BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

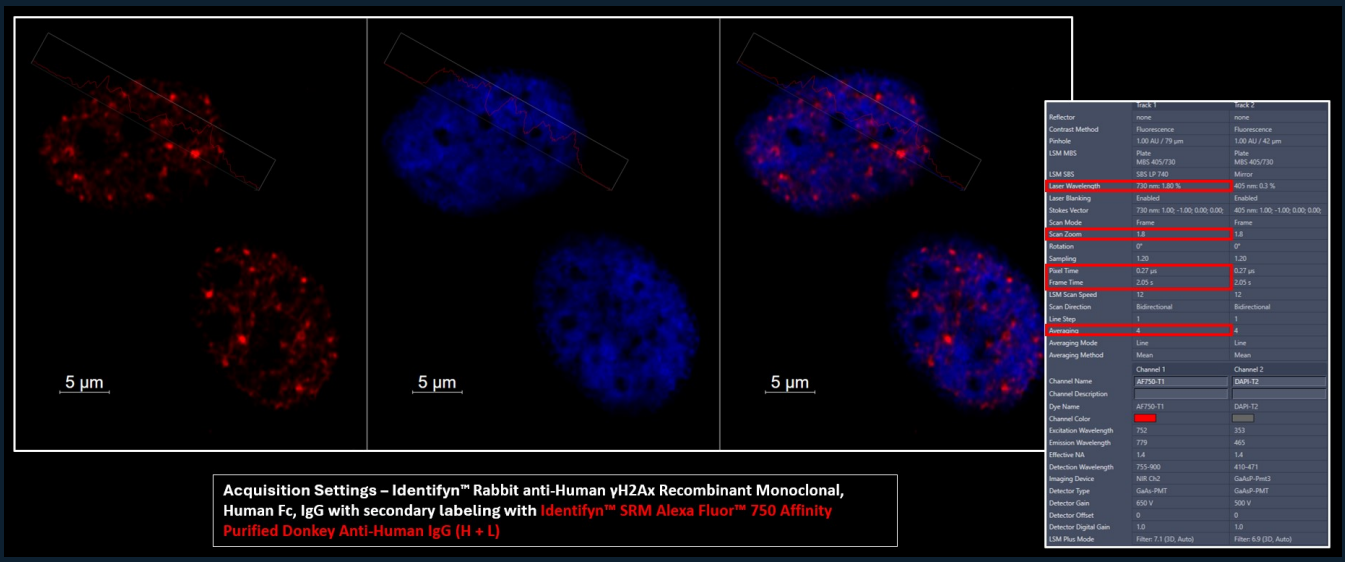
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

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

4	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

SA-000083 | 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 Donkey 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-000083 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-000083 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; 750	2; AF750-49007; 49 DAPI; 670 - 745; 335 - 383; 765 - 855; 420 - 470; 752
Confocal	405/DAPI; 750	2; None; 730nm @1.80%; 405nm @0.3%; 752; 353; 779; 465
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: 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 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): 543 X 810; Image Size (Pixels): 543 X 810; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 800ms; 25ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @80%; X-Cite Xylis Lamp Intensity @12%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 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: 15 Slices (2.80µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 749 X 791; Image Size (Pixels): 749 X 791; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 750ms; 150ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @70%; X-Cite Xylis Lamp Intensity @20%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 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: 82 Slices (4.10µm); Channels: 2; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.050µm; Image size (Pixels): 755 X 928; Image Size (Pixels): 755 X 928; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Channel 2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.27µs; Frame Time: 2.05s. Illumination: Laser Wavelength: 730nm @1.80%; 405nm @0.3%. Optical/scan: Pinhole: 1.00AU / 79µm; 1.00AU / 42µm; Scan Zoom: 1.8; LSM Scan Speed: 12; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.1(3D, Auto); Filter: 6.9 (3D, Auto). Structured source metadata values: 47.

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; 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): 1600 X 1104; Image Size (Pixels): 1600 X 1104; 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-000083 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

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

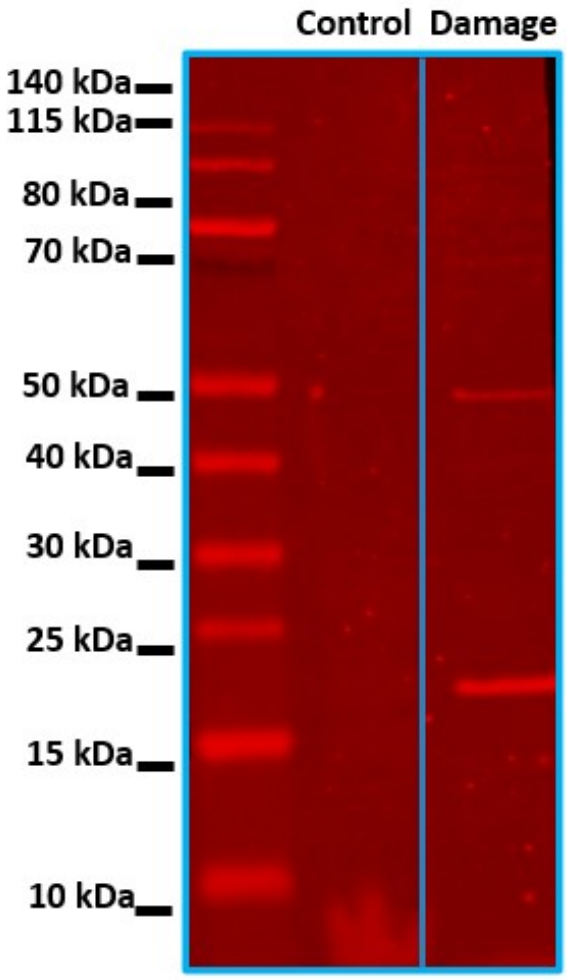
This dossier preserves the complete available evidence progression for SA-000083. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 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	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	730nm
Emission Filter	809BP81
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Identifyn® LLC

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Donkey Anti-Human, IgG (H + L)
Cat ID# - SA 000083

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™ 750 Affinity Purified Donkey Anti-Human, IgG (H + L) 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 = 730nm
Emission Filter = 809BP81
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

Copyright 2026 GMD12 LLC.

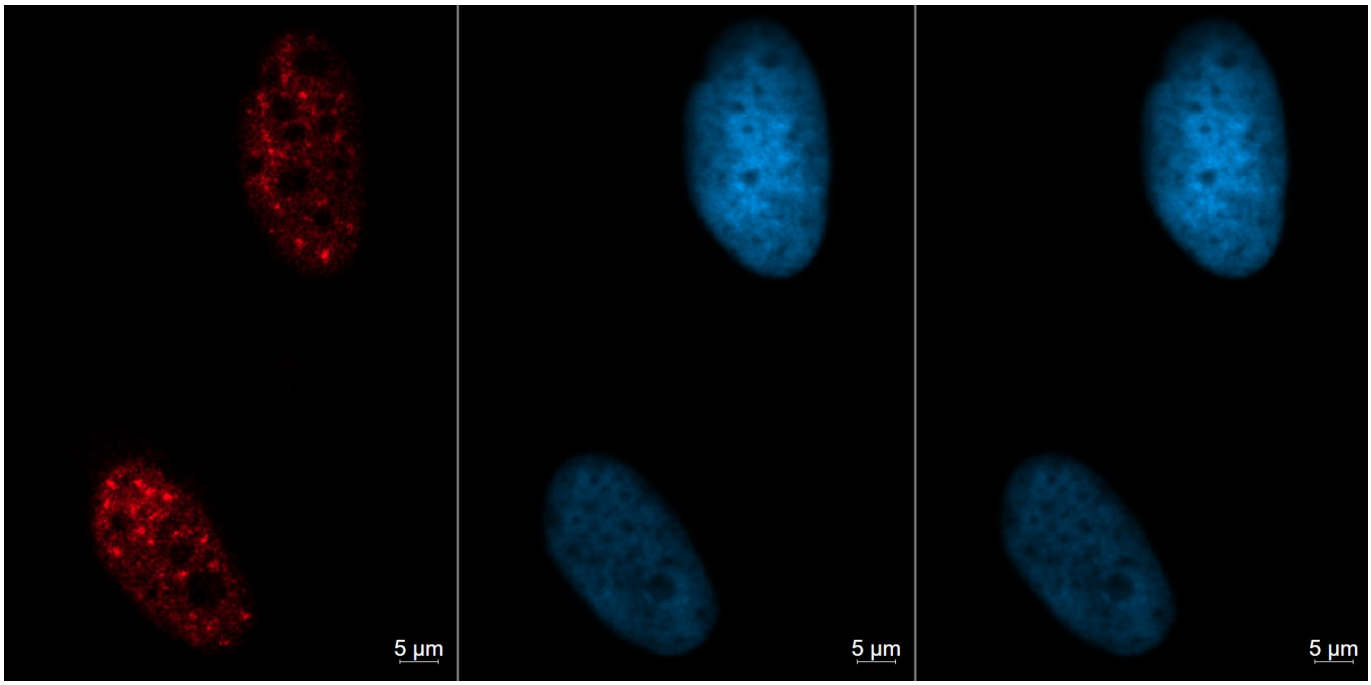
SOURCE PROVENANCE

Catalog	SA-000083

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	D8E23BC3AAC64CA4911A4D2E3F50FC04B4C9D4041D34C3A4B4BB51915A19BE5D
Method PDF SHA-256	5362A7DBA5FDF4A27F90EDD9E86313C0B010D5C2C9DAD6D22C1630EB839408C9

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	30

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 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)	543 X 810
Image Size (Scaled)	59.47µm X 88.71µm
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 @80% | X-Cite Xylis Lamp Intensity @12%
Exposure Time	800ms | 25ms
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.4
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 γH2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000083

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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) = 0.110μm X 0.110μm
Image size (Pixels) = 543 X 810
Image Size (Scaled) = 59.47μm X 88.71μm
Bit Depth = 16 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

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 @80%
Exposure Time = 800ms
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 = 25ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA =1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Post Processing

None

Image Correction

None

Image by J.K. Bennett

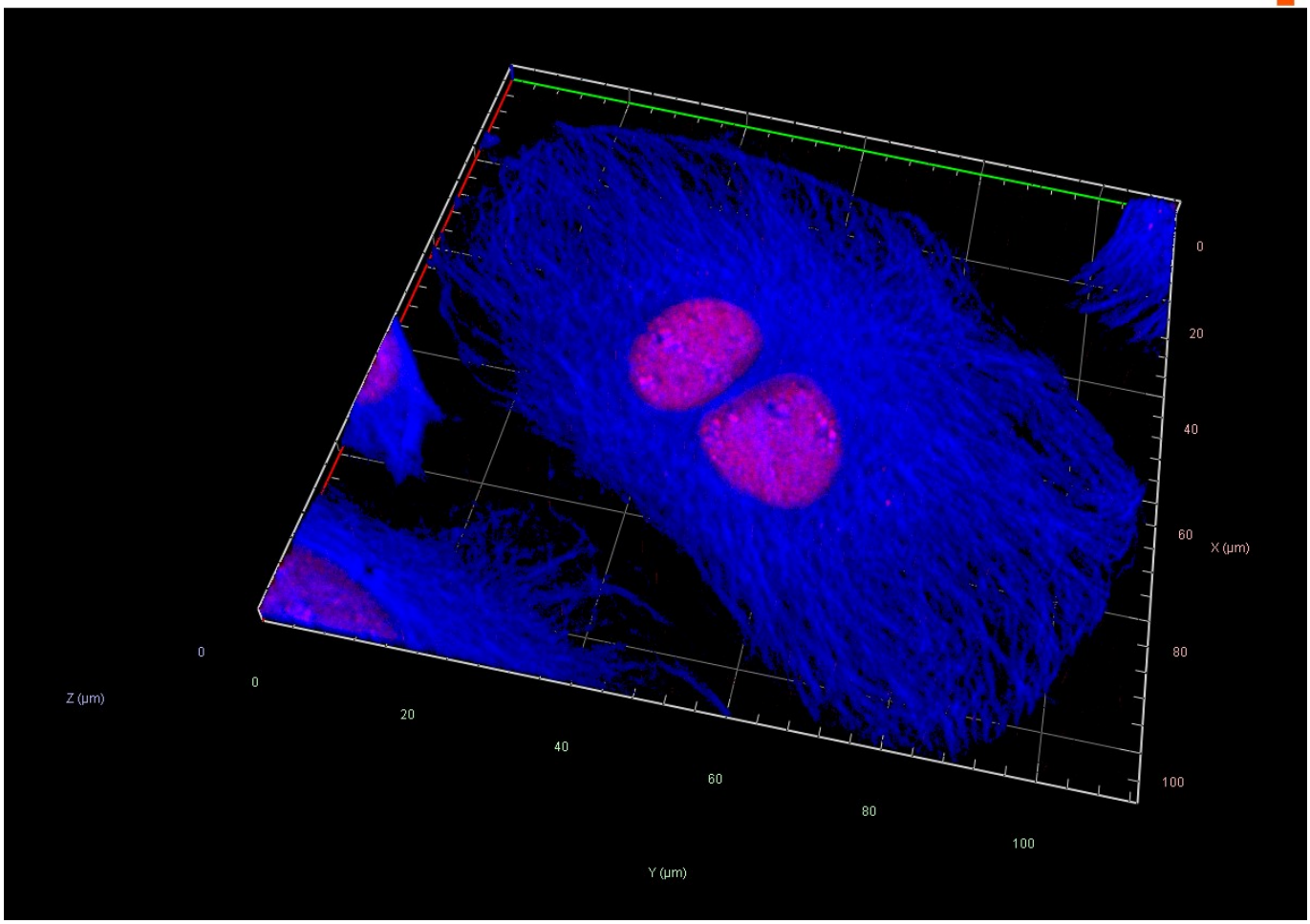
SOURCE PROVENANCE

Catalog	SA-000083

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 Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CD414AF0B50263718E137C861805A64A95827B76AA8D68FF0F233CC42DCDA2F9
Method PDF SHA-256	019201B654F96510C0137B5C254F673FE5A6194F5FCECF57E59E805E799D4C59

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; 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	15 Slices (2.80µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	749 X 791
Image Size (Scaled)	107.00µm X 113.00µm
Bit Depth	14 Bit
Image Center Position	X: 79.71mm, Y: 50.69mm
Stage Position	X: 79.71mm, Y: 50.69mm
ROI Center Offset	X: 1.14, Y: 0.00µm
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 @70% | X-Cite Xylis Lamp Intensity @20%
Exposure Time	750ms | 150ms
Excitation Wavelength	752 | 401
Emission Wavelength	779 | 422
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.51µm | 0.82µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 8% (750), Threshold 13% (405), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

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

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000083

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000001

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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® 405 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 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:

Z Stack - (3D)

Z-Stack = 15 Slices (2.80µm)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.200µm
Image Size (Pixels) = 749 X 791
Image Size (Scaled) = 107.00µm X 113.00µm
Bit Depth = 14 Bit
Image Center Position = X: 79.71mm, Y: 50.69mm
Stage Position = X: 79.71mm, Y: 50.69mm
ROI Center Offset = X: 1.14, Y: 0.00µm

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 @70%
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

405 -

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 @20%
Exposure Time = 150ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.82µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 8% (750), Threshold 13% (405), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

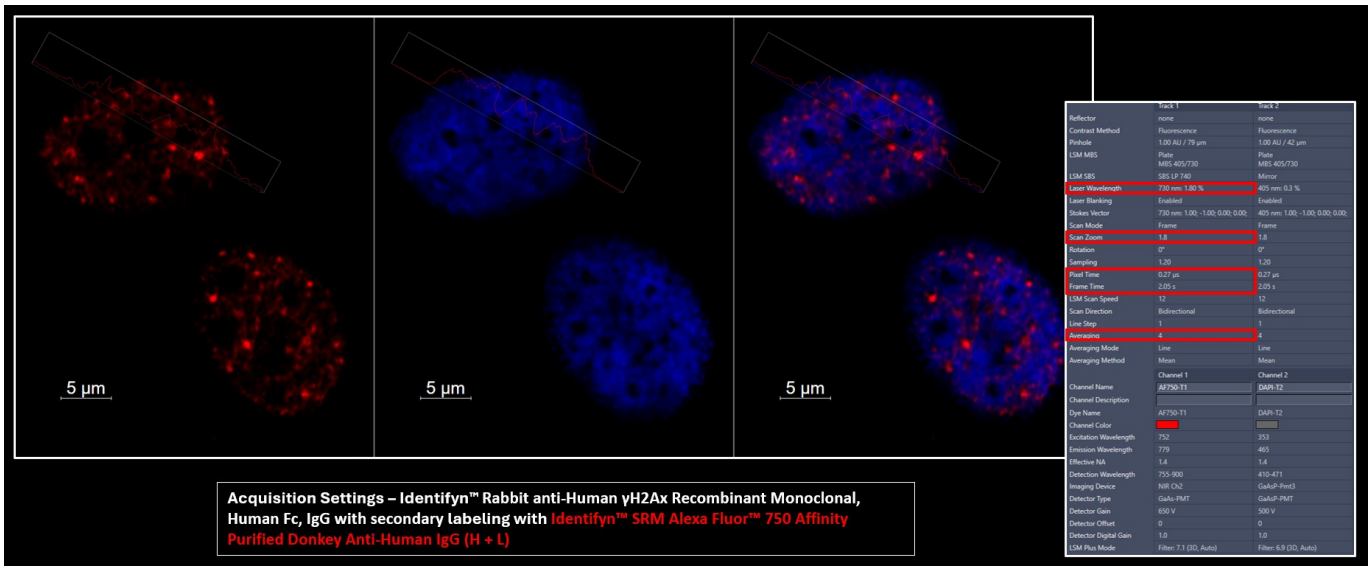
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	SA-000083
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	CB857A810F1B2F7123735490754A640E8D355D175A8A8C1C11CB71AA765E2814
Method PDF SHA-256	BB889D965A675A56A3518A67163874AB5054FC55185CFC7120E211E1529A121C

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	47

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	82 Slices (4.10µm)
Channels	2
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.050µm
Image Size (Pixels)	755 X 928
Image Size (Scaled)	44.41µm X 54.91µm
Bit Depth	16 Bit
Image Center Position	X: 76.85mm, Y: 51.67mm
Stage Position	X: 76.85mm, Y: 51.67mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 79µm | 1.00AU / 42µm
LMS MBS	Plate MBS 405/730
LMS SBS	SBS LP 740 | Mirror
Laser Wavelength	730nm @1.80% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	1.20
Pixel Time	0.27µs
Frame Time	2.05s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.4
Detection Wavelength	755 - 900 | 410 - 471
Imaging Device	NIR Channel 2 | GaAsP-Pmt3
Detector Type	GaAs-PMT | GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	650 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.1(3D, Auto) | Filter: 6.9 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axis were set to Auto - X (Min 0.0 and Max 26.4), Y (Min 18 and Max 18002)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

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

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 750 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000083

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

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 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 = 82 Slices (4.10μm)

Channels = 2
Scaling (Per Pixel) = 0.059μm X 0.059μm X 0.050μm
Image Size (Pixels) = 755 X 928
Image Size (Scaled) = 44.41μm X 54.91μm
Bit Depth = 16 Bit
Image Center Position = X: 76.85mm, Y: 51.67mm
Stage Position = X: 76.85mm, Y: 51.67mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

750 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 79µm
LMS MBS = Plate MBS 405/730
LMS SBS = SBS LP 740
Laser Wavelength = 730nm @1.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27µs
Frame Time = 2.05s
LSM Scan Speed = 12
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 = GaAs-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.1(3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 42µm
LMS MBS = Plate MBS 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27µs
Frame Time = 2.05s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 471
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.9 (3D, Auto)

Profile View Display

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

Profile View = X and Y axis were set to Auto - X (Min 0.0 and Max 26.4), Y (Min 18 and Max 18002)

Image Corrections

None

Image by J.K. Bennett

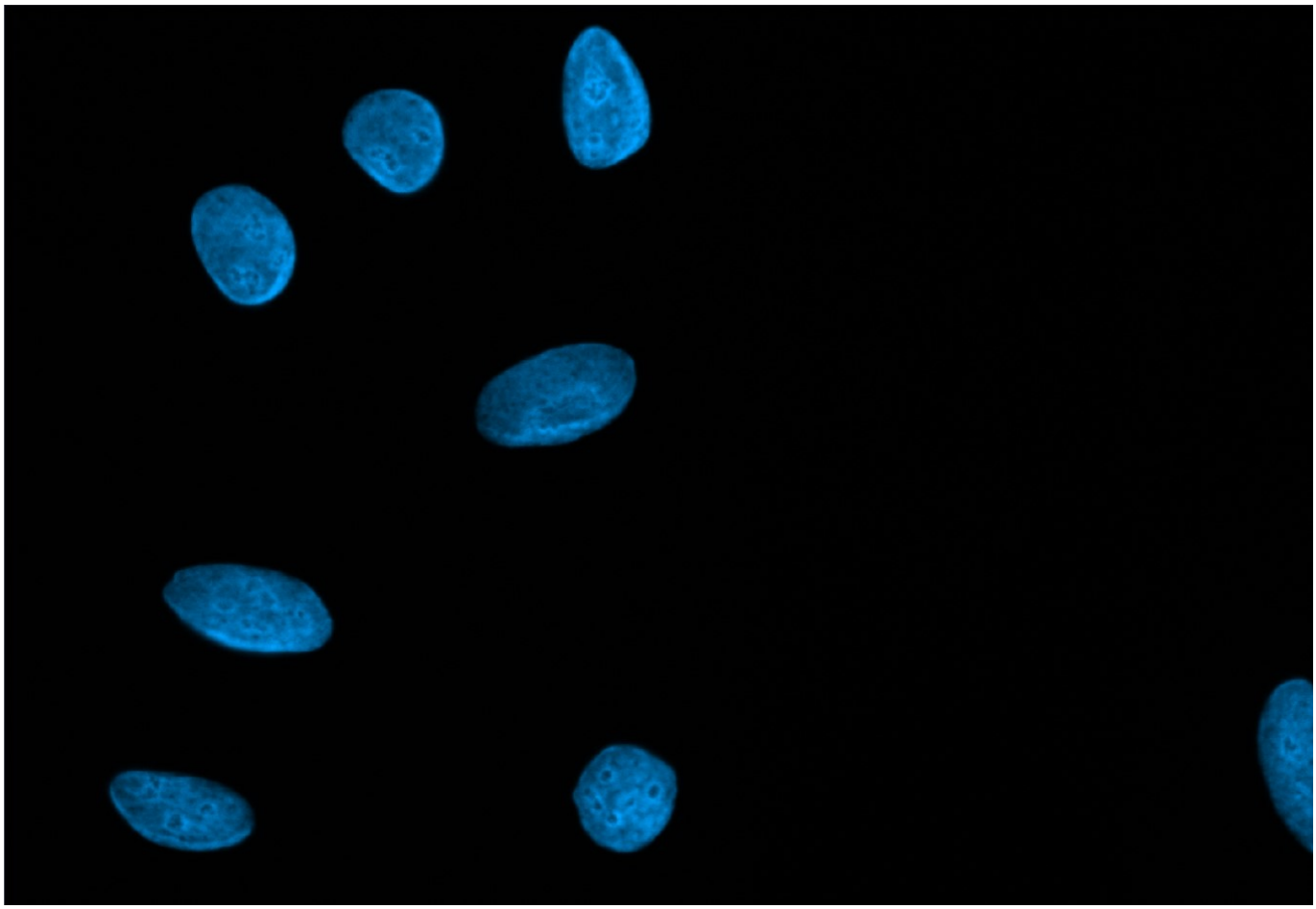
SOURCE PROVENANCE

Catalog	SA-000083
Stage	Confocal
Instrument	ZEISS LSM 980

SOURCE PROVENANCE

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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A22296C7A222577B35F4838182754409B6F34414BA6C55216E65D87F633A6D1A
Method PDF SHA-256	D49CA619A6E55CE3BD46254E4B576D6C620AE5EC4E796FA87BBD0F72D45C1F32

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)	1600 X 1104
Image Size (Scaled)	228.57µm X 157.71µm
Bit Depth	14 Bit
Image Center Position	X: 50.95mm, Y: 51.81mm
ROI Center Offset	X: 50.95µm, Y: 51.81µm
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 Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000083

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) = 1600 X 1104
Image Size (Scaled) = 228.57µm X 157.71µm
Bit Depth = 14 Bit
Image Center Position = X: 50.95mm, Y: 51.81mm
ROI Center Offset = X: 50.95µm, Y: 51.81µm

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

SOURCE PROVENANCE

Catalog	SA-000083
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	E97796BEB579FC7F55A372BCBDA16D96A4ED13E5F5E6E2947047831C96413AF5
Method PDF SHA-256	D304F6D7CF02D04CA82E60C90216C426A67944B692A8E85B801EEDD13D138495