

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

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

7

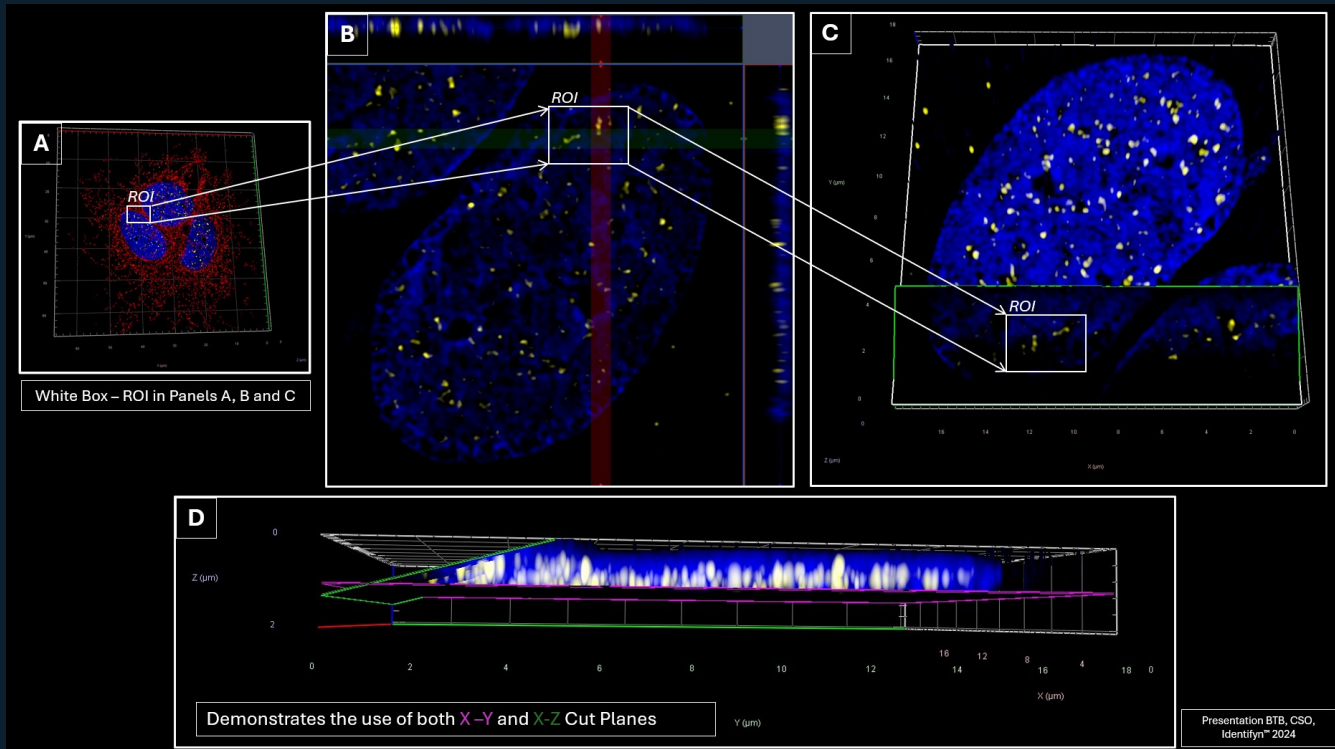
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000025 | 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
- 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™ 532 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-000025 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-000025 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	532	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 647	2; AF532-49014; 50 Cy5; 49 DAPI; 515- 545; 625 - 655; 335 - 383; 555 - 595
Widefield SIM — Apotome	405/DAPI; 532; 555; 647	2; AF532-49014; 50 Cy5; 515 - 545; 625 - 655; 555 - 595; 665 - 715; 528
Confocal	405/DAPI; 488; 532; 647; 750	3; None; 488nm @2.0%; 640nm @0.20%; 405nm @0.40%; 528; 653; 353
Airyscan	405/DAPI; 488; 532; 647	3; None; 488nm @1.0%; 640nm @0.20%; 405nm @1.0%; 528; 653; 353
SIM	405/DAPI; 488; 532	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; T3-Axiocam 820 #2-SR; 488; 405
SIM ²	405/DAPI; 488; 532; 647	3; 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; T3-Axiocam 820 #2-SR; 488
Control	405/DAPI; 532; 555	2; AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 528

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 532.

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, 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): 960 X 766; Image Size (Pixels): 960 X 766; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 705. Timing: Exposure Time: 150ms; 80ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @25%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 38.

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; 532; 555; 647.

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: 16 Slices (3.0µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 1114 X 852; Image Size (Pixels): 1114 X 852; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 50ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @40%. 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; 532; 555; 647.

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 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: 80 Slices (3.95µm); Channels: 3; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.050µm; Image size (Pixels): 1037 X 1003; Image Size (Pixels): 1037 X 1003; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-PMT 2; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.65µs; Frame Time: 4.36s. Illumination: Laser Wavelength: 488nm @2.0%; 640nm @0.20%. Optical/scan: Pinhole: 1.00 AU / 47µm; 1.00 AU / 57µm; Scan Zoom: 1.2; LSM Scan Speed: 8; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 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: 62 Slices (3.05µm); Channels: 3; Scaling (Per Pixel): 35.30nm X 35.30nmµm X 50.00nm; Image size (Pixels): 1849 X 2022; Image Size (Pixels): 1849 X 2022; 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: 488nm @1.0%; 640nm @0.20%. Optical/scan: Pinhole: 5.00 AU / 229µm; 5.00 AU / 280µm; Scan Zoom: 1.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: AiryScan Mode: Super Resolution 9.6 (3D, Auto); Super Resolution 9.5 (3D, Auto). Structured source metadata values: 59.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 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: 77 Slices (2.28µm); Channels: 2; Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm; Image size (Pixels): 1466 X 1050; Image Size (Pixels): 1466 X 1050; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 488nm @8.00%; 405nm @4.0%; Illumination Wavelength: 488; 405. 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; 532.

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: 69 Slices (2.04µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 1359X 1376; Image Size (Pixels): 5056 X 5056; 1359X 1376; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820. Timing: Exposure Time: 120ms; 100ms; Frame Time: 1.59s; 1.33s. Illumination: Laser Wavelength: 488nm @8.00%; 640nm @2.00%; Illumination Wavelength: 488; 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: 77.

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; 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): 1130 X 882; Image Size (Pixels): 1130 X 882; 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; 532; 555.

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-000025 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 μ m X 0.03530 μ m X 0.05000 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)=1849 X 2022; Image Size (Scaled)=65.27 μ m X 71.37 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 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)=1466 X 1050; Image Size (Scaled)=30.95 μ m X 22.17 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 30.00nm -> 0.01320 μ m X 0.01320 μ m X 0.03000 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)=5056 X 5056; Image Size (Scaled)=66.72 μ m X 66.72 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

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.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

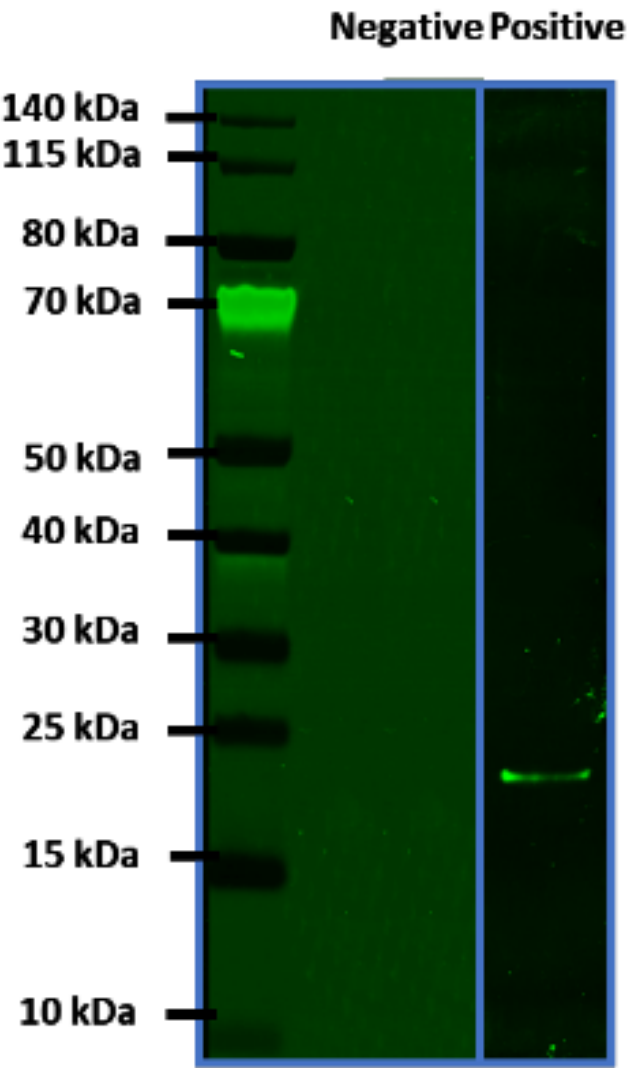
This dossier preserves the complete available evidence progression for SA-000025. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	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	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	HeLa
DETECTED DYES	532
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	Avalanche Photodiode
Intensity	L2
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™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

Etoposide-treated (2 μ M) 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 γ H2AX chimeric antibody (Human Fc) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 532 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 = 572 $\pm$ 28nm

Detector = Avalanche Photodiode

Intensity = L2

Pixel size = 100 μ m

Scan Speed = High

Quality = High

Focus = Membrane (0 μ m in Z-axis)

Post Processing

None

Image: Identifyn® LLC

Copyright 2026 GMD12 LLC.

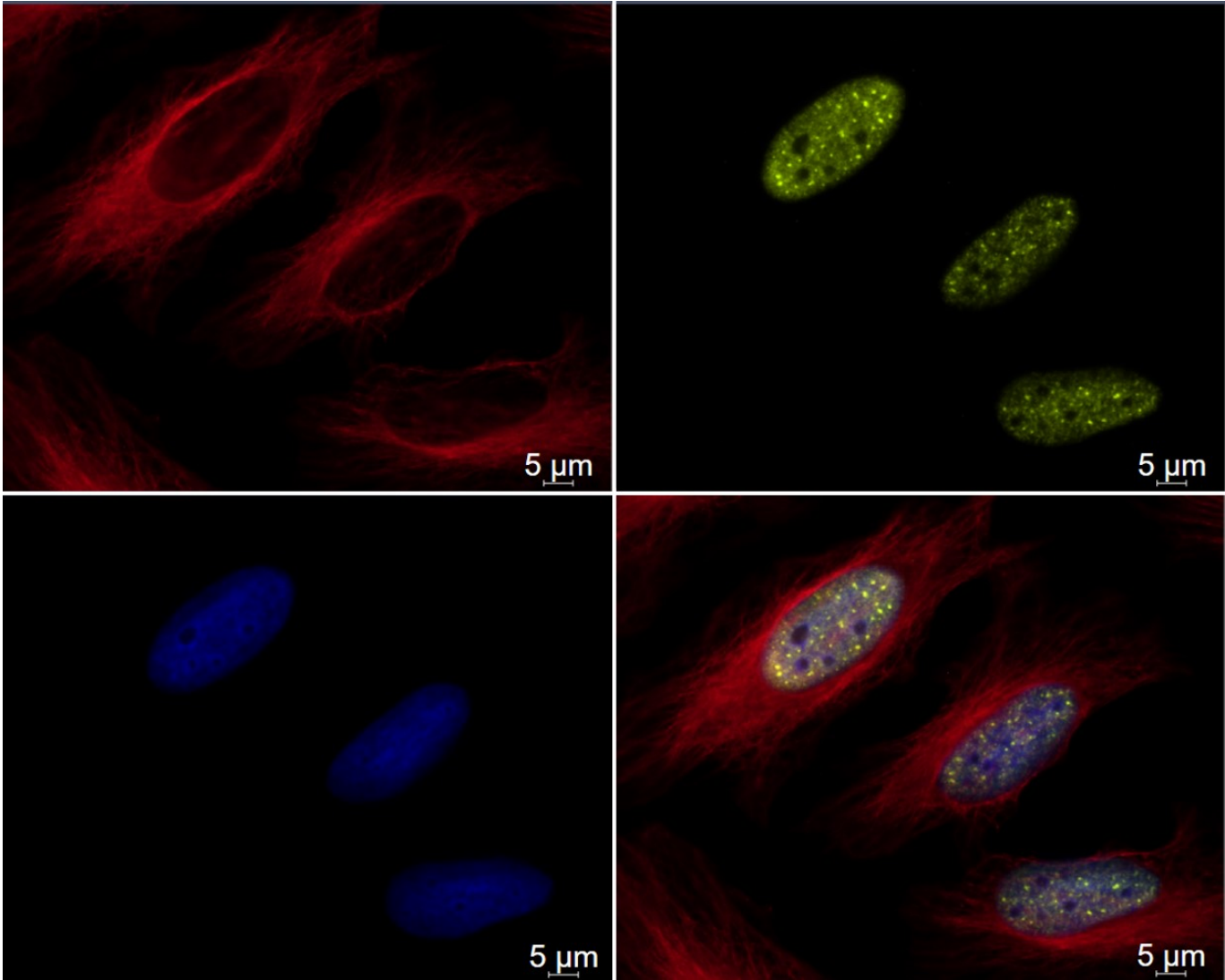
SOURCE PROVENANCE

Catalog	SA-000025
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	6C7E78C97FABF78EEDE6312CC26DFDF18F99A98A92B591E98792AD73503F9A0
Method PDF SHA-256	8238824A5F186BFD85DEFADA3CDB2C08ECACADC9F48500274C9C2E0A45B7E326

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	960 X 766
Image Size (Scaled)	105.14µm X 84.00µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	AF532-49014 50 Cy5 49 DAPI
Beam Splitter	550 660 395
Filter Excitation Wavelength	515- 545 625 - 655 335 - 383
Filter Emission Wavelength	555 - 595 665 - 715 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	150ms 80ms
Excitation Wavelength	528 653 353
Emission Wavelength	553 668 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.86µm 1.03µm 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000025

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 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 Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 μ m X 0.110 μ m
Image size (Pixels) = 960 X 766
Image Size (Scaled) = 105.14 μ m X 84.00 μ m
Bit Depth = 14 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

532 -

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 @20%
 Exposure Time = 150ms
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.86µm
 Binning Mode = 2,2

647 -

Reflector = 50 Cy5
 Beam Splitter = 660
 Filter Excitation Wavelength = 625 - 655
 Filter Emission Wavelength = 665 - 715
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25%
 Exposure Time = 150ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 1.03µm
 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 @8.0%
 Exposure Time = 80ms
 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

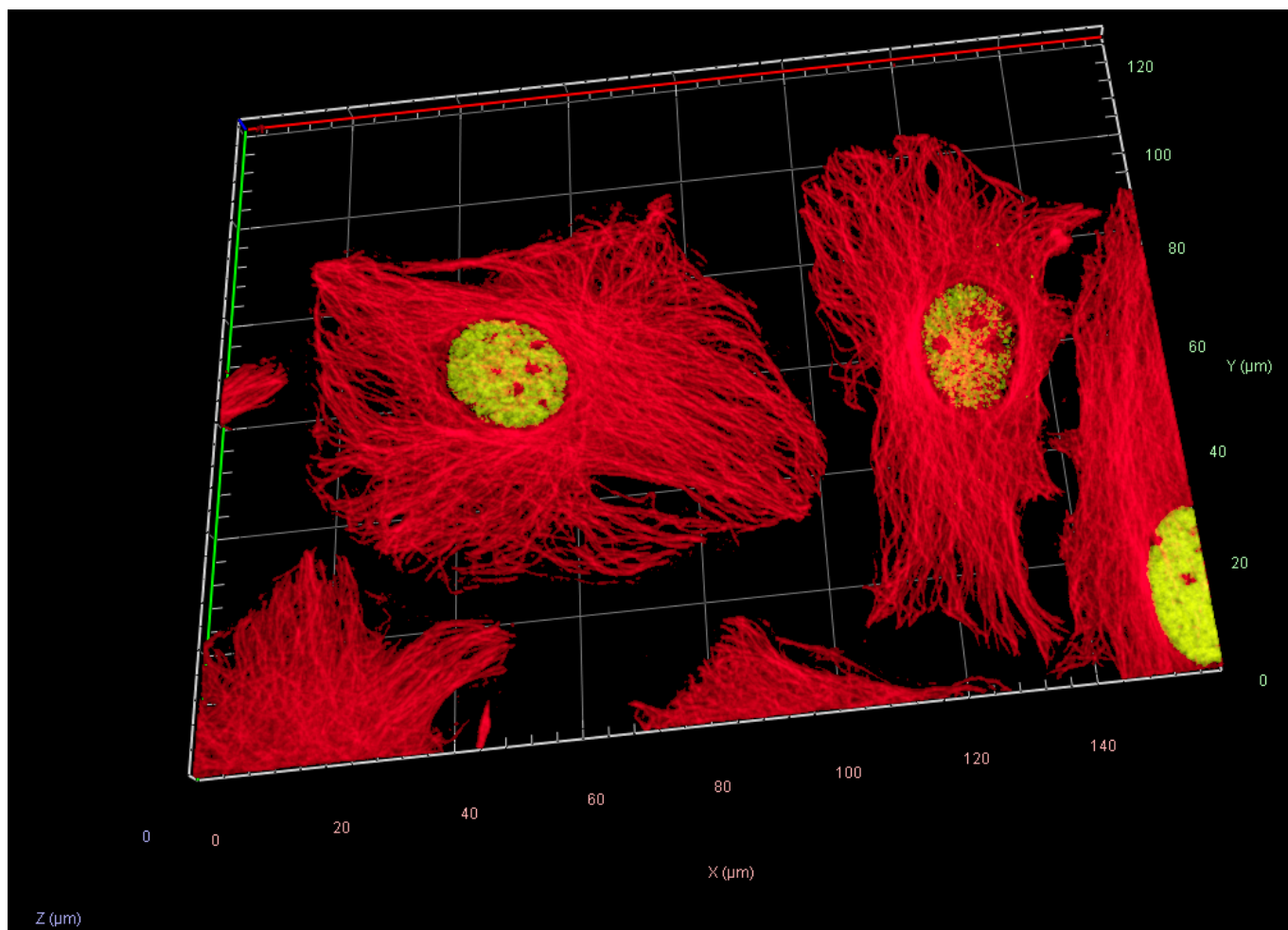
Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000025
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	008A72771E8C83206972077967B680B3D0DABC9E0F32F1907E50F5D3DBEBFCE1
Method PDF SHA-256	F7D97FE8379C6E6A87CACFAC7F6576A625EC52F97D3AF0B2B59B200050DCFACB

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; 532; 555; 647
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	16 Slices (3.0µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	1114 X 852
Image Size (Scaled)	159.14µm X 121.71µm
Bit Depth	14 Bit
Image Center Position	X: 78.84mm, Y: 47.28mm
Stage Position	X: 78.84mm, Y: 47.28mm
ROI Center Offset	X: 1.14, Y: 0.00
Reflector	AF532-49014 50 Cy5
Beam Splitter	550 660
Filter Excitation Wavelength	515 - 545 625 - 655
Filter Emission Wavelength	555 - 595 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @40%
Exposure Time	50ms 200ms
Excitation Wavelength	528 653
Emission Wavelength	553 668
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.07µm 1.30µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100%
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

Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG

Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000064

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 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 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 = 16 Slices (3.0µm)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.200µm

Image Size (Pixels) = 1114 X 852

Image Size (Scaled) = 159.14µm X 121.71µm

Bit Depth = 14 Bit

Image Center Position = X: 78.84mm, Y: 47.28mm

Stage Position = X: 78.84mm, Y: 47.28mm

ROI Center Offset = X: 1.14, Y: 0.00

532 -

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 @20%

Exposure Time = 50ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.25

Imaging Device = AxioCam 807

Camera Adapter = 1X

Depth of Focus = 1.07µm

Binning Mode = 2,2

647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @40%

Exposure Time = 200ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = AxioCam 807

Camera Adapter = 1X

Depth of Focus = 1.30µm

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% all channels, Ramp 0% all channels, Maximum 100%

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

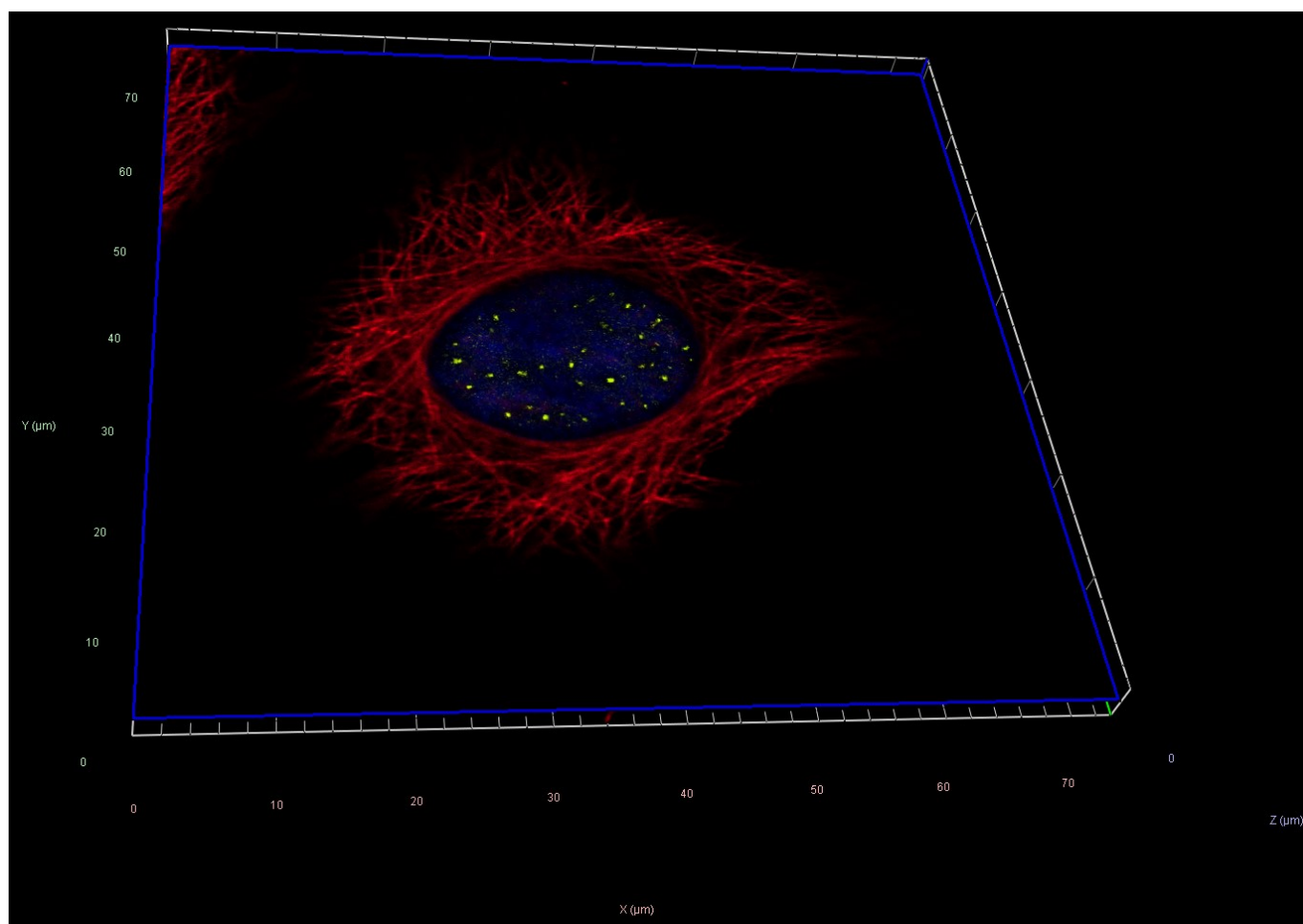
Image by E.F Simon

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000025
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	BC260978A2471E327CB95049E71D14DA012355D5E672CBEEACECCDE7AD495D93
Method PDF SHA-256	6750C1B871349DB3BE900CBD60CC49D54764186603AF0F2AED5E42B4C1B7D437

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647; 750
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 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	80 Slices (3.95µm)
Channels	3
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.050µm
Image Size (Pixels)	1037 X 1003
Image Size (Scaled)	73.21µm X 70.81µm
Bit Depth	16 Bit
Image Center Position	X: 90.61mm, Y: 51.29mm
Stage Position	X: X: 90.61mm, Y: 51.29mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 47µm 1.00 AU / 57µm 1.00 AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @2.0% 640nm @0.20% 405nm @0.40%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.2
Rotation	17.11°
Sampling	1.00
Pixel Time	0.65µs
Frame Time	4.36s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	528 653 353
Emission Wavelength	553 668 465
Effective NA	1.4
Detection Wavelength	533 - 600 656 - 700 410 - 470
Imaging Device	Airyscan GaAsP-PMT 2 GaAsP-PMT 1
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	750 V 650 V
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (532), Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness100%, 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. A blue outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.
Position of Clip	7%
Clip Y	0°
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

Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG

Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000064

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 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 = 80 Slices (3.95µm)

Channels = 3

Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.050µm

Image Size (Pixels) = 1037 X 1003

Image Size (Scaled) = 73.21µm X 70.81µm

Bit Depth = 16 Bit

Image Center Position = X: 90.61mm, Y: 51.29mm

Stage Position = X: 90.61mm, Y: 51.29mm

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

532 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00 AU / 47µm

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)

Laser Wavelength = 488nm @2.0%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.2

Rotation = 17.11°

Sampling = 1.00

Pixel Time = 0.65µs

Frame Time = 4.36s

LSM Scan Speed = 8

Scan Direction = Bidirectional

Line Step = 1

Averaging = 4

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.4

Detection Wavelength = 533 - 600

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 = 1.00 AU / 57µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.20%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 17.11°
 Sampling = 1.00
 Pixel Time = 0.65µs
 Frame Time = 4.36s
 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 2
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 37µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.40%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 17.11°
 Sampling = 1.00
 Pixel Time = 0.65µs
 Frame Time = 4.36s
 LSM Scan Speed = 8
 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-PMT 1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (532), Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness100%, 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 both the Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG and the Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

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

Position of Clip = 7%

Clip Y = 0°

Clip Z = 0°

Image Corrections -

None

Image by P. Raut

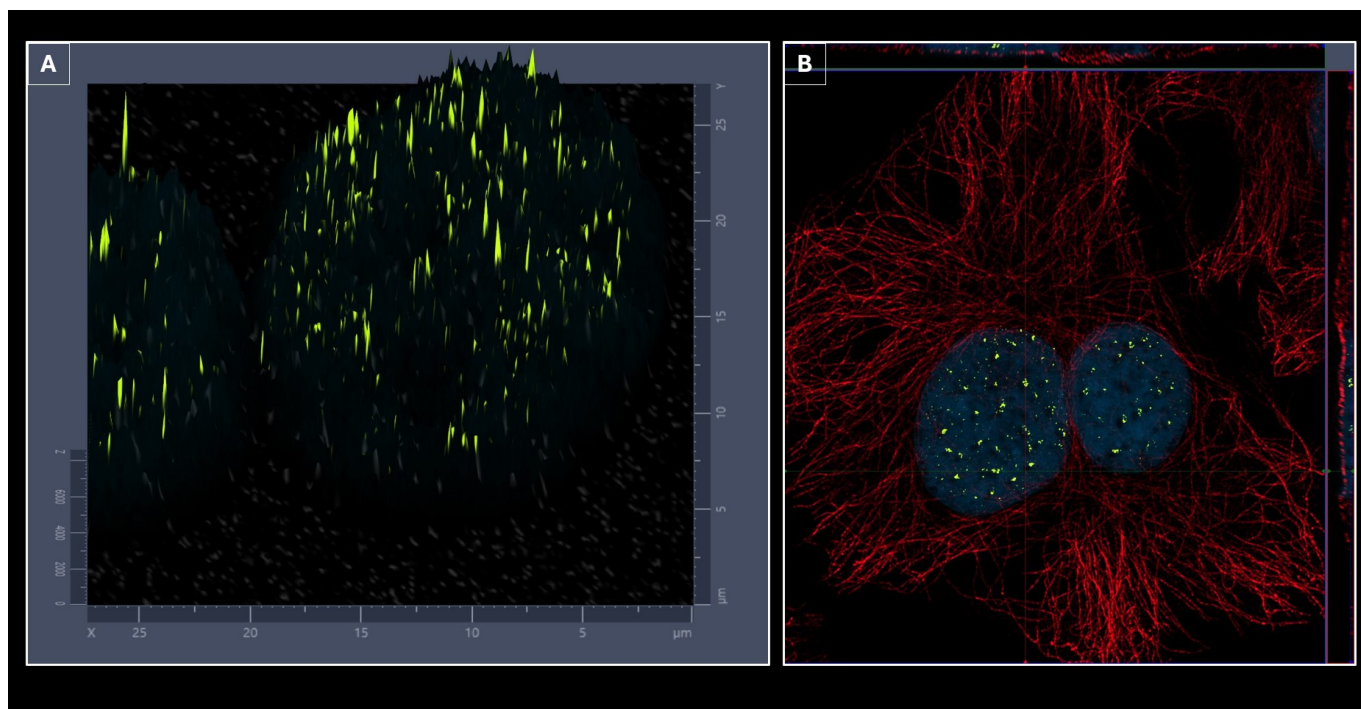
Copyright 2026 GMD12 LLC..

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000025
Stage	Confocal
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	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1D65CD226957D41656B8ED1251F24C6D5622AC86064122AFBDD7B241B0E0360C
Method PDF SHA-256	D810764EC3AEB11ADA00505F05B4E27C6994E9153F5A8A5264E923729B5A984D

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	59

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 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	62 Slices (3.05µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.05000 µm
Image Size (Pixels)	1849 X 2022
Image Size (Scaled)	65.27µm X 71.37µm
Bit Depth	16 Bit
Image Center Position	X: 83.71mm, Y: 51.30mm
Stage Position	X: X: 83.71mm, Y: 51.30mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 229µm 5.00 AU / 280µm 5.00 AU / 181µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @1.0% 640nm @0.20% 405nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	-41.07°
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	528 653 353
Emission Wavelength	553 668 465
Effective NA	1.4
Detection Wavelength	515 - 580 635 - 700 400 - 465
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 9.6 (3D, Auto) Super Resolution 9.5 (3D, Auto) Super Resolution 8.7 (3D, Auto)
Render Mode	Surface
Grid distance	1
Angle X	45
Angle Y	-180
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0
Cut Lines	X 825, Y 1366, Z 8
Line Width	X 1, Y 1, Z 1
Cut Line Opacity	50

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, Human Fc, IgG

Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000064

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 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 = 62 Slices (3.05µm)

Channels = 3

Scaling (Per Pixel) = 35.30nm X 35.30nmµm X 50.00nm

Image Size (Pixels) = 1849 X 2022

Image Size (Scaled) = 65.27µm X 71.37µm

Bit Depth = 16 Bit

Image Center Position = X: 83.71mm, Y: 51.30mm

Stage Position = X: X: 83.71mm, Y: 51.30mm

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

532 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 229µm

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)

Laser Wavelength = 488nm @1.0%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.0

Rotation = -41.07°

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 = 528

Emission Wavelength = 553

Effective NA = 1.4

Detection Wavelength = 515 - 580

Imaging Device = Airyscan

Detector Type = GaAsP-PMT

Detector Gain = 850 V

Detector Offset = 0

Detector Digital Gain = 1.0

AiryScan Mode = Super Resolution 9.6 (3D, Auto)

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 280µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.20%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = -41.07°
 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 = 635 - 700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.5 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 181µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = -41.07°
 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 = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400 - 465
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.7 (3D, Auto)

2.5D Image Processing - Panel A

Zeiss Zen Software converts a two-dimensional image into a height map called a 2.5D Image. The greatest extension in the Z-direction represents the intensity values, creating a pseudo-three-dimensional image representing fluorescent localization or colocalization of the collected signal and its intensity displayed in arbitrary units.

2.5D Display

Render Mode = Surface
 Grid distance = 1
 Pallete and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 45
 Angle Y = -180
 Z Scaling = 1.0
 Ambient = 50
 Specular = 20
 Shininess = 50
 Light Height = 2.0

Orthogonal View, Panel B - Uses Image 8 of the Z-Stack 62 Images.

Cut Lines = X 825, Y 1366, Z 8
 Line Width = X 1, Y 1, Z 1
 Cut Line Opacity = 50

Maximum Intensity Opacity (MIP) and Measure 3D Distance NOT selected

Summary

Panel A employs a 2.5D view to demonstrate signal localization of the Identifyn® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG and Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L) within the nuclear compartment and also to demonstrate the high brightness the 532 displays on the Y axis, with the average signal exceeding 6000 A.U. for a nuclear target such as this DNA damage-induced phosphorylation event.

Panel B uses an orthogonal view to demonstrate nuclear localization and signal within the nuclear compartment in the X, Y, and Z axes again.

Image Corrections -

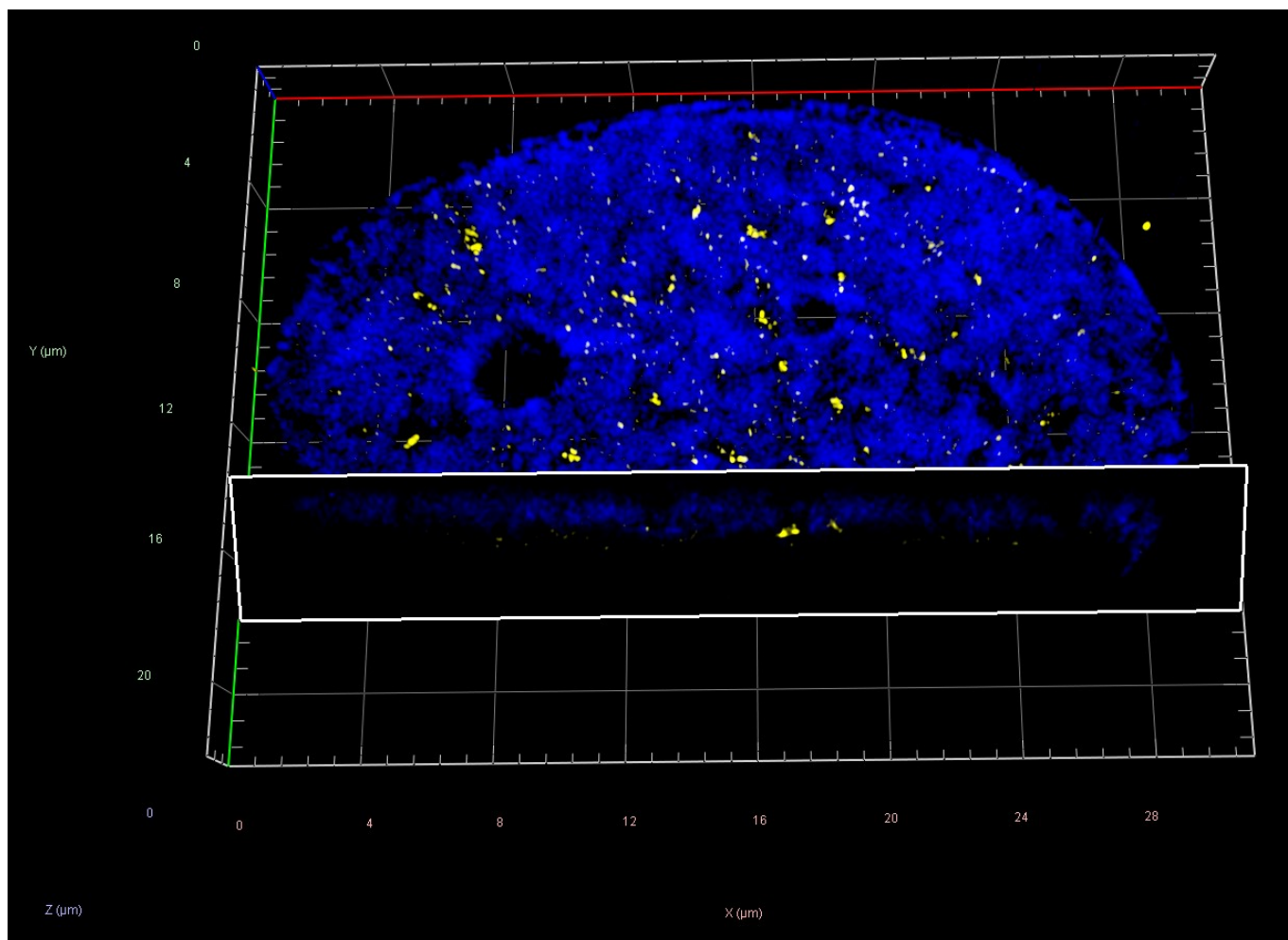
None

Image by P. Raut

Copyright 2026 GMD12 LLC..
 Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE	
Catalog	SA-000025
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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5D6C7B20DF1AD915098C11A5108D38B2287AD5F5CF4607B6656CF2E252E9C936
Method PDF SHA-256	2B59F05B34C55876C5E179EFF465157BDF87F7C28E16436E92F5E86CCDFF7175

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
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	77 Slices (2.28µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1466 X 1050
Image Size (Scaled)	30.95µm X 22.17µm
Bit Depth	16 Bit
Image Center Position	X: 57.11mm, Y: 34.86mm
Stage Position	X: 57.11mm, Y: 34.86mm
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	488 405
Channel Name	T2-Axiocam 820 #2-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	488 405
Emission Wavelength	525 445
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	120ms 50ms
Depth of Focus	0.81µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @8.00% 405nm @4.0%
Frame Time	1.59s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.0704 (532), 9.5905 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Maximum Projection	Precise
Appearance	Threshold 4% (532), Threshold 4% DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	The Clipping plane is introduced to demonstrate the specificity of the Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.
X - Z	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A white outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.
Position of Clip	36%
Clip Y	-60°
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

Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000025

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 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 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 = 77 Slices (2.28 μ m)

Channels = 2
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 1466 X 1050
Image Size (Scaled) = 30.95 μ m X 22.17 μ m
Bit Depth = 16 Bit
Image Center Position = X: 57.11mm, Y: 34.86mm
Stage Position = X: 57.11mm, Y: 34.86mm
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, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503-546, 576-617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 488nm @8.00%
 Frame Time = 1.59s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @4.0%
 Frame Time = 676.00ms
 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 = 11.0704 (532), 9.5905 (DAPI)

Fitting = Fast Fit

Histogram = Scale to Min/Max

Baseline = Cut

3D Image Processing

3D Maximum Projection = Precise

Appearance = Threshold 4% (532), Threshold 4% DAPI, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = The Clipping plane is introduced to demonstrate the specificity of the Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

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

Position of Clip = 36%

Clip Y = -60°

Clip Z = 0°

Image Corrections

None

Image by P. Raut

Copyright 2026 GMD12 LLC.

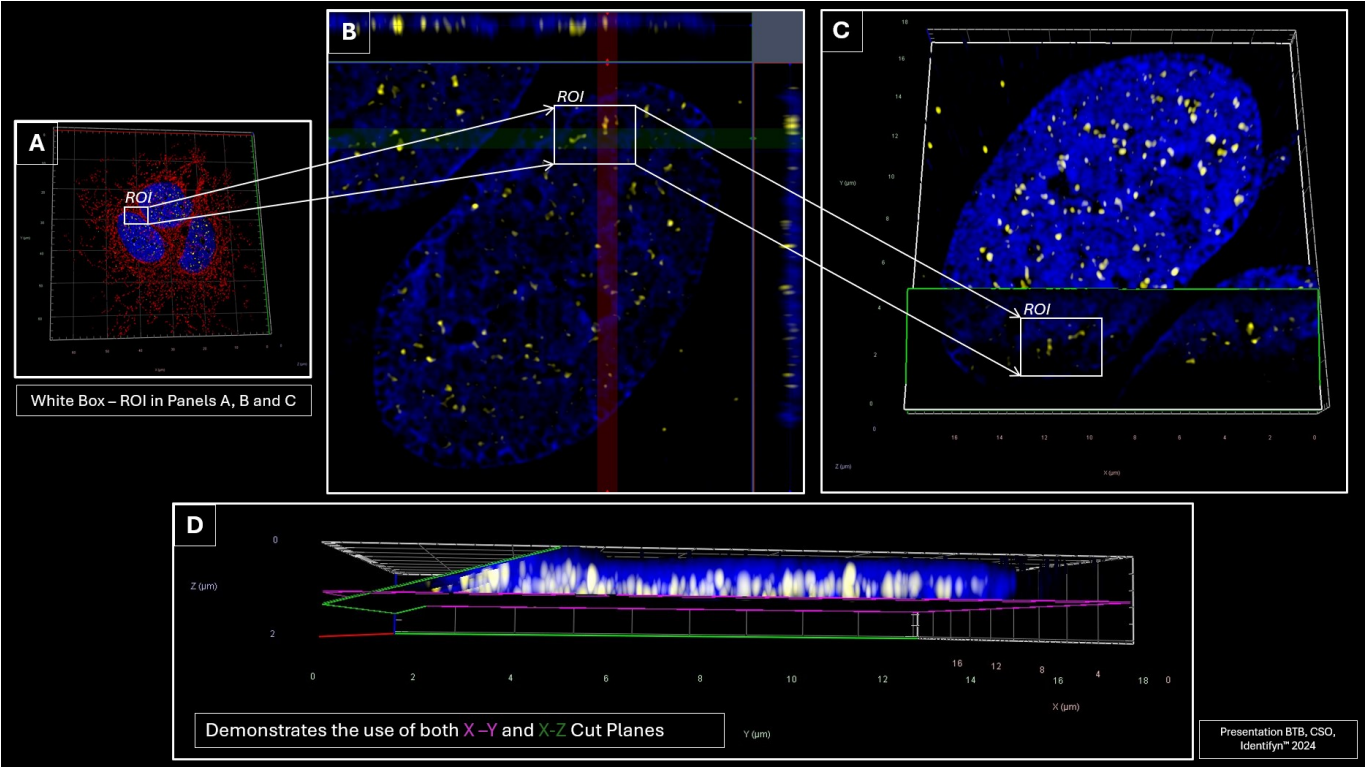
SOURCE PROVENANCE

Catalog	SA-000025
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:

SOURCE PROVENANCE

Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8EEAB3EE9850A7850D6DFE48341E1791151D0D22AE81718D6D06D8D1413FD841
Method PDF SHA-256	D9DEAD0C1D2D4F777B69A15760D855A8B28A2A427FF574106E8A105E2A63A57E

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	77

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	69 Slices (2.04µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 1359X 1376
Image Size (Scaled)	66.72µm X 66.72µm 17.93µm X 18.16µm
Bit Depth	16 Bit
Image Center Position	X: 59.10mm, Y: 30.97mm
Stage Position	X: 59.10mm, Y: 30.97mm
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	488 640 405
Channel Name	T2-Axiocam 820 #2-SR T1-Axiocam 820-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	488 640 405
Emission Wavelength	525 729 445
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 Axiocam 820
Camera Adapter	1X
Exposure Time	120ms 100ms 50ms
Depth of Focus	0.81µm 1.13µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @8.00% 640nm @2.00% 405nm @4.0%
Frame Time	1.59s 1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.5552 (532), 8.7195 (647), 7.5474 (DAPI)
Order Combination	Weiner Filter
Regularization	None
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Maximum Projection	Precise
Appearance	Threshold 1% (532), Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Cut Lines	X 896, Y 240, Z 19
Line Width	X 65, Y 65, Z 0
Cut Line Opacity	20
X - Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A violet outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.
Position of Clip	5% -61%
Clip Y	0° 75°
Clip Z	0°
X - Z	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and gamma-H2AX foci. A green outline of the clipped perimeter was shown, the Clip Front was Selected, and the Clip Back was NOT selected.
Note	The 647 channel will now be omitted from the data.

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, Human Fc, IgG

Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000064

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 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 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, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017), 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) - Panel A, original acquisition field

Z-Stack = 69 Slices (2.04µm)

Channels = 3

Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 66.72µm X 66.72µm

Bit Depth = 16 Bit

Image Center Position = X: 59.10mm, Y: 30.97mm

Stage Position = X: 59.10mm, Y: 30.97mm

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

Z Stack - (3D) - Panel B/C/D represent the Region of Interest taken as shown in Panel A

Z-Stack = 69 Slices (2.04µm)

Channels = 3

Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm

Image Size (Pixels) = 1359X 1376

Image Size (Scaled) = 17.93µm X 18.16µm

Bit Depth = 16 Bit

Image Center Position = X: 59.10mm, Y: 30.97mm

Stage Position = X: 59.10mm, Y: 30.97mm

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

532 -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 488

Channel Name = T2-Axiocam 820 #2-SR

Excitation Wavelength = 488

Emission Wavelength = 525

Effective NA = 1.4

Detection Wavelength = 419-470, 503-546, 576-617, 657-800

Imaging Device = Axiocam 820 #2

Camera Adapter = 1X

Exposure Time = 120ms

Depth of Focus = 0.81µm

Binning Mode = 2,2

Laser Wavelength = 488nm @8.00%

Frame Time = 1.59s

Scan Direction = Unidirectional

Grating 36.5µm 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 = 100ms
 Depth of Focus = 1.13µm
 Binning Mode = 2,2
 Laser Wavelength = 640nm @2.00%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @4.0%
 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 = 3
 Algorithm = SIM2
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 8.5552 (532), 8.7195 (647), 7.5474 (DAPI)
 Order Combination = Weiner Filter
 Regularization = None
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Histogram = Scale to Min/Max

3D Image Processing Panel A -

3D Maximum Projection = Precise
 Appearance = Threshold 1% (532), Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing, Panels, B, C, and D -

3D Maximum Projection = Precise
 Appearance = Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Orthogonal View, Panel B - Uses Image 19 of the Z-Stack 69 Images.

Cut Lines = X 896, Y 240, Z 19
 Line Width = X 65, Y 65, Z 0
 Cut Line Opacity = 20

Maximum Intensity Opacity (MIP) and Measure 3D Distance NOT selected

Clipping Image Process Panels C and D -

The Clipping plane is introduced to demonstrate the specificity of both the Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG and the Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Human, IgG (H + L) to the DNA damage-induced gamma-H2AX foci within the nuclear compartment.

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

Position of Clip = 5%

Clip Y = 0°

Clip Z = 0°

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

Position of Clip = -61%

Clip Y = 75°

Clip Z = 0°

Summary -

Panel A - Shows the original size and orientation of the acquisition, presented in 3-dimensional Maximum Projections. The white box demonstrates the Region of Interest (ROI) Chosen to Investigate the specificity of the Identifyn® primary and 532 secondary antibodies.

Note: The 647 channel will now be omitted from the data.

Panel B - Orthogonal view using the settings described above (see orthogonal view) to further enhance and identify the signal specificity under review. The X and Y axes are highlighted by a red and green intersecting line, respectively.

Panel C - Demonstrates the 3-dimensional Maximum Projection of the ROI, using both an X-Y and X-Z cut plane to isolate the signal specificity further.

Panel D - Provides a detailed view of the cut planes employed from the Z-angle so one may be orientated to the image.

Image Corrections

None

Image analysis and Presentation by Dr. Brian T. Bennett

Copyright 2026 GMD12 LLC.

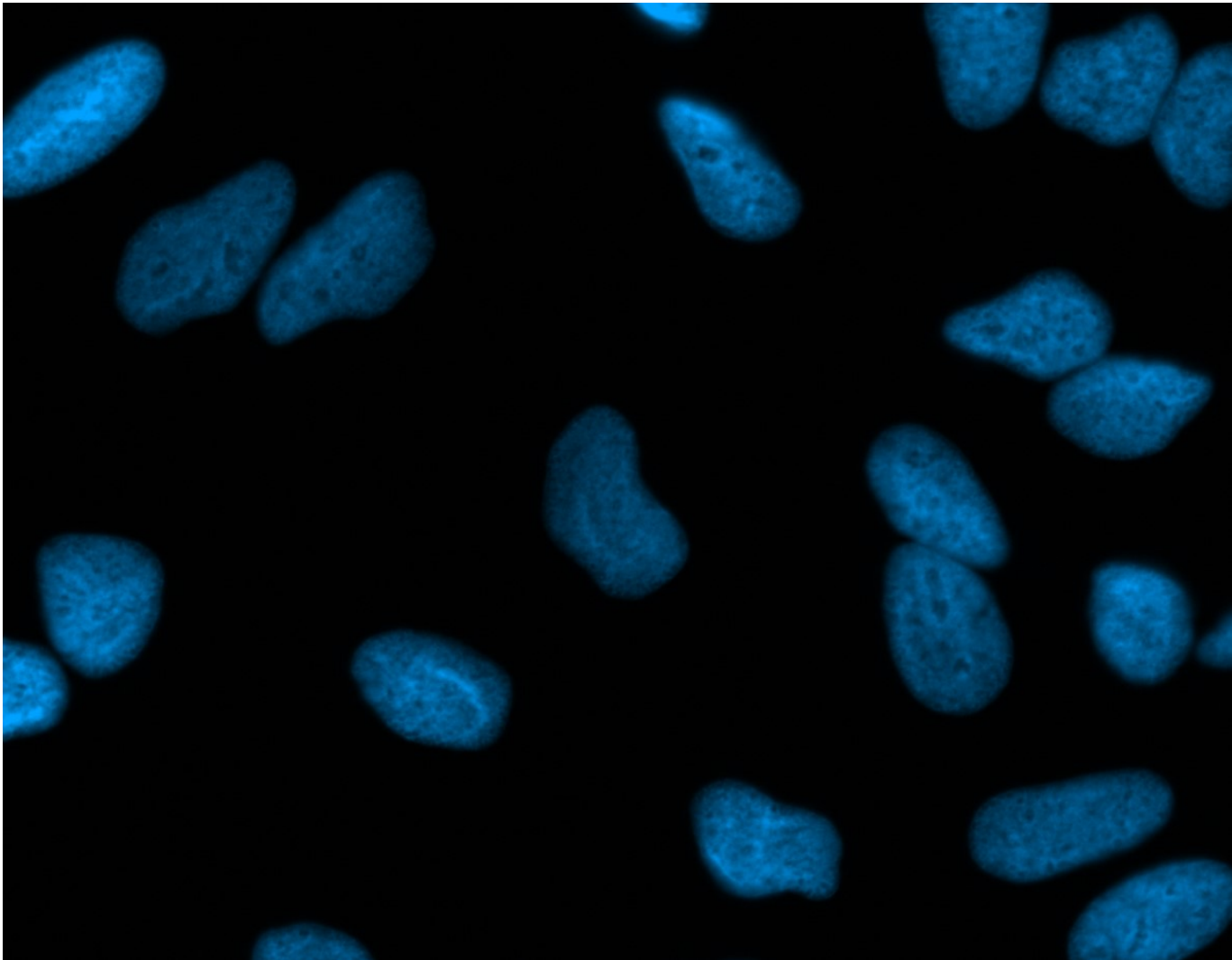
SOURCE PROVENANCE	
Catalog	SA-000025
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	77
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4971BD47B9CE751EFC9BCBB8F0521E68A025F9232FDD81373CDA6440C31271BF

SOURCE PROVENANCE

Method PDF SHA-256

3DF1BBAE9B99AE42A42021042D94C0572EACF95809010768291B714868C55ED5

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555
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)	1130 X 882
Image Size (Scaled)	161.43µm X 126.00µm
Bit Depth	14 Bit
Image Center Position	X: 51.51mm, Y: 50.47mm
Stage Position	X: 51.51mm, Y: 50.47mm
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 @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	528 353
Emission Wavelength	553 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.07µm 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™ 532 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000025

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® 532 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) = 1130 X 882

Image Size (Scaled) = 161.43µm X 126.00µm

Bit Depth = 14 Bit

Image Center Position = X: 51.51mm, Y: 50.47mm

Stage Position = X: 51.51mm, Y: 50.47mm

ROI Center Offset = X: 1.14µm, Y: 0.00µm

532 -

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 @25%

Exposure Time = 200ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.07µm

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

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000025
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	952FE400F194C0849B9CE2CDEACF2EB9EB9B78B9F34AD144A93B295FBB0A876A
Method PDF SHA-256	5C43FFBC7377BCA86D356E0A295A2C91849F7C73796128AE83830AB5ED8B06A0