BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

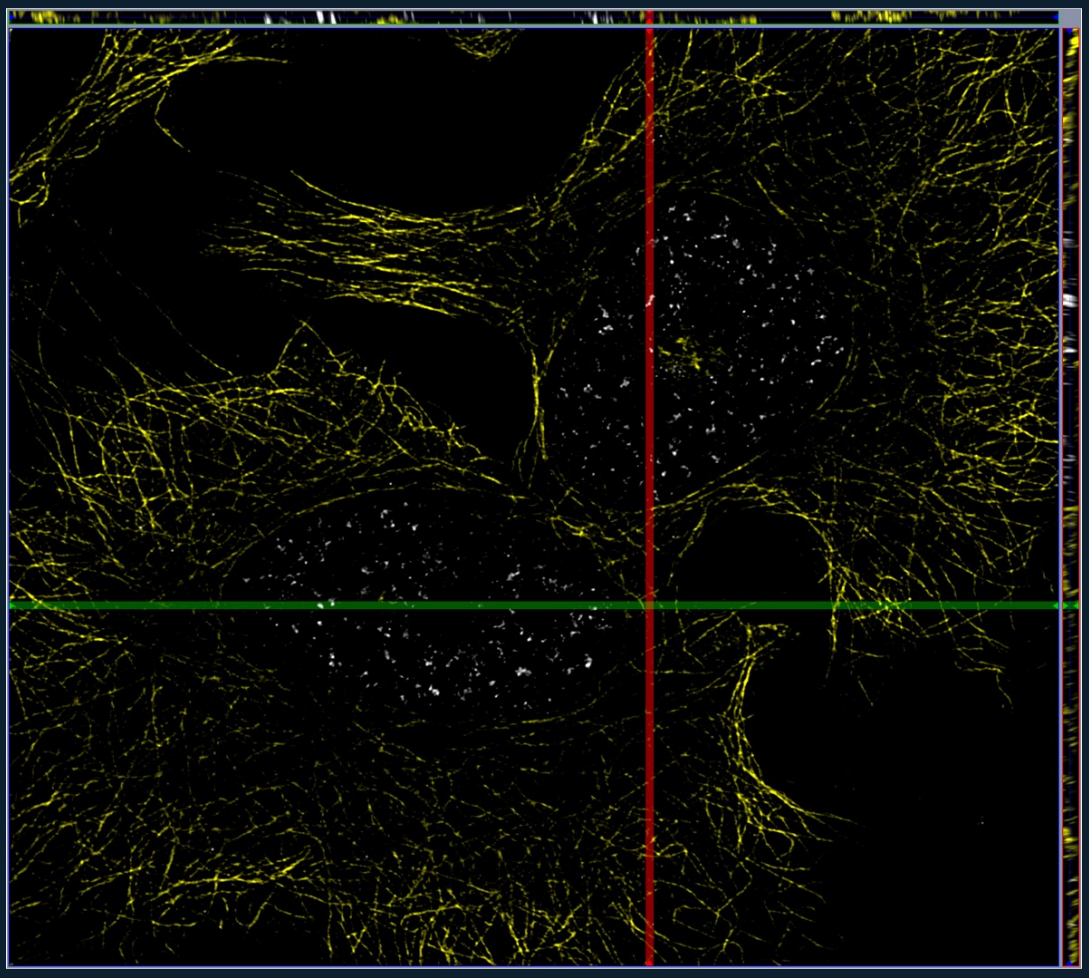
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 405 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-000003 | 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™ 405 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-000003 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-000003 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	405/DAPI	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 750	2; AF405-49027; 372 - 408; 417 - 445; 401; 422
Widefield SIM — Apotome	405/DAPI; 555	2; AF405-49027; AF532-49014; 372 - 408; 515 - 545; 417 - 445; 555 - 595; 401
Confocal	405/DAPI; 488; 532; 750	2; 405nm @0.5%; 488nm @1.0%; 401; 528; 422; 553; None
Airyscan	405/DAPI; 488; 532; 750	2; 405nm @1.0%; 488nm @1.0%; 401; 528; 422; 553; None
SIM	405/DAPI; 488; 532; 555	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; 405; 561
SIM²	405/DAPI; 488; 555	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; 405
Control	405/DAPI; 647	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401

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 405/DAPI.

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 750, 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): 875 X 913; Image Size (Pixels): 875 X 913; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 21.

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; 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

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: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 875 X 864; Image Size (Pixels): 875 X 864; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 700ms; 150ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; 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 Widefield SIM — Apotome. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 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: 27 Slices (1.82µm); Channels: 2; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.070µm; Image size (Pixels): 586 X 1280; Image Size (Pixels): 586 X 1280; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsp-PMT1; Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.59µs; 0.70µs; Frame Time: 17.76s; 4.02s. Illumination: Laser Wavelength: 405nm @0.5%; 488nm @1.0%. Optical/scan: Pinhole: 1.00 AU / 37µm; 1.00AU / 46µm; Scan Zoom: 0.80; 1.3; LSM Scan Speed: 7; 8; Scan Direction: Bidirectional; Averaging: 8; 4; Effective NA: 1.4. Processing: Projection: View Angle 54°, Scale Z 93%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 52.

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; 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 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: 33 Slices (2.24µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 70.00nm; Image size (Pixels): 2371 X 2371; Image Size (Pixels): 2371 X 2371; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.88µs; Frame Time: 11.85s. Illumination: Laser Wavelength: 405nm @1.0%; 488nm @1.0%. Optical/scan: Pinhole: 5.00 AU / 179µm; 5.00AU / 228µm; Scan Zoom: 1.2; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 116%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.7 (3D, Auto); Super Resolution 8.9 (3D, Auto). Structured source metadata values: 47.

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; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 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): 2391 X 2483; Image Size (Pixels): 2391 X 2483; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820#2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 676.00ms; 1.33s. Illumination: Laser Wavelength: 405nm @3.0%; 561nm @1.0%; Illumination Wavelength: 405; 561. 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: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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: 36 Slices (1.05µm); Channels: 2; 1; Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm; Image size (Pixels): 3363 X 3001; Image Size (Pixels): 3363 X 3001; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820#2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 676.00ms; 1.33s. Illumination: Laser Wavelength: 405nm @3.0%; 561nm @1.0%; Illumination Wavelength: 405; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4. Structured source metadata values: 53.

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; 555.

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 Imager.M1; 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): 1186 X 977; Image Size (Pixels): 1186 X 977; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 650ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 29.

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 Imager.M1; ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 647.

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-000003 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): 35.29nm X 35.29nm X 70.00nm -> 0.03529 µm X 0.03529 µm X 0.07000 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)=2371 X 2371; Image Size (Scaled)=83.66µm X 83.66µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2391 X 2483; Image Size (Scaled)=50.48µm X 52.42µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3363 X 3001; Image Size (Scaled)=71.00µm X 63.36µm; 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

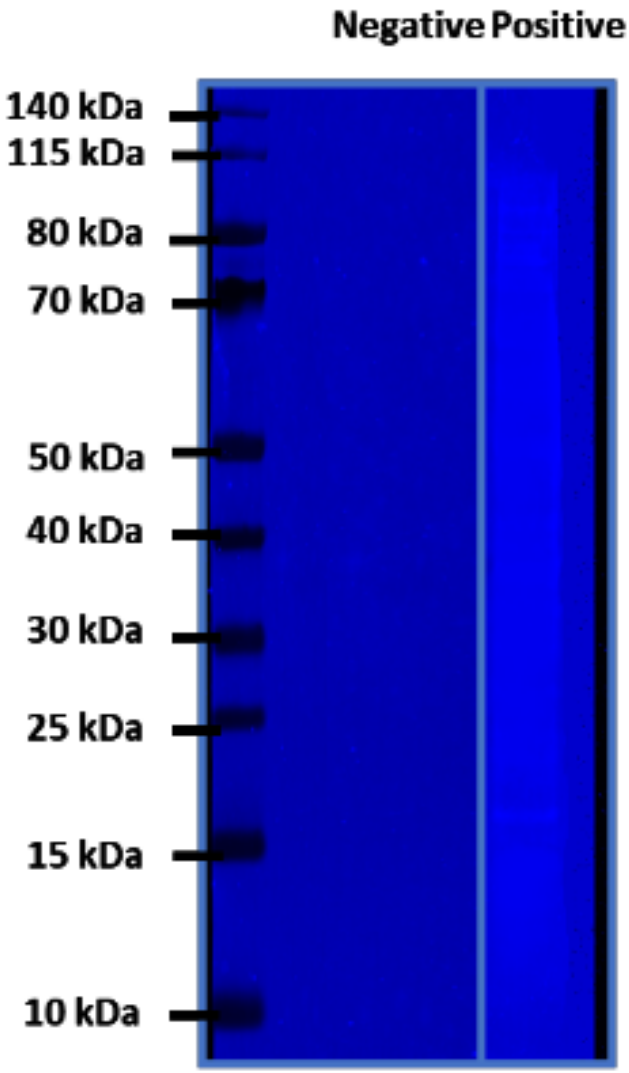
This dossier preserves the complete available evidence progression for SA-000003. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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	405/DAPI
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	375nm
Emission Filter	452±45nm
Detector	PMT
Intensity	10
Pixel size	100 µm
Scan Speed	Medium
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™ 405 Affinity Purified Goat Anti-Human IgG (H + L)
Cat ID# - SA 000003

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™ 405 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 = 375nm
Emission Filter = 452±45nm
Detector = PMT
Intensity = 10
Pixel size = 100 µm
Scan Speed = Medium
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

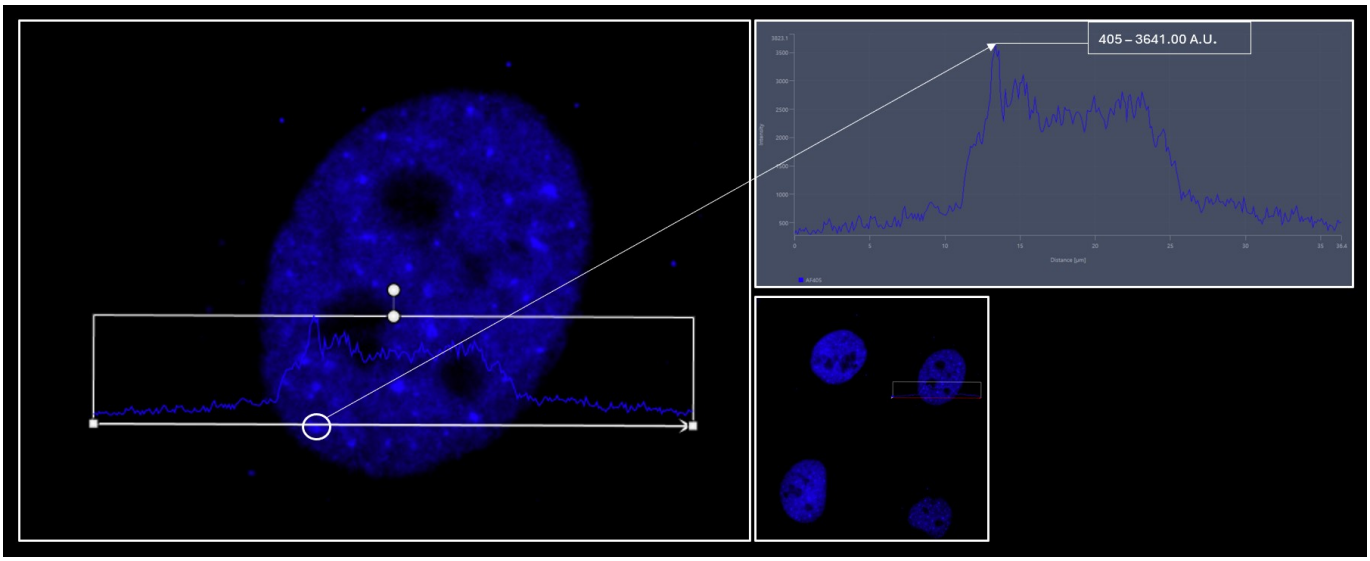
SOURCE PROVENANCE

Catalog	SA-000003

SOURCE PROVENANCE

Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	42ADFECC5FB4EC55EFFED613617282C2611E73954784912D54776D0A388999EC
Method PDF SHA-256	1DFCE1EBA0D5430FB4DD29D19C3FD5311E0D80EF42BC4DB49DA99F1E97F4790F

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	21

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 750, 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)	875 X 913
Image Size (Scaled)	95.83µm X 100.00µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	AF405-49027
Beam Splitter	412
Filter Excitation Wavelength	372 - 408
Filter Emission Wavelength	417 - 445
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20%
Exposure Time	300ms
Excitation Wavelength	401
Emission Wavelength	422
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.65µm
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 H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

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, Identifyn® Rabbit anti-Human 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® 405 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 cat#36961.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, 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) = 875 X 913
Image Size (Scaled) = 95.83μm X 100.00μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

405 -

Reflector = AF405-49027
Beam Splitter = 412
Filter Excitation Wavelength = 372 - 408
Filter Emission Wavelength = 417 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 300ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.65µm
Binning Mode = 2,2

Post Processing

None

Summary

The image shows a 2-dimensional field from which a single nucleus was chosen and a line profile created crossing the nuclear boundaries. Note that the 405/gamma-H2AX signal is contained within the nuclear compartment as expected, and in this widefield application, using an sCMOS camera, we cross a foci whose signal is 3641.00 A.U. intensity.

Image Correction

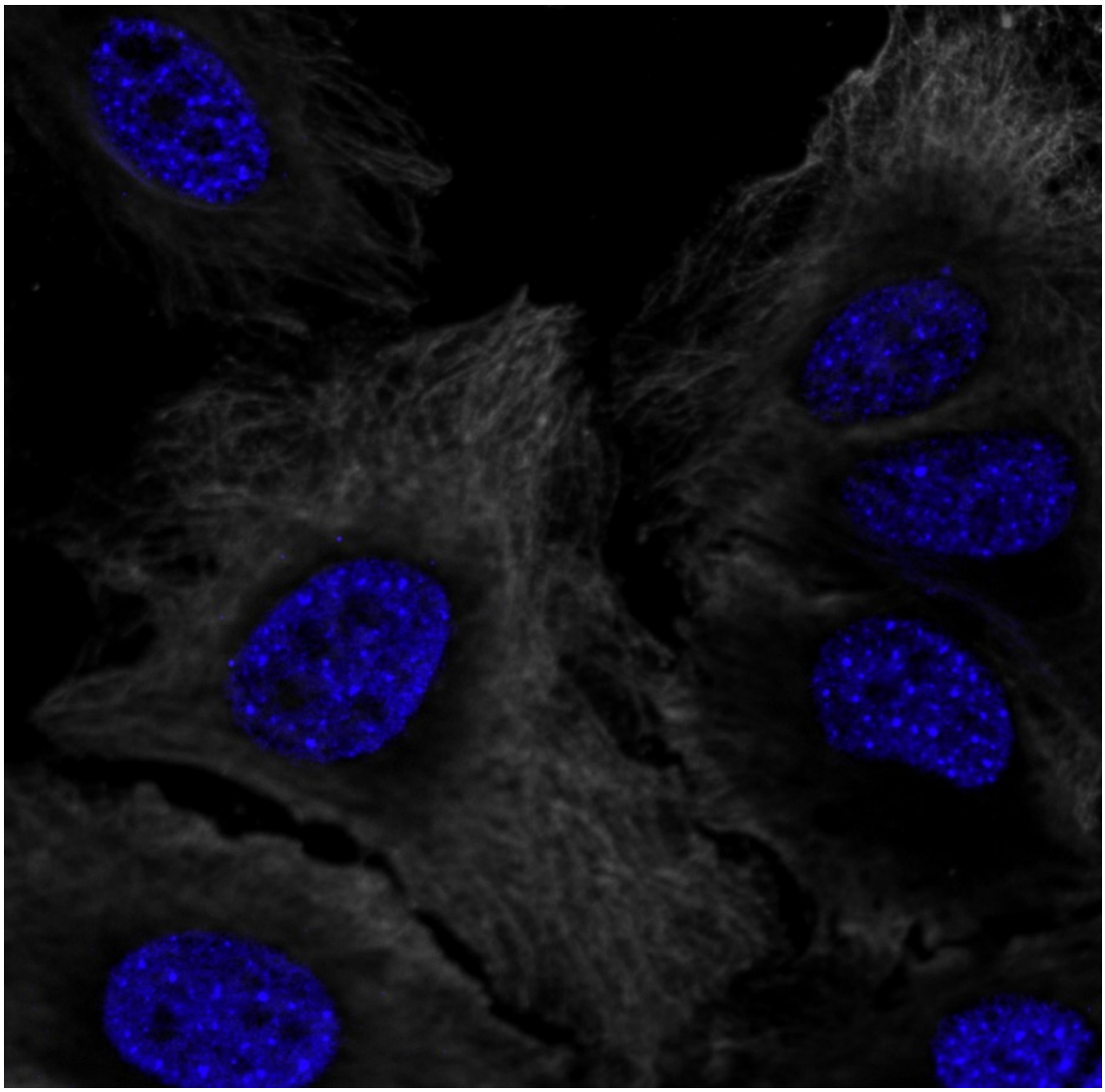
None

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000003
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	21
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	06D38CBFB3EBB5CD6CBB70CAFB6C722639F0BCDA444EA3C44D14027431C7FB68
Method PDF SHA-256	5E3AA6BB6CE3D00AC1C335CEE2D75A87A5D8DACA8426220B31838F3B9A100FC2

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
METADATA VALUES	32

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
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	875 X 864
Image Size (Scaled)	125.00µm X 123.43µm
Bit Depth	14 Bit
Image Center Position	X: 90.23mm, Y: 50.31mm
Stage Position	X: 90.23mm, Y: 50.31mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF405-49027 | AF532-49014
Beam Splitter	412 | 550
Filter Excitation Wavelength	372 - 408 | 515 - 545
Filter Emission Wavelength	417 - 445 | 555 - 595
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	700ms | 150ms
Excitation Wavelength	401 | 553
Emission Wavelength	422 | 568
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	0.82µm | 1.10µm
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 Primary Antibody

Identifyn® Rabbit anti-Human H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000037

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, Identifyn® Rabbit anti-Human 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, and Identifyn® 405 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® 555 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 cat#36961.

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) - Widefield SIM optical sectioning was used to locate a single plane where the nuclear compartment/405 was in optical focus, leaving the 555 (tubulin) in it's non-optical plane, hence out of focus.

Channels = 2
Scaling (Per Pixel) = 0.143μm X 0.143μm
Image Size (Pixels) = 875 X 864
Image Size (Scaled) = 125.00μm X 123.43μm
Bit Depth = 14 Bit
Image Center Position = X: 90.23mm, Y: 50.31mm

Stage Position = X: 90.23mm, Y: 50.31mm
ROI Center Offset = X: 1.14, Y: 0.00µm

405-

Reflector = AF405-49027
Beam Splitter = 412
Filter Excitation Wavelength = 372 - 408
Filter Emission Wavelength = 417 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 700ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.82µm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10%
Exposure Time = 150ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10µm
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.

Image Corrections

Identifyn® SRM Alexa Fluor™ 555 was pseudo-colored in Zen Software from orange, the default color, to dark grey to provide visual contrast between the 405/555 channels

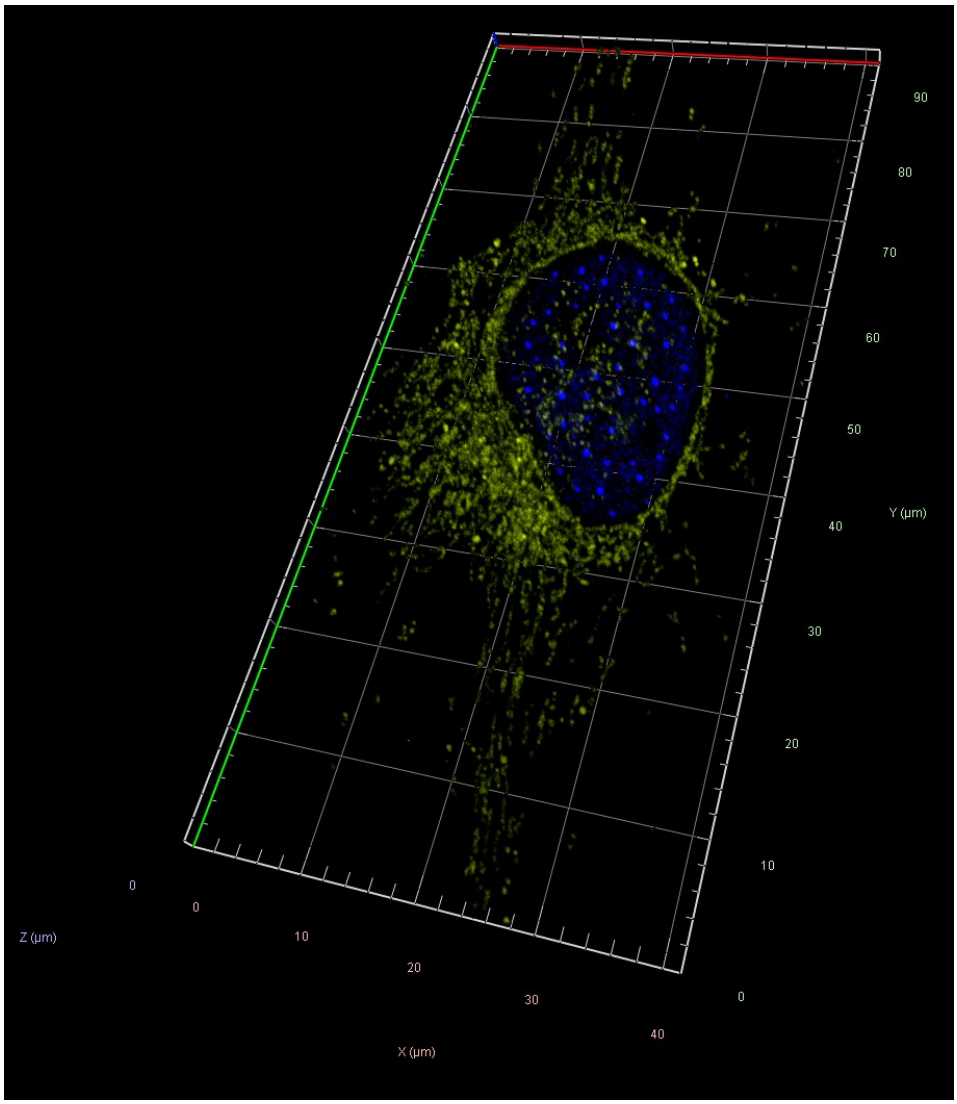
---

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000003
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	32
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	172A807CFED2CB283E303160F63446FF1EB24C48EFF4BD235788F92A6DD8CABE
Method PDF SHA-256	A0361106FB498A81F3D74D83E1958465B5839D13E7F4C4F7DDE950E342E980A6

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 750
METADATA VALUES	52

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	27 Slices (1.82µm)
Channels	2
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.070µm
Image Size (Pixels)	586 X 1280
Image Size (Scaled)	41.37µm X 90.36µm
Bit Depth	16 Bit
Image Center Position	X: 77.77mm, Y: 53.53mm
Stage Position	X: 77.77mm, Y: 53.53mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Pinhole	1.00 AU / 37µm | 1.00AU / 46µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	405nm @0.5% | 488nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	0.80 | 1.3
Rotation	0° | 65.21°
Sampling	1.00
Pixel Time	0.59µs | 0.70µs
Frame Time	17.76s | 4.02s
LSM Scan Speed	7 | 8
Scan Direction	Bidirectional
Line Step	1
Averaging	8 | 4
Excitation Wavelength	401 | 528
Emission Wavelength	422 | 553
Effective NA	1.4
Detection Wavelength	410-470 | 510 - 580
Imaging Device	GaAsp-PMT1 | Airyscan
Detector Type	GaAsp-PMT
Detector Gain	650 V | 750 V
Detector Offset	0

FIELD	SOURCE-DOCUMENTED VALUE
Detector Digital Gain	1.0
Reflector	None
Contrast Method	Fluorescence
3D Maximum Projection	Precise
Appearance	Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness130%, Enabled Tone Mapping
Projection	View Angle 54°, Scale Z 93%, 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 H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000026

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, Identifyn® Rabbit anti-Human 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, and Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02), which 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 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 = 27 Slices (1.82µm)

Channels = 2
Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.070µm
Image Size (Pixels) = 586 X 1280
Image Size (Scaled) = 41.37µm X 90.36µm
Bit Depth = 16 Bit
Image Center Position = X: 77.77mm, Y: 53.53mm
Stage Position = X: 77.77mm, Y: 53.53mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.80
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59µs
Frame Time = 17.76s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsp-PMT1
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 46µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = 65.21°
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 = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 510 - 580
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background = Black
Light = Brightness130%, Enabled Tone Mapping
Projection = View Angle 54°, Scale Z 93%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

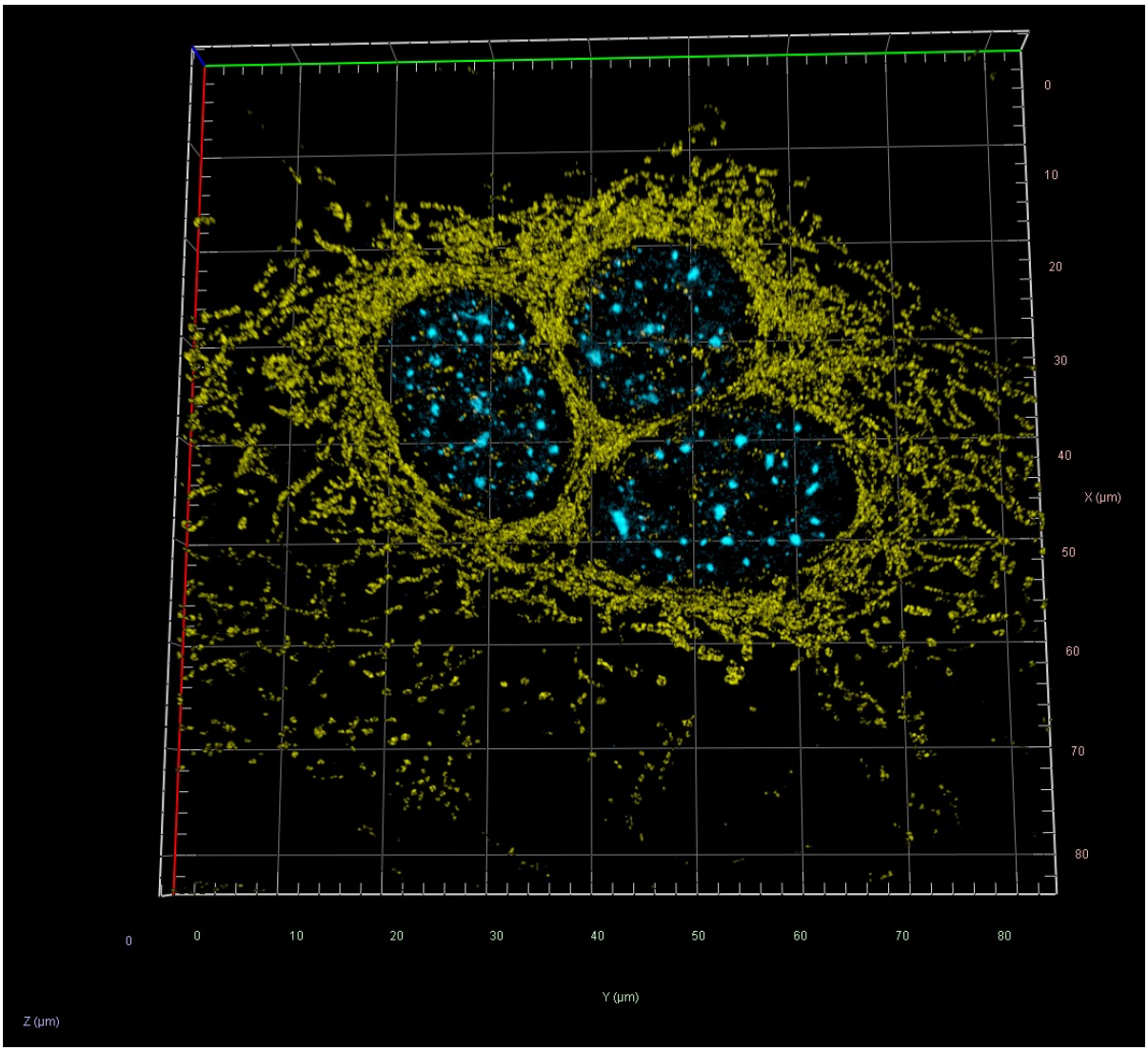
SOURCE PROVENANCE

Catalog	SA-000003
Stage	Confocal

SOURCE PROVENANCE

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	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DBED8428AFFF7E8E6EA5F49128F809E61F6326CED252A912DE2C38BAD582C057
Method PDF SHA-256	7BBF0B67987D24317FCB6570C9C06BBB17BB104A41D53906DBA1262A8191462A

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 750
METADATA VALUES	47

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 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	33 Slices (2.24µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.07000 µm
Image Size (Pixels)	2371 X 2371
Image Size (Scaled)	83.66µm X 83.66µm
Bit Depth	16 Bit
Image Center Position	X: 78.33mm, Y: 52.30mm
Stage Position	X: 78.33mm, Y: 52.30mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Pinhole	5.00 AU / 179µm | 5.00AU / 228µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	405nm @1.0% | 488nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.2
Rotation	0°
Sampling	2.00
Pixel Time	0.88µs
Frame Time	11.85s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	401 | 528
Emission Wavelength	422 | 553
Effective NA	1.4
Detection Wavelength	400-451 | 510 - 580
Imaging Device	Airyscan
Detector Type	GaAsp-PMT
Detector Gain	650 V | 750 V
Detector Offset	0

FIELD	SOURCE-DOCUMENTED VALUE
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.7 (3D, Auto) | Super Resolution 8.9 (3D, Auto)
Reflector	None
Contrast Method	Fluorescence
3D Maximum Projection	Precise
Appearance	Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness110%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 116%, 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 H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000026

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, Identifyn® Rabbit anti-Human 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, and Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02), which 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 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 = 33 Slices (2.24µm)

Channels = 2
Scaling (Per Pixel) =35.29nm X 35.29nm X 70.00nm
Image Size (Pixels) = 2371 X 2371
Image Size (Scaled) = 83.66µm X 83.66µm
Bit Depth = 16 Bit
Image Center Position = X: 78.33mm, Y: 52.30mm
Stage Position = X: 78.33mm, Y: 52.30mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 179µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.88µs
Frame Time = 11.85s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 400-451
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.7 (3D, Auto)

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 228µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.88µs
Frame Time = 11.85s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 510 - 580
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.9 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background = Black
Light = Brightness110%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 116%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from blue, the default color, to turquoise to provide visual contrast between the 405/532 channels

---

Image by P. Raut

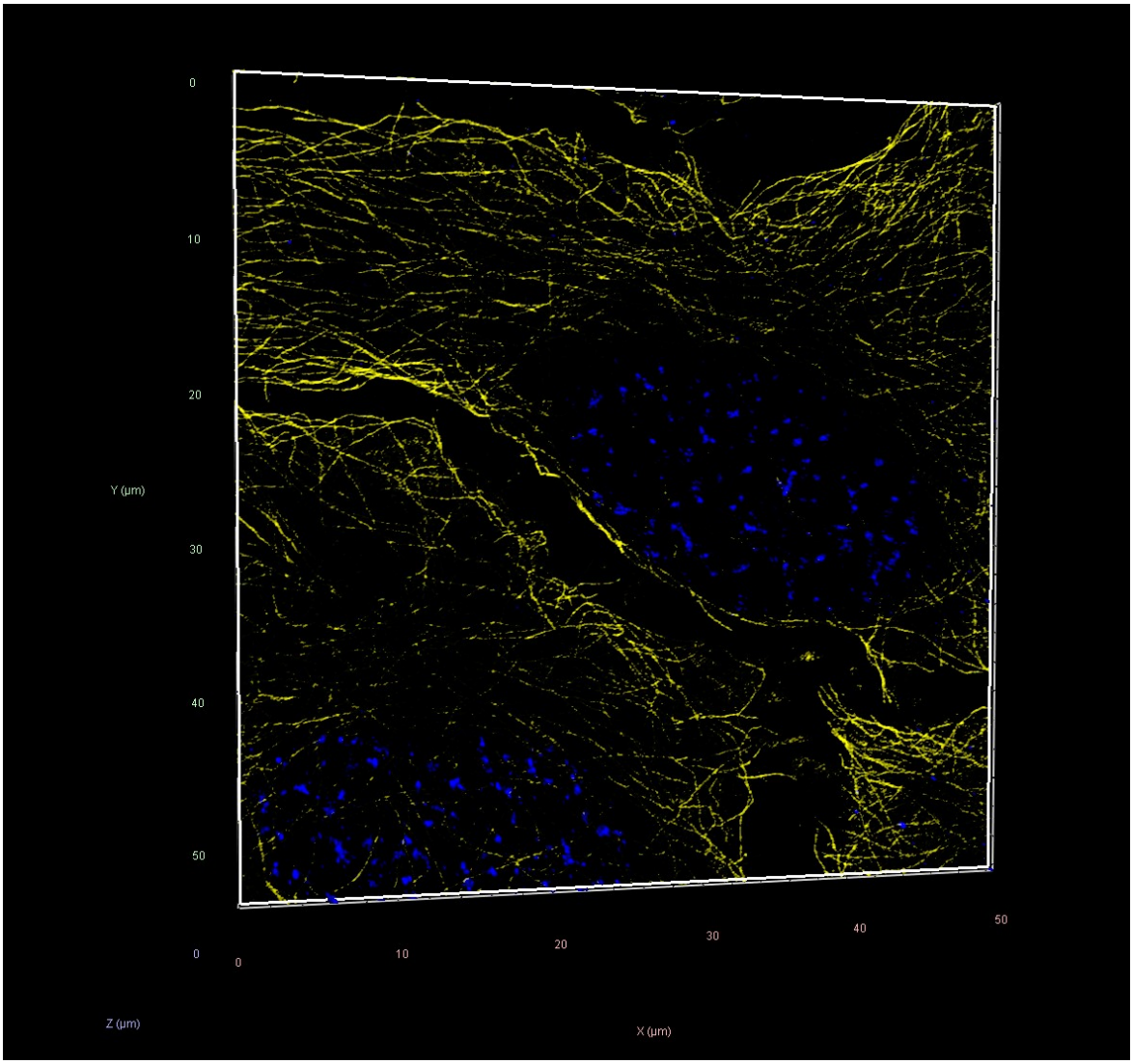
SOURCE PROVENANCE

Catalog	SA-000003

SOURCE PROVENANCE

Stage	Airyscan
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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CCC187C7AB5176630C927E0C0CE32492361CED88F25DDEEFF36E861F26F99305
Method PDF SHA-256	4EE07B04DD1F9E496B2B26A52BF13AD091348DEF477FE4CB5F32B6BC0E997BDF

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 555
METADATA VALUES	55

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)	2391 X 2483
Image Size (Scaled)	50.48µm X 52.42µm
Bit Depth	16 Bit
Image Center Position	X: 55.66mm, Y: 52.42mm
Stage Position	X: 55.66mm, Y: 52.42mm
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	405 | 561
Channel Name	T2-Axiocam 820#2-SR | T1-Axiocam 820-SR
Excitation Wavelength	405 | 561
Emission Wavelength	445 | 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 | 120ms
Depth of Focus	0.69µm | 0.92µm
Binning Mode	2,2
Laser Wavelength	405nm @3.0% | 561nm @1.0%
Frame Time	676.00ms | 1.33s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.4348 (405), 11.1267 (532)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Maximum Projection	Precise
Appearance	Threshold 1% (405), Threshold 1% (555), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 130%, 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™ 405 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 white Outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.
Position of Clip	-54%
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

Identifyn® Rabbit anti-Human H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000037

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, Identifyn® Rabbit anti-Human 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, and Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 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 (2.28µm)

Channels = 2
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 2391 X 2483
Image Size (Scaled) = 50.48µm X 52.42µm
Bit Depth = 16 Bit
Image Center Position = X: 55.66mm, Y: 52.42mm
Stage Position = X: 55.66mm, Y: 52.42mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

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 = T2-Axiocam 820#2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @3.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 32.0µm grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820-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 = 120ms
Depth of Focus = 0.92µm
Binning Mode = 2,2
Laser Wavelength = 561nm @1.0%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm 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 = 9.4348 (405), 11.1267 (532)
Fitting = Fast Fit
Histogram = Scale to Min/Max
Baseline = Cut

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% (405), Threshold 1% (555), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 130%, 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™ 405 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 white Outline of the clipped perimeter was shown, the Clip Back was Selected, and the Clip Front was NOT selected.

Position of Clip = -54%
Clip Y = -0°
Clip Z =0°

Image Corrections

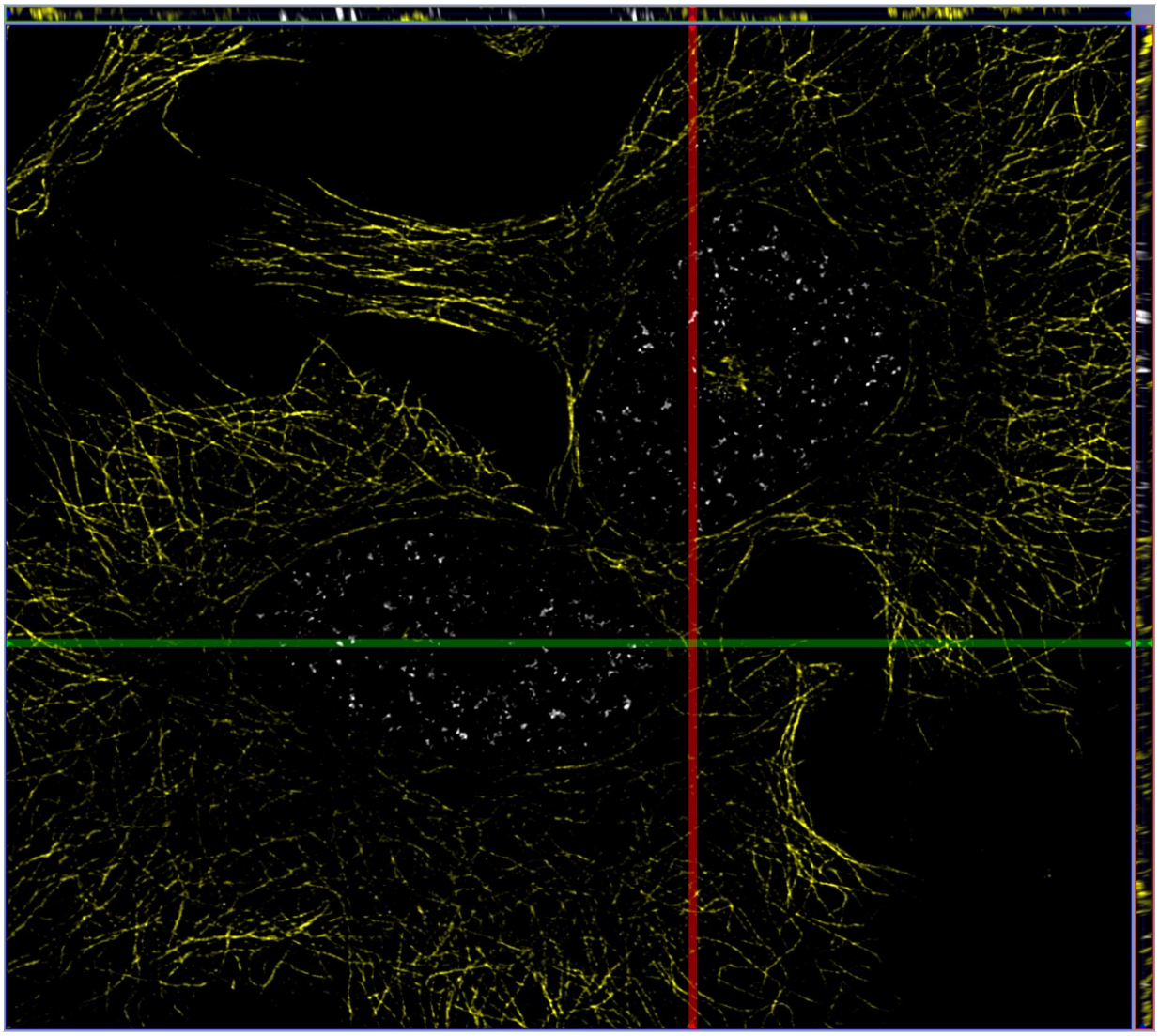
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000003
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	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3F2974EB35DA93117744E0A4E54D8C385AABF3955B84EB68CED80EB4334A6600
Method PDF SHA-256	EB6986FDBB2AE0DBE7DC05126801F627426664C0F19E3A89E1BE6B9FFB398249

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	53

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	36 Slices (1.05µm)
Channels	2 | 1
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	3363 X 3001
Image Size (Scaled)	71.00µm X 63.36µm
Bit Depth	16 Bit
Image Center Position	X: 52.55mm, Y: 37.54mm
Stage Position	X: 52.55mm, Y: 37.54mm
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	405 | 561
Channel Name	T2-Axiocam 820#2-SR | T1-Axiocam 820-SR
Excitation Wavelength	405 | 561
Emission Wavelength	445 | 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 | 120ms
Depth of Focus	0.69µm | 0.92µm
Binning Mode	2,2
Laser Wavelength	405nm @3.0% | 561nm @1.0%
Frame Time	676.00ms | 1.33s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.8786 (405), 10.4966 (555)
Order Combination	Wiener Filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
Cut Lines	X 2054, Y 1848, Z 21
Line Width	X 25, Y 25, Z 0
Cut Line Opacity	27

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 H2AX Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000037

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, Identifyn® Rabbit anti-Human 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, and Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 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 = 36 Slices (1.05µm)

Channels = 2
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 3363 X 3001
Image Size (Scaled) = 71.00µm X 63.36µm
Bit Depth = 16 Bit
Image Center Position = X: 52.55mm, Y: 37.54mm
Stage Position = X: 52.55mm, Y: 37.54mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

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 = T2-Axiocam 820#2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @3.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 32.0µm grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820-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 = 120ms
Depth of Focus = 0.92µm
Binning Mode = 2,2
Laser Wavelength = 561nm @1.0%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm 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 = 8.8786 (405), 10.4966 (555)
Order Combination = Wiener Filter
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

Orthogonal View - Uses Image 21 of the Z-Stack 36 Images.

Cut Lines = X 2054, Y 1848, Z 21
Line Width = X 25, Y 25, Z 0
Cut Line Opacity = 27
Maximum Intensity Opacity (MIP) and Measure 3D Distance NOT selected

Image Corrections -

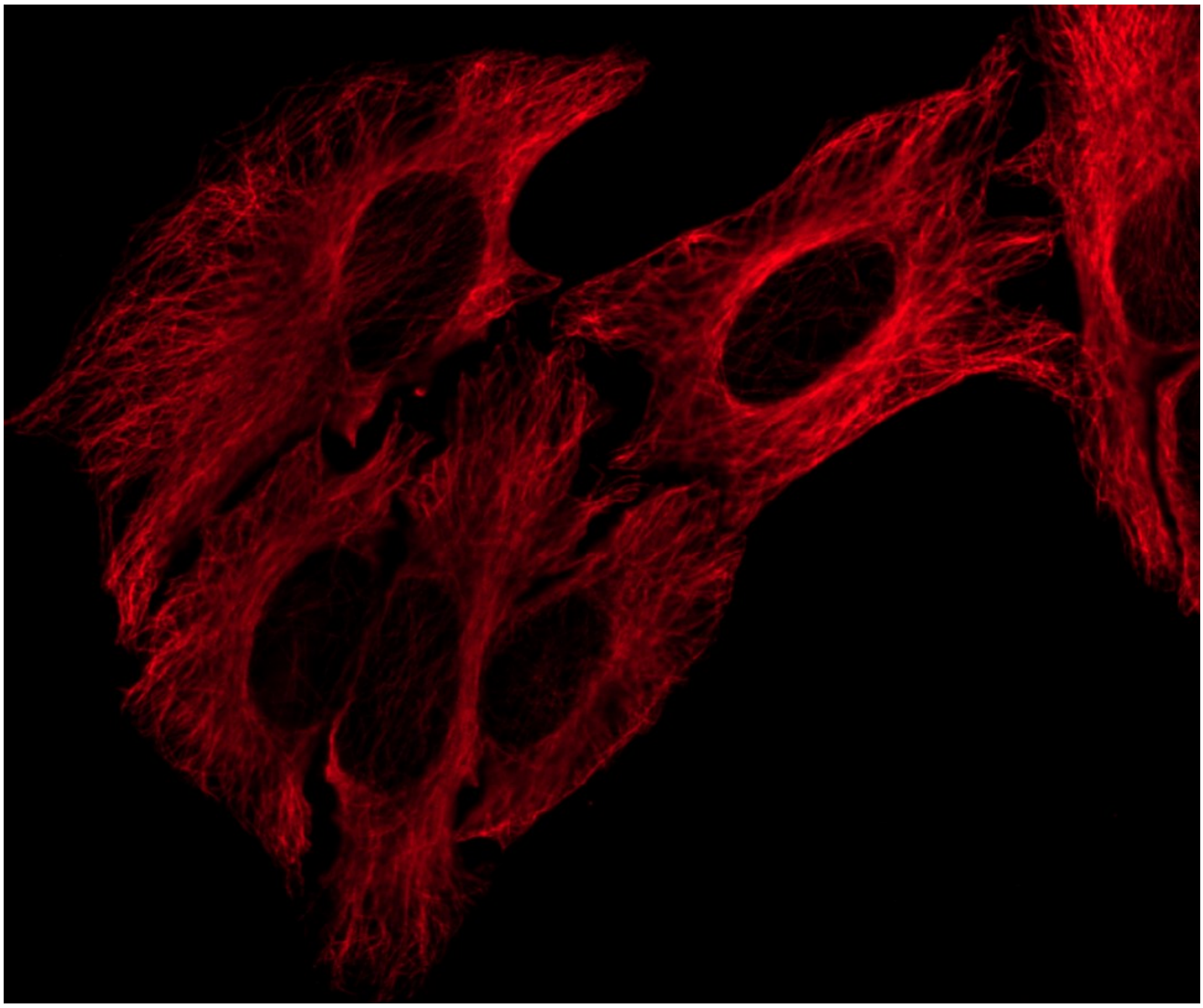
Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from blue, the default color, to white to provide visual contrast between the 405/555 channels.

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000003
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	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B0FDCAB9F6D1F2A4FAEC8587D5CC252D08EB95B049918FDD13666E735C9D9170
Method PDF SHA-256	D70F0B05FF3CA076B46B5ABA789D7A480658620BCA313CF39F1AD388E9C6D988

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	29

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)	1186 X 977
Image Size (Scaled)	169.43µm X 139.57µm
Bit Depth	14 Bit
Image Center Position	X: 98.87mm, Y: 54.24mm
ROI Center Offset	X: 98.87µm, Y: 54.24µm
Reflector	AF405-49027 | 50 Cy5
Beam Splitter	412 | 660
Filter Excitation Wavelength	372 - 408 | 625 - 655
Filter Emission Wavelength	417 - 445 | 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25%
Exposure Time	650ms | 200ms
Excitation Wavelength	401 | 653
Emission Wavelength	422 | 668
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	0.82µm | 1.30µm
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® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Human, IgG (H + L)
Cat ID# - SA 000003

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

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® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

To demonstrate the presence of cells in the sample, minus DAPI (which would overlap the 405), a 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 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 cat#36961.

Note that the lamp intensity and exposure times were set to the 647 control samples.

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) = 1186 X 977
Image Size (Scaled) = 169.43µm X 139.57µm
Bit Depth = 14 Bit
Image Center Position = X: 98.87mm, Y: 54.24mm
ROI Center Offset = X: 98.87µm, Y: 54.24µm

405-

Reflector = AF405-49027
Beam Splitter = 412
Filter Excitation Wavelength = 372 - 408
Filter Emission Wavelength = 417 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 650ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.82µm
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 = 200ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

Post Processing -

None

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 Axio Imager.M1 Widefield. 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 data suggests the secondary antibody is highly specific and free of unconjugated (free) dye.

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000003
Stage	Control
Instrument	ZEISS Axio Imager.M1; 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	29
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D79C0974CEEE1863A1BF0F7BD4EBFC7C5E38168FCD7D55D258BC1C2C35A5E8B8
Method PDF SHA-256	75C95F268C1411035FAA69EC4A813FC2210E940B239D1CB6BBB93FA252BD798B