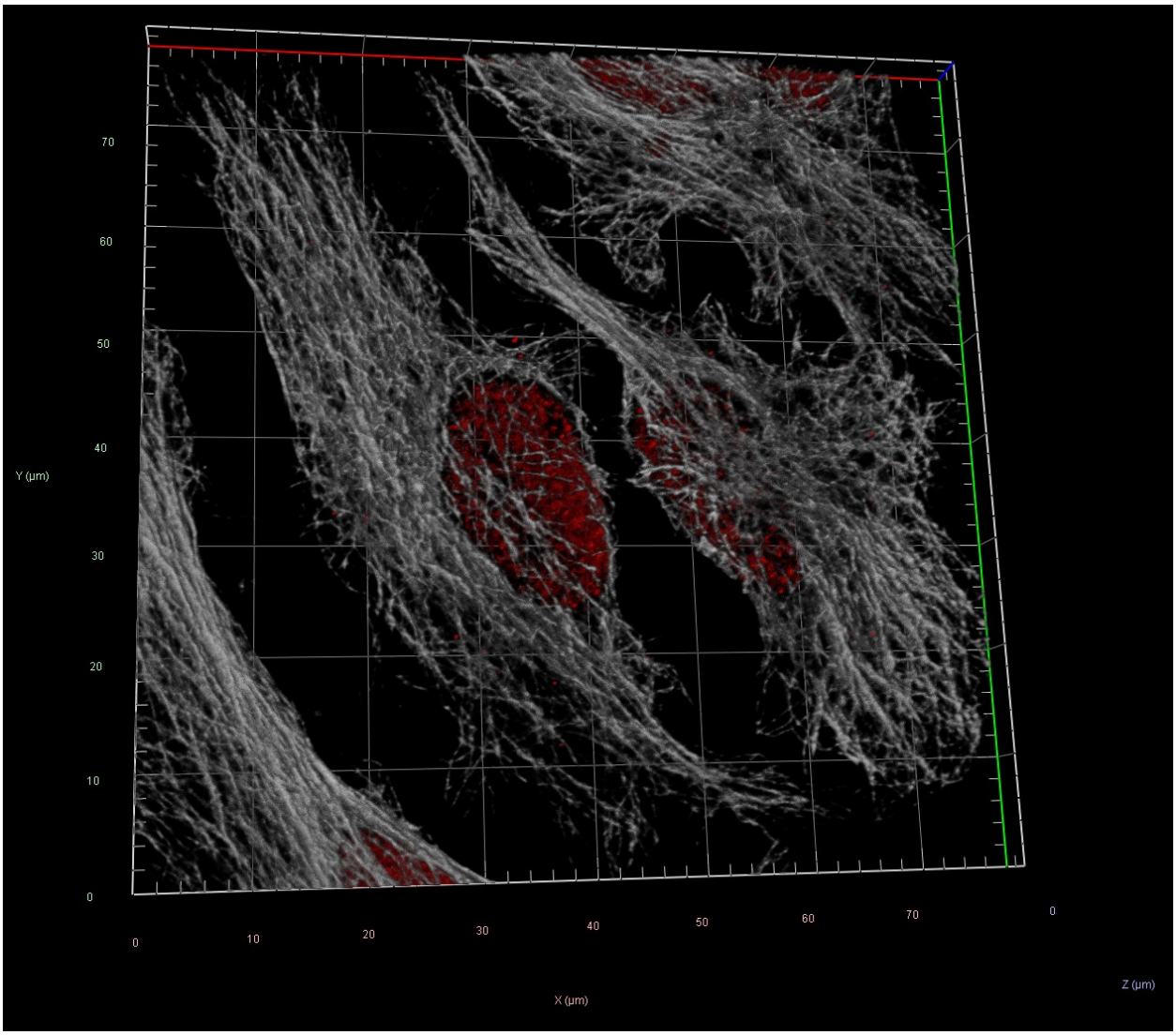
SOURCE PROVENANCE

Catalog	SA-000047
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	0FEC9CCA84084FB260F6F65BE44E901B74676779C7E106F97E20AF9F030E9AD9
Method PDF SHA-256	57893E47B8E9741FCA63CB02244182120F4B014C0D0555800FC3CFF150013DA9

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647; 750
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	56 Slices (3.85µm)
Channels	2
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.070µm
Image Size (Pixels)	1105 X 1105
Image Size (Scaled)	78.01µm X 78.01µm
Bit Depth	16 Bit
Image Center Position	X: 85.76mm, Y: 59.97mm
Stage Position	X: 85.76mm, Y: 59.97mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 51µm | 1.00AU / 57µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.2% | 640nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	-11.13°
Sampling	1.00
Pixel Time	0.70µs
Frame Time	4.02s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	590 | 653
Emission Wavelength	618 | 668
Effective NA	1.4
Detection Wavelength	580 - 645 | 656 - 700
Imaging Device	Airyscan | GaAsP-PMT
Detector Type	GaAsP-PMT
Detector Gain	750 V | 700 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Volume Projection	Precise
Appearance	Threshold 2% (594), Threshold 2% (647), Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000064

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack =56 Slices (3.85µm)

Channels = 2
Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.070µm
Image Size (Pixels) = 1105 X 1105
Image Size (Scaled) = 78.01µm X 78.01µm
Bit Depth = 16 Bit
Image Center Position = X: 85.76mm, Y: 59.97mm
Stage Position = X: 85.76mm, Y: 59.97mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = -11.13°
Sampling = 1.00
Pixel Time = 0.70µs
Frame Time = 4.02s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 580 - 645
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 57µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = -11.13°
Sampling = 1.00
Pixel Time = 0.70µs
Frame Time = 4.02s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsP-PMT
Detector Type = GaAsP-PMT
Detector Gain = 700 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 2% (594), Threshold 2% (647), 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)

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast between the 594/647 channels.

Image by P. Raut

Copyright 2026 GMD12 LLC.

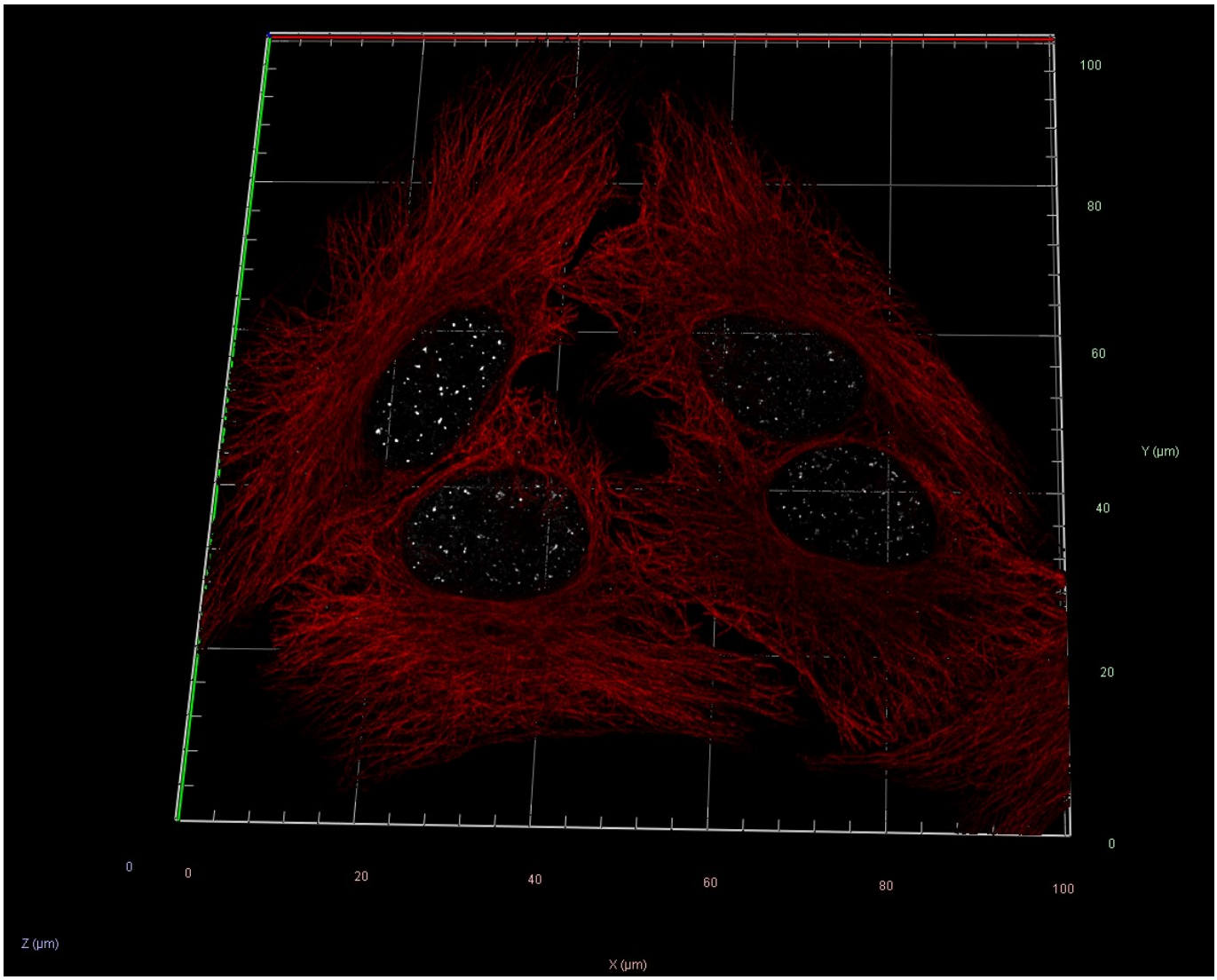
SOURCE PROVENANCE

Catalog	SA-000047

SOURCE PROVENANCE

Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CEC6C2FD8155D48FB8A8052A0FE54C8AAC01054F288D2A7B66DAEE74BEEB53D9
Method PDF SHA-256	6DD6804AF11D6028F2E58A1CBEB48470548EB5C9A0CFFAF3116081F6CBC3A60B

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	21 Slices (1.00µm) - Subset of 63 Slices taken in Acquisition
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.05000 µm
Image Size (Pixels)	2849 X 2849
Image Size (Scaled)	100.57µm X 100.57µm
Bit Depth	16 Bit
Image Center Position	X: 84.03mm, Y: 60.09mm
Stage Position	X: 84.03mm, Y: 60.09mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 252µm | 5.00 AU / 281µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @1.0% | 640nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	20.33°
Sampling	2.00
Pixel Time	0.73µs
Frame Time	14.22s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	590 | 653
Emission Wavelength	618 | 668
Effective NA	1.4
Detection Wavelength	571-635 | 647-700
Imaging Device	Airyscan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 9.3 (3D, Auto) | Super Resolution 8.9 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (594), Threshold 1% (488), Ramp 0% (594), Ramp 0% (488), 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)

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

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000064

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 21 Slices (1.00µm) - Subset of 63 Slices taken in Acquisition

Channels = 2
Scaling (Per Pixel) = 35.30nm X 35.30nm X 50.00nm
Image Size (Pixels) = 2849 X 2849
Image Size (Scaled) = 100.57µm X 100.57µm
Bit Depth = 16 Bit
Image Center Position = X: 84.03mm, Y: 60.09mm
Stage Position = X: 84.03mm, Y: 60.09mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 252µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.0
Rotation = 20.33°
Sampling = 2.00
Pixel Time = 0.73µs
Frame Time = 14.22s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 571-635
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.3 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 281µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.0
Rotation = 20.33°
Sampling = 2.00
Pixel Time = 0.73µs
Frame Time = 14.22s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 647-700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.9 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% (594), Threshold 1% (488), Ramp 0% (594), Ramp 0% (488), 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)

Image Corrections -

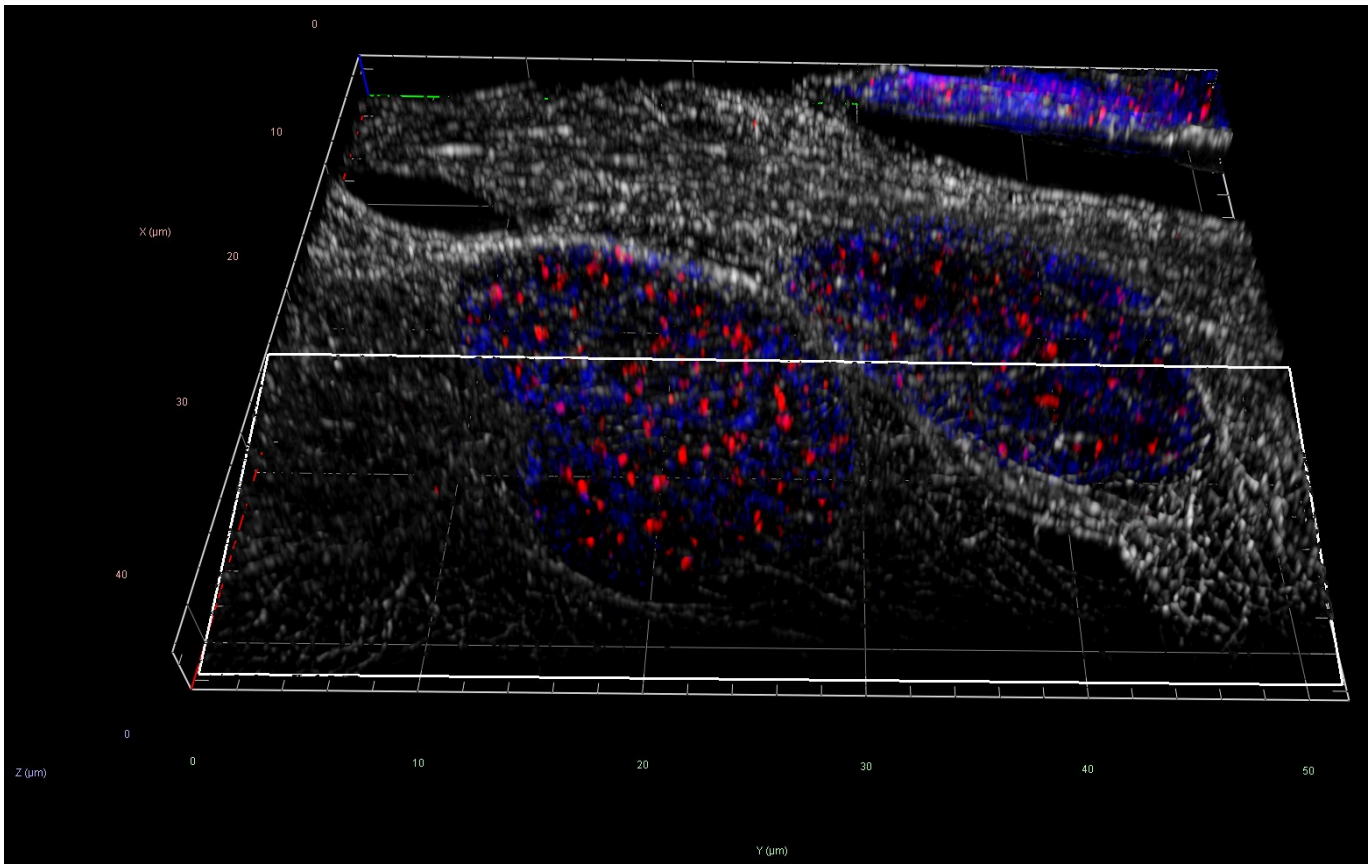
Image by P. Raut

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000047
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AC6C4369781CBE9CE94FC808C76F45A69EF7AC1E87F0A34D6FC7EB374A983955
Method PDF SHA-256	176B29576FB9CF45CC1EE5FB8DB9E11513360BD6D1AE91D43217CD48CC352818

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 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	60 Slices (2.95nµm)
Channels	2
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	2515 X 3066
Image Size (Scaled)	42.48µm X 51.79µm
Bit Depth	16 Bit
Image Center Position	X: 65.44mm, Y: 30.78mm
Stage Position	X: 65.44mm, Y: 30.78mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 640
Channel Name	T2-Axiocam 820 #2-SR | T1-Axiocam 820 -SR
Excitation Wavelength	561 | 640
Emission Wavelength	597 | 729
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	100ms | 120ms
Depth of Focus	0.92µm | 1.13µm
Binning Mode	2,2
Laser Wavelength	561nm @1.50% | 640nm @2.0%
Frame Time	1.33s | 1.59s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.2977 (647), 9.7149 (594)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	The Clipping plane is introduced to demonstrate the specificity of the Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.
Y - Z	Active was selected, and an invisible filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A white Outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.
Position of Clip	40%
Clip Y	-80°
Clip Z	0°

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000064

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 60 Slices (2.95nµm)
Channels = 2
Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm
Image Size (Pixels) = 2515 X 3066
Image Size (Scaled) = 42.48µm X 51.79µm
Bit Depth = 16 Bit
Image Center Position = X: 65.44mm, Y: 30.78mm
Stage Position = X: 65.44mm, Y: 30.78mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

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 = T2-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 @1.50%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @2.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.2977 (647), 9.7149 (594)
Fitting = Fast Fit
Histogram = Scale to Min/Max
Baseline = Cut

3D Image Processing

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

Clipping Image Process = The Clipping plane is introduced to demonstrate the specificity of the Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

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

Position of Clip = 40%
Clip Y = -80°
Clip Z =0°

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast between the 594/647 channels.

Image by P. Raut

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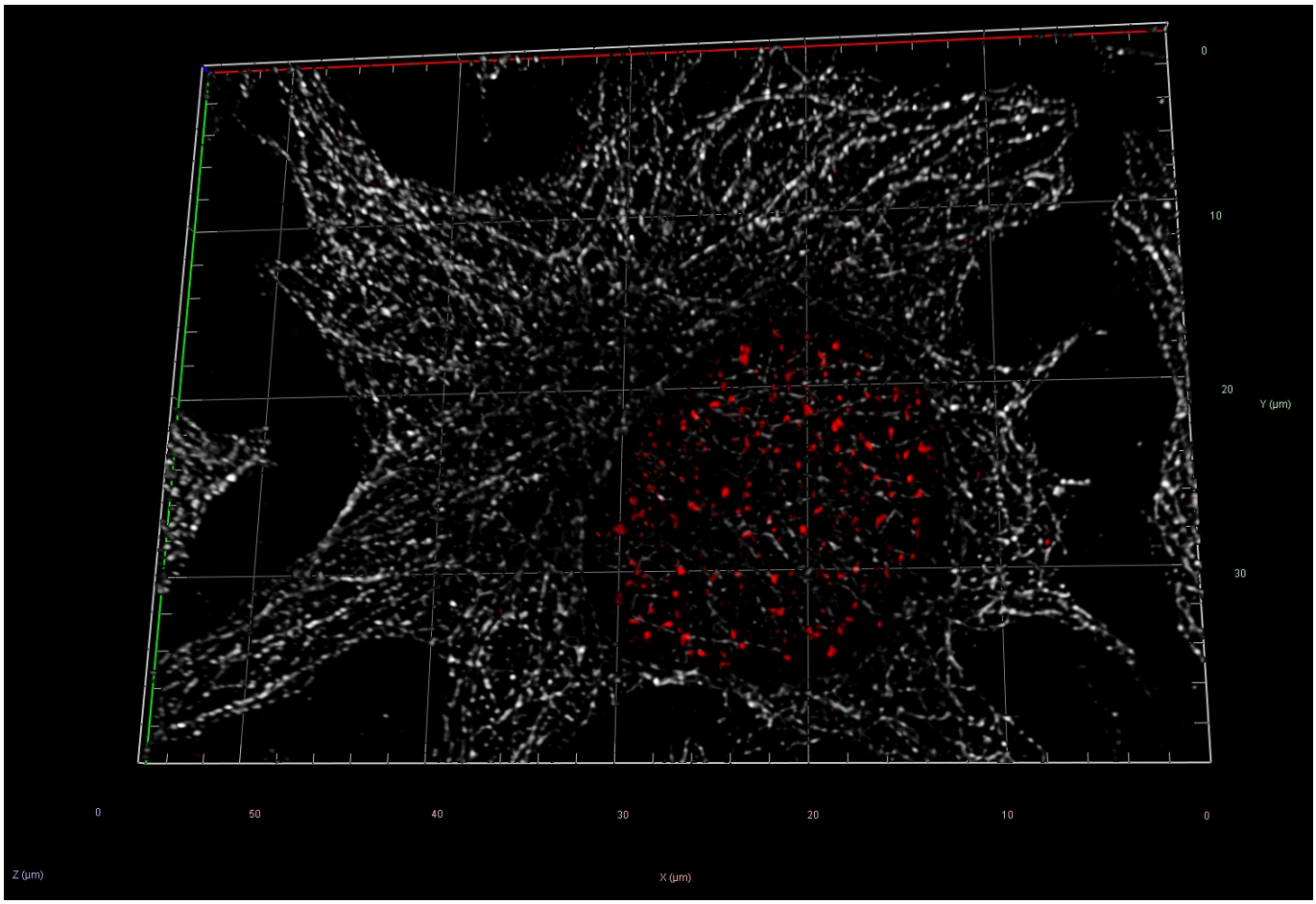
SOURCE PROVENANCE

Catalog	SA-000047
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	372B64ACEA585A8A56E44020E2D75174B34FD2390CA11D783636DAEAFD56F19A
Method PDF SHA-256	921A5BFA2400FE7084674A0BF08C0D3B27966F72FBF0DD09DF84406B78066E24

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 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	18 Slices (850nm) - Subset of 53 Slices taken in Acquisition
Channels	2 | 1
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 47.22222 µm
Image Size (Pixels)	3265 X 2365
Image Size (Scaled)	55.15µm X 39.95µm
Bit Depth	16 Bit
Image Center Position	X: 62.53mm, Y: 34.51mm
Stage Position	X: 62.53mm, Y: 34.51mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 | 640
Channel Name	T2-Axiocam 820 #2-SR | T1-Axiocam 820 -SR
Excitation Wavelength	561 | 640
Emission Wavelength	597 | 729
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	100ms | 120ms
Depth of Focus	0.92µm | 1.13µm
Binning Mode	2,2
Laser Wavelength	561nm @1.50% | 640nm @2.0%
Frame Time	1.33s | 1.59s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.3259 (647), 8.8265 (594)
Order Combination	Wiener Filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Maximum Projection	Precise
Appearance	Threshold 1% 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)

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

Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000064

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 18 Slices (850nm) - Subset of 53 Slices taken in Acquisition

Channels = 2
Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm
Image Size (Pixels) = 3265 X 2365
Image Size (Scaled) = 55.15µm X 39.95µm
Bit Depth = 16 Bit
Image Center Position = X: 62.53mm, Y: 34.51mm
Stage Position = X: 62.53mm, Y: 34.51mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

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 = T2-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 @1.50%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @2.0%
Frame Time = 1.59s
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 = 9.3259 (647), 8.8265 (594)
Order Combination = Wiener Filter
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

3D Image Processing

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

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast between the 594/647 channels.

Image by P. Raut

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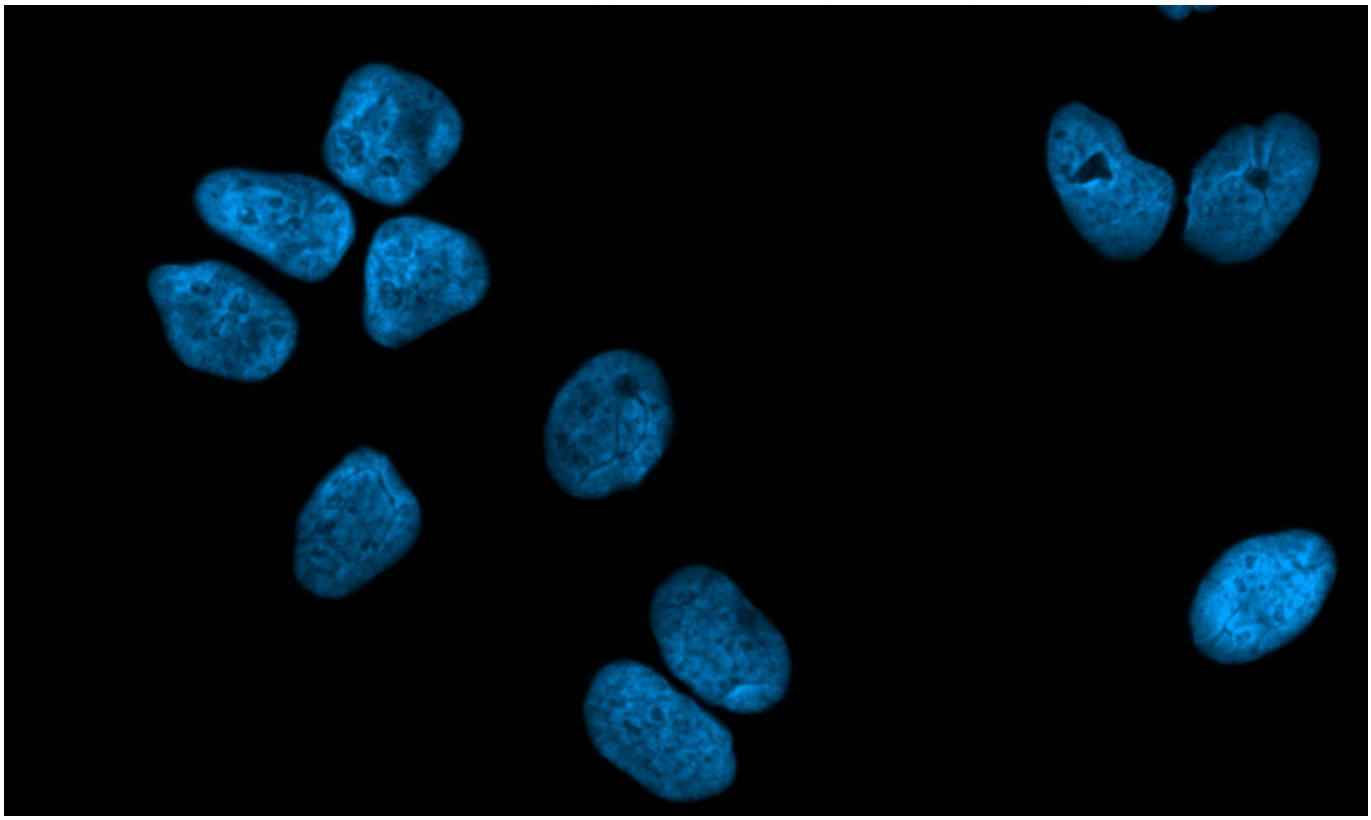
SOURCE PROVENANCE

Catalog	SA-000047
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7819CD8389ED1A18210B9F0E016E5AB9DDAABEE9ABB331CFE14EA95E8184D903
Method PDF SHA-256	892991D6036351C31FAD97BE843CECBE75BB14E27462775D0C33D0E6500E9EA4

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1393 X 829
Image Size (Scaled)	199.00µm X 118.43µm
Bit Depth	14 Bit
Image Center Position	X: 48.85mm, Y: 48.72mm
Stage Position	X: 48.85mm, Y: 48.72mm
ROI Center Offset	X: 1.14µm, 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	200ms | 30ms
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.20µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000047

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. In the absence of a primary antibody, the Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 1393 X 829
Image Size (Scaled) = 199.00µm X 118.43µm
Bit Depth = 14 Bit
Image Center Position = X: 48.85mm, Y: 48.72mm
Stage Position = X: 48.85mm, Y: 48.72mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

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 @25%
Exposure Time = 200ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20µm
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%
Exposure Time = 30ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

Image Corrections

None

Control Summary -

In the absence of a primary antibody, Identifyn Standards for Optical Microscopy Methods™ were applied using standard staining methodology and image acquisition settings using the Zeiss Apotome SIM. With no image correction, the image demonstrates that the secondary antibody, minus a primary target, had no off-target binding and no significant presence of free dye within the sample.

The control data suggests the secondary antibody is highly specific and free of unconjugated (free) dye post-conjugation.

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000047
Stage	Control
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	32

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D6A9316B2A974C891B4E4FAA2E485B91F7C60052B01A2A38ECC25C6D92850565
Method PDF SHA-256	73BFF0EC66764630A7BFAC0CC5B6FDB5A9E0019FD3A5CCE7ED738D919EE3CF48