

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

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

4

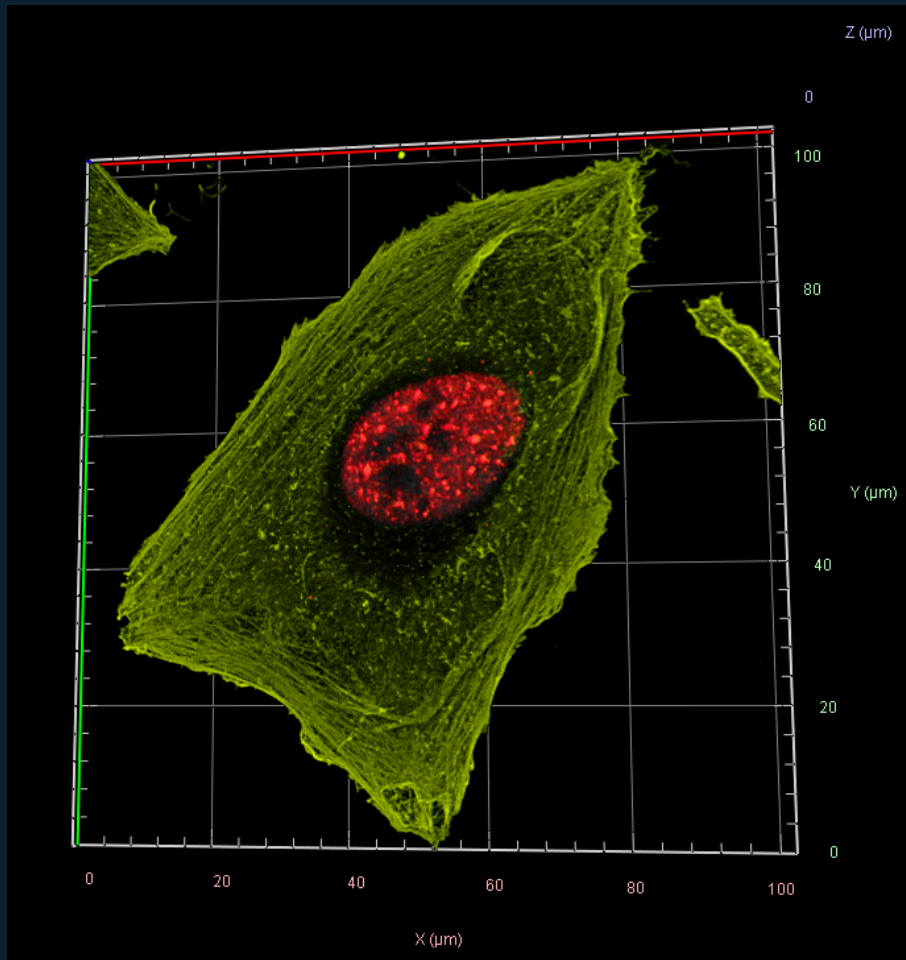
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

SA-000080 | 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
- 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™ 750 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 -> Control

BENNETT ASSESSMENT

The preserved SA-000080 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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-000080 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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 4 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	750	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 750	2; AF750-49007; 49 DAPI; 660 - 745; 335 - 383; 765 - 855; 420 - 470; 752
Widefield SIM — Apotome	405/DAPI; 488; 750	2; AF750-490007; 39 HE eGFP; 670 - 745; 450 - 490; 765 - 855; 500 - 550; 752
Confocal	405/DAPI; 488; 532; 750	3; None; 730nm @2.0%; 488nm @0.2%; 405nm @0.2%; 752; 528; 353
Control	405/DAPI; 750	2; AF750-49007; 49 DAPI; 670 - 745; 335-383; 765 - 855; 420-470; 752

MODALITY ASSESSMENT / PART 1 OF 5

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: 10.

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 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

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 5

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 sCMOS, 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): 867 X 767; Image Size (Pixels): 867 X 767; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 705. Timing: Exposure Time: 850ms; 20ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @75%; X-Cite Xylis Lamp Intensity @12%. Optical/scan: Effective NA: 1.4; .5. Structured source metadata values: 31.

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 5

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: 10 Slices (1.80µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 1236 X 825; Image Size (Pixels): 1236 X 825; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 750ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @80%; X-Cite Xylis Lamp Intensity @30%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 45%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 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

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 5

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 46 Slices (2.25µm) - Subset of Z Stack109 Slices (5.40µm); Channels: 3; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.050µm; Image size (Pixels): 1737 X 1737; Image Size (Pixels): 1737 X 1737; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Channel 2; GaAsP-Pmt3; Detector Type: GaAsP-Pmt; GaAsP-Pmt. Timing: Pixel Time: 0.22µs; Frame Time: 3.25s. Illumination: Laser Wavelength: 730nm @2.0%; 488nm @0.2%. Optical/scan: Pinhole: 1.00AU / 79µm; 1.00AU / 52µm; Scan Zoom: 1.3; LSM Scan Speed: 11; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 6.9 (3D, Auto). 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 980, 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 5

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): 996 X 1013; Image Size (Pixels): 996 X 1013; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono; AxioCam 807. Timing: Exposure Time: 800ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @80%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 31.

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; 750.

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-000080 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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

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

No derivative source correction is recorded for this dossier.

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

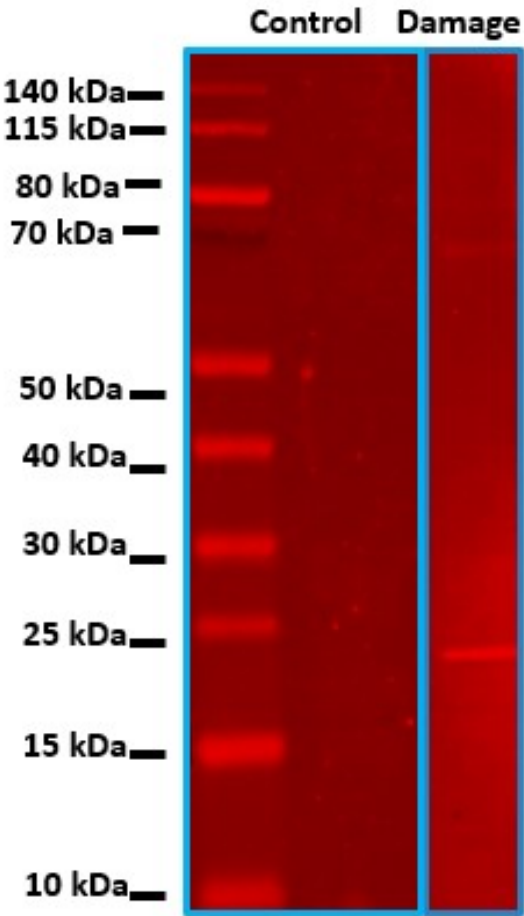
This dossier preserves the complete available evidence progression for SA-000080. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 980	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	750
METADATA VALUES	10

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	730nm
Emission Filter	809BP81
Detector	Avalanche Photodiode
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)

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™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000080

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 80 oC 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 Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG for 1 hour, followed by 5X PBS washes.

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Goat Anti-Human, IgG (H + L) was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 min 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 = 730nm

Emission Filter = 809BP81

Detector = Avalanche Photodiode

Intensity = 3

Pixel size = 100 μm

Scan Speed = High

Quality = High

Focus = Membrane (0 μm in Z-axis)

Post Processing

None

Image by K. Small

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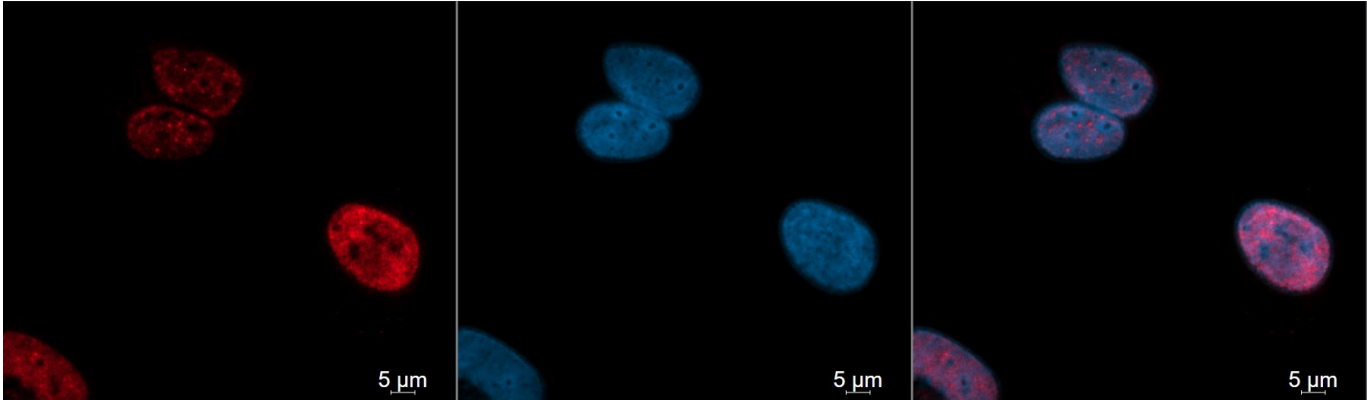
SOURCE PROVENANCE

Catalog	SA-000080
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)

SOURCE PROVENANCE

Structured source metadata values	10
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9A511DFF649C9255270D375159642C0F25451050965D6945527EA24FC7D4D178
Method PDF SHA-256	0EAC3E5AA7345A9A4391E1ED9B40B3F072E5F384E5A4F2EEC23744DCF7A19643

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	31

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 sCMOS, 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)	867 X 767
Image Size (Scaled)	94.96µm X 84.00µm
Bit Depth	16 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	AF750-49007 49 DAPI
Beam Splitter	760 395
Filter Excitation Wavelength	660 - 745 335 - 383
Filter Emission Wavelength	765 - 855 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @75% X-Cite Xylis Lamp Intensity @12%
Exposure Time	850ms 20ms
Excitation Wavelength	752 353
Emission Wavelength	779 465
Effective NA	1.4 .5
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.21µm 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

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

Cat ID# - PA-RR-000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000080

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

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705 sCMOS, 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) = 867 X 767

Image Size (Scaled) = 94.96 μ m X 84.00 μ m

Bit Depth = 16 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

750 -

Reflector = AF750-49007
 Beam Splitter = 760
 Filter Excitation Wavelength = 660 - 745
 Filter Emission Wavelength = 765 - 855
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @75%
 Exposure Time = 850ms
 Excitation Wavelength = 752
 Emission Wavelength = 779
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 1.21µm
 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 @12%
 Exposure Time = 20ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = .5
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 2,2

Image Correction

Note - The limitations of using an LED light source and a Widefield microscope outfitted with a custom filter on small foci targets lead to limited light collection and pixelation for this target. We present this image to demonstrate the specificity of both the Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG and Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Goat Anti-Human, IgG (H + L), however, imaging 750 on small nuclear targets using this set up is not optimal nor recommended.

Image by J.K. Bennett

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SOURCE PROVENANCE

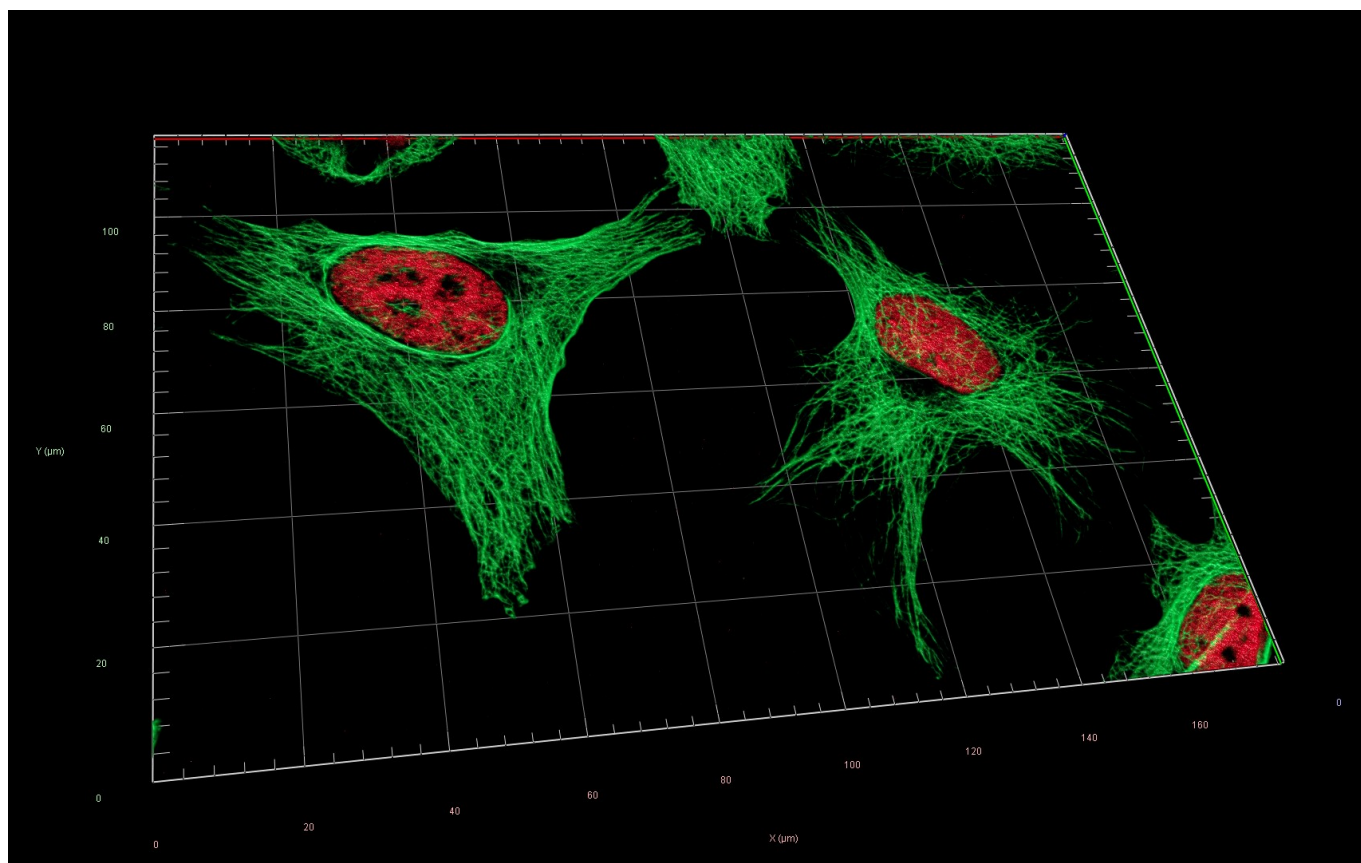
Catalog

SA-000080

SOURCE PROVENANCE

Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705 sCMOS, and X-Cite Xylys Lamp was used with the following parameters for each dye:
Structured source metadata values	31
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5658777ECC8D056D75A16C58221D34003F2011D639C70B1F465FE7D2B48FFD8A
Method PDF SHA-256	9E9E2DE5A5E99A4695FEEB87671791D9AE6B9D08CDA302191B2B06C90E306BE8

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 750
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	10 Slices (1.80µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	1236 X 825
Image Size (Scaled)	176.57µm X 117.86µm
Bit Depth	14 Bit
Image Center Position	X: 78.45mm, Y: 48.32mm
Stage Position	X: 78.45mm, Y: 48.32mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF750-490007 39 HE eGFP
Beam Splitter	760 495
Filter Excitation Wavelength	670 - 745 450 - 490
Filter Emission Wavelength	765 - 855 500 - 550
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @80% X-Cite Xylis Lamp Intensity @30%
Exposure Time	750ms 100ms
Excitation Wavelength	752 493
Emission Wavelength	779 517
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.51µm 1.00µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% (488), Threshold 13% (750), Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 89%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 45%, 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™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000080

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

Cat ID# - SA 000015

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® 750 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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

Microscope

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

Z Stack - (3D)

Z-Stack = 10 Slices (1.80µm)

Channels = 2

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

Image Size (Pixels) = 1236 X 825

Image Size (Scaled) = 176.57µm X 117.86µm

Bit Depth = 14 Bit

Image Center Position = X: 78.45mm, Y: 48.32mm

Stage Position = X: 78.45mm, Y: 48.32mm

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

750 -

Reflector = AF750-490007

Beam Splitter = 760

Filter Excitation Wavelength = 670 - 745

Filter Emission Wavelength = 765 - 855

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @80%

Exposure Time = 750ms

Excitation Wavelength = 752

Emission Wavelength = 779

Effective NA = 1.25

Imaging Device = AxioCam 807

Camera Adapter = 1X

Depth of Focus = 1.51µm

Binning Mode = 2,2

488 -

Reflector = 39 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 - 550

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @30%

Exposure Time = 100ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.25

Imaging Device = AxioCam 807

Camera Adapter = 1X

Depth of Focus = 1.00µm

Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 13% (488), Threshold 13% (750), Ramp 50% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 89%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 45%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

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

Image Corrections

None

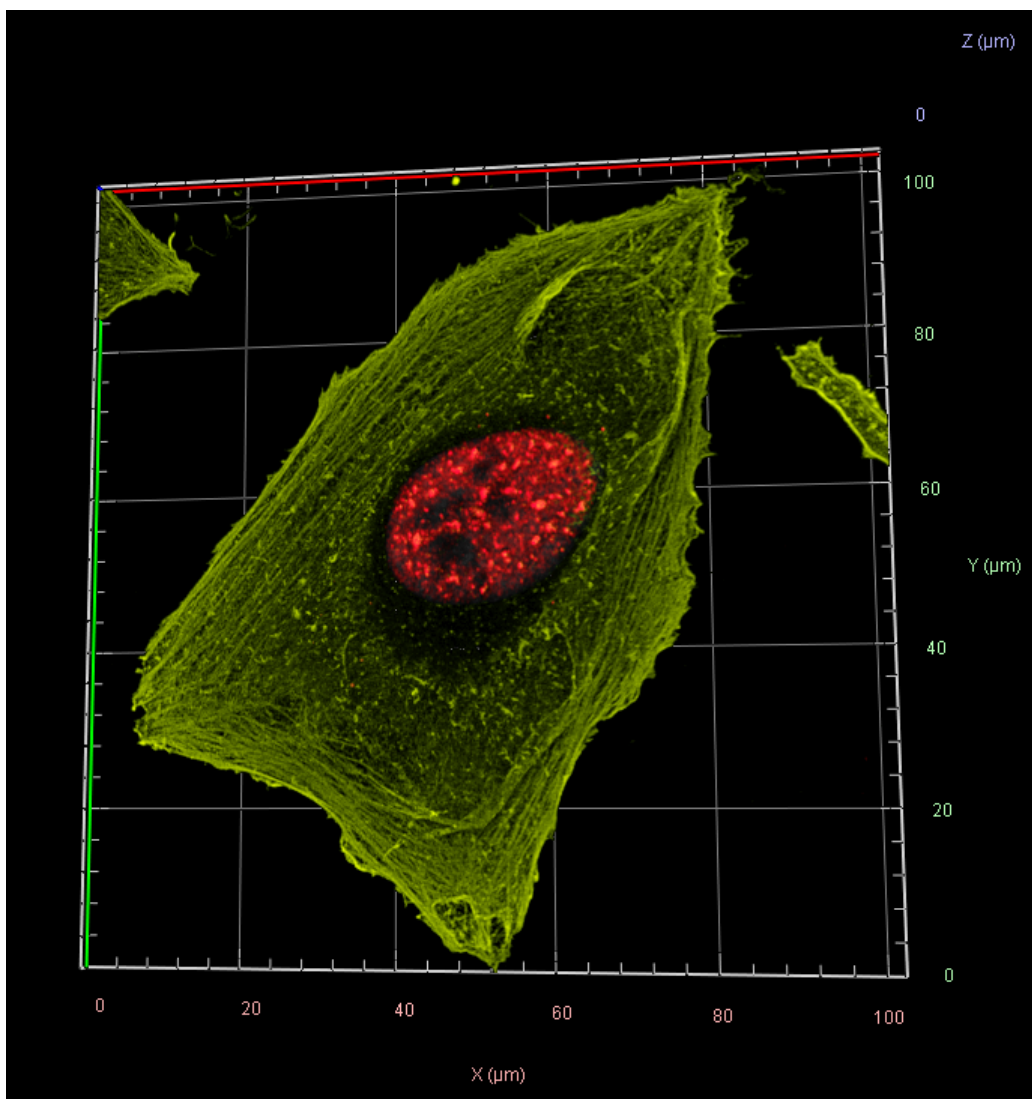
Image by E.F. Simon

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SOURCE PROVENANCE

Catalog	SA-000080
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	0A3BA8346D837B927E33EE41C7E7BC4ADA20D914246C47CBA83EC4D8A8639A4E
Method PDF SHA-256	FBFCED3B8421A459B501B67C9210C7D74194845C6C0F491C624669D743F89002

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	46 Slices (2.25µm) - Subset of Z Stack109 Slices (5.40µm)
Channels	3
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.050µm
Image Size (Pixels)	1737 X 1737
Image Size (Scaled)	102.19µm X 102.19µm
Bit Depth	16 Bit
Image Center Position	X: 70.60mm, Y: 52.94mm
Stage Position	X: 70.60mm, Y: 52.94mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 79µm 1.00AU / 52µm 1.00AU / 42µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 740 Mirror
Laser Wavelength	730nm @2.0% 488nm @0.2% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	0°
Sampling	1.20
Pixel Time	0.22µs
Frame Time	3.25s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	752 528 353
Emission Wavelength	779 553 465
Effective NA	1.4
Detection Wavelength	755 - 900 525 - 570 410 - 470
Imaging Device	NIR Channel 2 GaAsP-Pmt3 GaAsP-Pmt3
Detector Type	GaAsP-Pmt GaAsP-Pmt

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	700 V 550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) Filter: 6.9 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (750), Threshold 1% (532), Threshold 1% (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)

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™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000080

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

Cat ID# - SA 000024

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® 750 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 46 Slices (2.25µm) - Subset of Z Stack109 Slices (5.40µm)

Channels = 3

Scaling (Per Pixel) = 0.059µm X 0.059µm X 0.050µm

Image Size (Pixels) = 1737 X 1737

Image Size (Scaled) = 102.19µm X 102.19µm

Bit Depth = 16 Bit

Image Center Position = X: 70.60mm, Y: 52.94mm

Stage Position = X: 70.60mm, Y: 52.94mm

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

750-

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 79µm

LMS MBS = MBS 488/639 & 405/730

LMS SBS = SBS LP 740

Laser Wavelength = 730nm @2.0%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.3

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.22µs

Frame Time = 3.25s

LSM Scan Speed = 11

Scan Direction = Bidirectional

Line Step = 1

Averaging - 4

Excitation Wavelength = 752

Emission Wavelength = 779

Effective NA = 1.4

Detection Wavelength = 755 - 900

Imaging Device = NIR Channel 2

Detector Type = GaAsP-Pmt

Detector Gain = 700 V

Detector Offset = 0

Detector Digital Gain = 1.0

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

532-

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 52µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 488nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.22µs
 Frame Time = 3.25s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Detection Wavelength = 525 - 570
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-Pmt
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.2 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 42µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.22µs
 Frame Time = 3.25s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsp-Pmt3
 Detector Type = GaAsp-Pmt
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.9 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (750), Threshold 1% (532), Threshold 1% (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)

Image Corrections

None

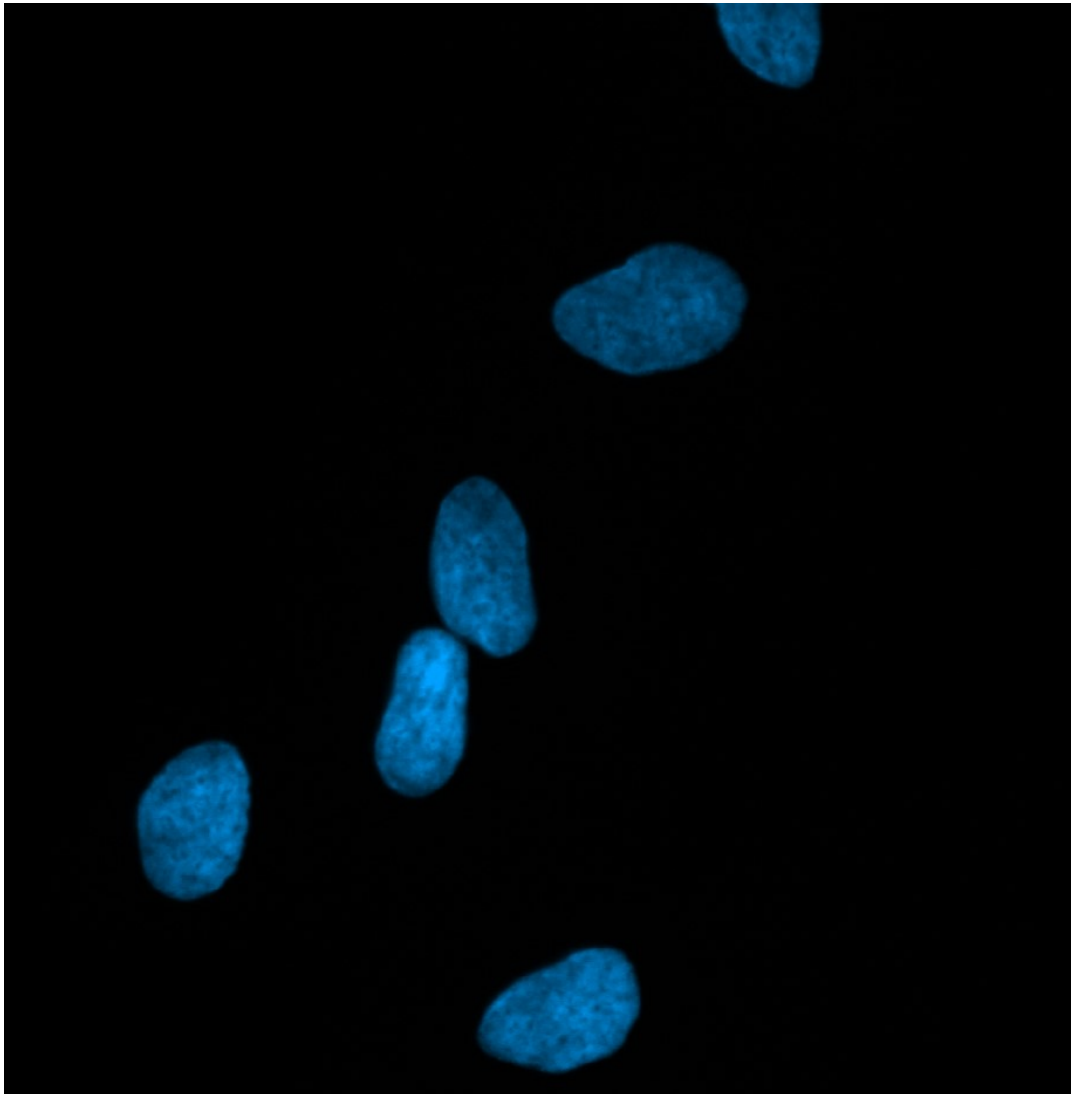
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000080
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E4E528F64DF629F690DE0D6E6D999D1859B46A34EEC17A4FCE1F50946CEB01B3
Method PDF SHA-256	539514FD88FE4D89D99D26F5F7C25F346D2D377F7ABEA557D9CE4492DAFD612D

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; 750
METADATA VALUES	31

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)	996 X 1013
Image Size (Scaled)	142.29µm X 144.71µm
Bit Depth	14 Bit
Image Center Position	X: 47.05mm, Y: 52.36mm
ROI Center Offset	X: 47.05µm, Y: 52.36µm
Reflector	AF750-49007 49 DAPI
Beam Splitter	760 395
Filter Excitation Wavelength	670 - 745 335-383
Filter Emission Wavelength	765 - 855 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @80% X-Cite Xylis Lamp Intensity @10%
Exposure Time	800ms 30ms
Excitation Wavelength	752 353
Emission Wavelength	779 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono Axiocam 807
Camera Adapter	1X
Depth of Focus	1.51µm 0.90µm
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™ 750 Affinity Purified Goat Anti-Human, IgG (H + L)

Cat ID# - SA 000080

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® 750 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) = 996 X 1013

Image Size (Scaled) = 142.29µm X 144.71µm

Bit Depth = 14 Bit

Image Center Position = X: 47.05mm, Y: 52.36mm

ROI Center Offset = X: 47.05µm, Y: 52.36µm

750 -

Reflector = AF750-49007

Beam Splitter = 760

Filter Excitation Wavelength = 670 - 745

Filter Emission Wavelength = 765 - 855

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @80%

Exposure Time = 800ms

Excitation Wavelength = 752

Emission Wavelength = 779

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.51µm

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
 Camera Adapter = 1X
 Depth of Focus = 0.90µm
 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

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SOURCE PROVENANCE

Catalog	SA-000080
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	31
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	88F735BBFB5077CDD0AD928A0D6AC0DE862F2C805C257A7AD50DFF79B49F4F8E
Method PDF SHA-256	3973266C7584EF7F5EC8F54B74ACE11194C43A39BB5420C5FF74713B2DF80197