BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

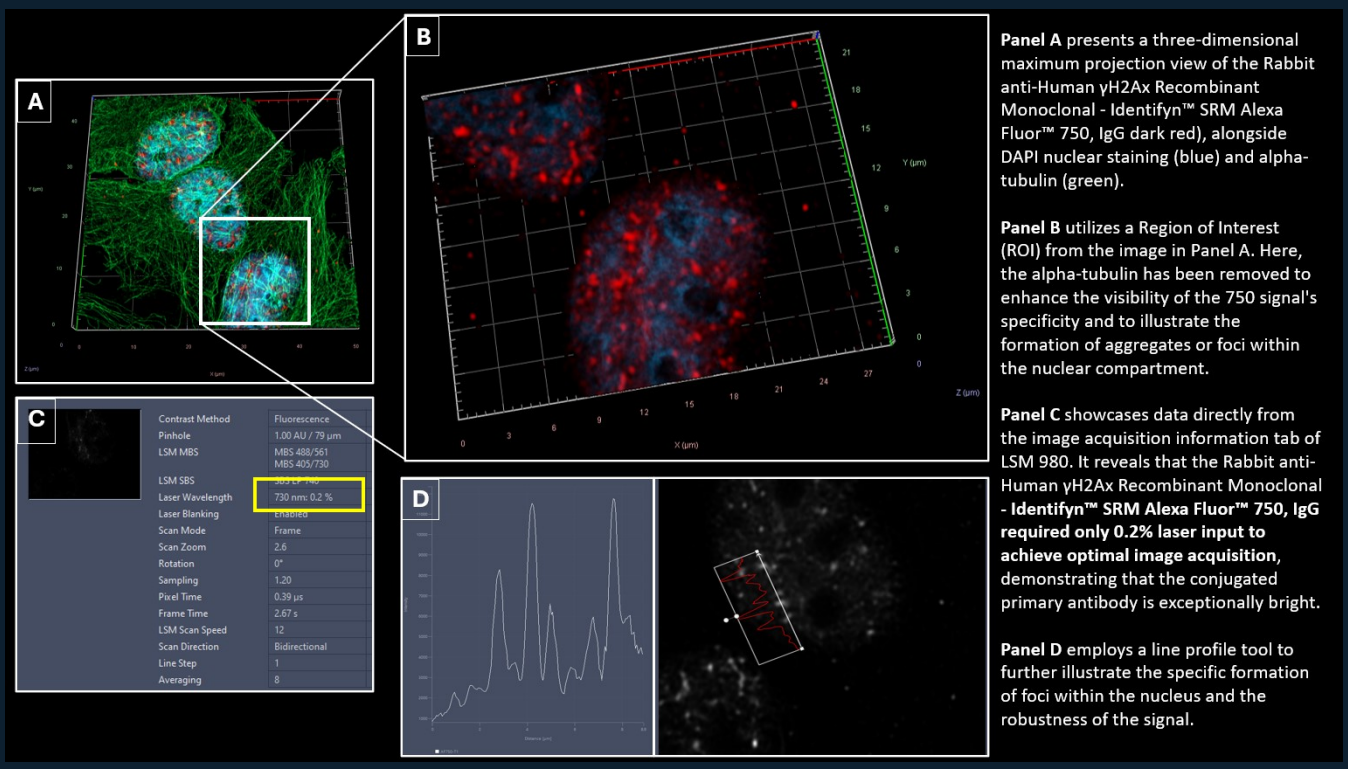
H2AX

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750

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

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A presents a three-dimensional maximum projection view of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750, IgG dark red), alongside DAPI nuclear staining (blue) and alpha-tubulin (green).

Panel B utilizes a Region of Interest (ROI) from the image in Panel A. Here, the alpha-tubulin has been removed to enhance the visibility of the 750 signal's specificity and to illustrate the formation of aggregates or foci within the nuclear compartment.

Panel C showcases data directly from the image acquisition information tab of LSM 980. It reveals that the Rabbit anti-Human γH2Ax Recombinant Monoclonal - **Identifyn™ SRM Alexa Fluor™ 750, IgG required only 0.2% laser input to achieve optimal image acquisition**, demonstrating that the conjugated primary antibody is exceptionally bright.

Panel D employs a line profile tool to further illustrate the specific formation of foci within the nucleus and the robustness of the signal.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

DC-000031 | 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
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for H2AX, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal

BENNETT ASSESSMENT

The preserved DC-000031 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 DC-000031 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

No separate control record is preserved; none is inferred from method or template language.

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; AF750-490007; 49 DAPI; 672 - 748; 335-383; 765 - 855; 420-470; 752
Confocal	405/DAPI; 488; 750	3; None; 730nm @0.2%; 488nm @0.07%; 405nm @0.2%; 752; 493; 353

MODALITY ASSESSMENT / PART 1 OF 4

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 4

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 833 X 698; Image Size (Pixels): 833 X 698; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250ms; 20ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%. Optical/scan: Effective NA: 1.4; .5. Structured source metadata values: 32.

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 4

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 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 69 Slices (6.80µm); Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm X 0.100µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705; Axiocam 807 mono. Timing: Exposure Time: 200ms; 18ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @18%; X-Cite Xylis Lamp Intensity @10%. 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: 48.

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 4

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: 109 Slices (3.24µm) - Main Image Acquisition; Channels: 3; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.030µm; Image size (Pixels): 857 X 802; Image Size (Pixels): 857 X 802; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Channel 2; GaAsP-Pmt3; Detector Type: GaAs-Pmt; GaAsP-Pmt. Timing: Pixel Time: 0.39µs; Frame Time: 2.67s. Illumination: Laser Wavelength: 730nm @0.2%; 488nm @0.07%. Optical/scan: Pinhole: 1.00AU / 79µm; 1.00AU / 49µm; Scan Zoom: 2.6; LSM Scan Speed: 12; 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: 6.8 (3D, Auto); Filter: 6.5 (3D, Auto). Structured source metadata values: 60.

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; 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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000031 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 / AUTO-RECONCILED

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

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 2µM -> Etoposide = 2 µM. The governing scientist identified every 200 µM occurrence as a technician transcription error. The canonical historical concentration is 2 µM. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 139. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human γH2Ax (Serine 139) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750

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. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

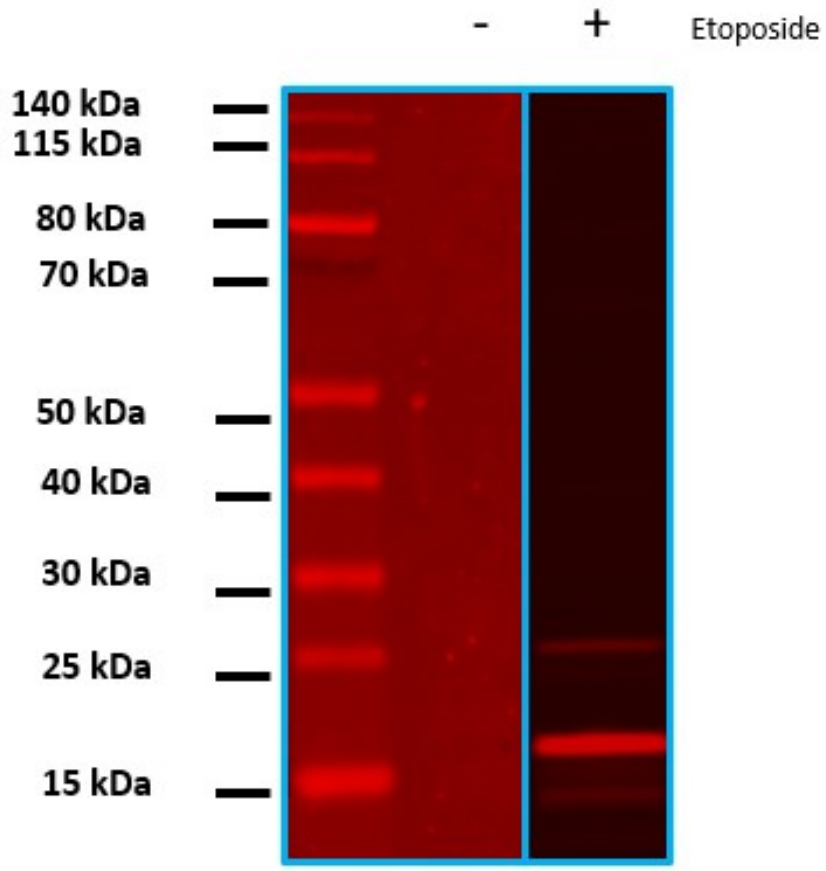
This dossier preserves the complete available evidence progression for DC-000031. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	HEK293
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	829±62 nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Kyle Small

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™

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate
Cat ID# - 000031

HEK 293 cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down the cell debris. The lysate supernatant (100ug) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C 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 the 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 - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

Control Data - Primary Unconjugated Antibody

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 = 829±62 nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Kyle Small

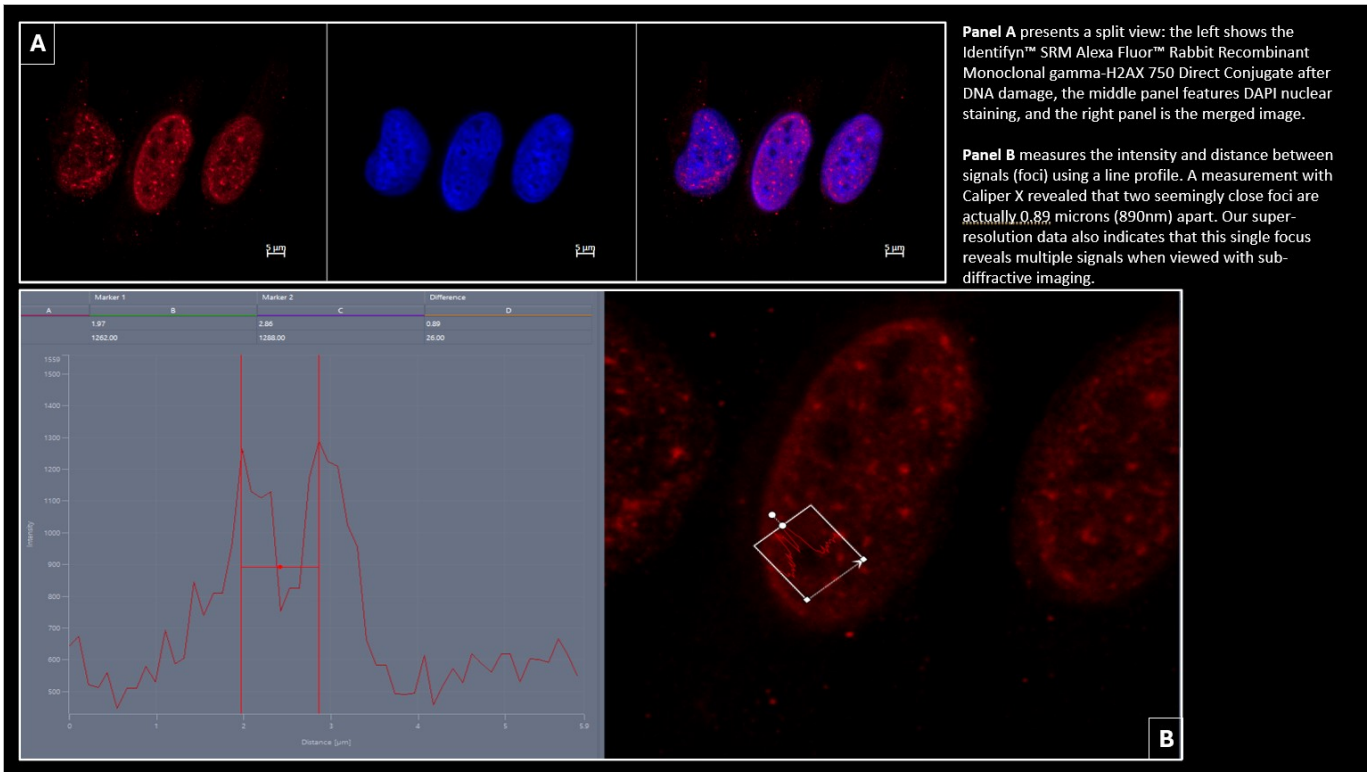
SOURCE PROVENANCE

Catalog	DC-000031

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	HEK293
Image SHA-256	039BA498285E9E256891C4D50404DA8BD33B0A8EB86229AA5AFA73FAD42381F9
Method PDF SHA-256	B3B9212F68726FE2A8B3E1922584FC2025A3D4FF18D02A68817E987A0D7E29E6

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A presents a split view: the left shows the Identifyn™ SRM Alexa Fluor™ Rabbit Recombinant Monoclonal gamma-H2AX 750 Direct Conjugate after DNA damage, the middle panel features DAPI nuclear staining, and the right panel is the merged image.

Panel B measures the intensity and distance between signals (foci) using a line profile. A measurement with Caliper X revealed that two seemingly close foci are actually 0.89 microns (890nm) apart. Our super-resolution data also indicates that this single focus reveals multiple signals when viewed with sub-diffractive imaging.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	833 X 698
Image Size (Scaled)	91.23µm X 76.45µm
Bit Depth	14 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 @15%
Exposure Time	250ms | 20ms
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.4 | .5
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.09µm | 0.72µm
Binning Mode	2,2
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 5.9), Y (Min 429 and Max 1559)

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™

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate
Cat ID# - 000031

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 specifically 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 - Identifyn® SRM Alexa Fluor™ 750- Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110μm X 0.110μm
Image size (Pixels) = 833 X 698
Image Size (Scaled) = 91.23μm X 76.45μm
Bit Depth = 14 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 @15%
Exposure Time = 250ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 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

Split View - Panel A

The left panel shows the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate after DNA damage, the middle panel features DAPI nuclear staining, and the right panel is the merged image.

Profile View Display - Panel B

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 5.9), Y (Min 429 and Max 1559)

Summary

The Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate demonstrates a significant nuclear signal following etoposide-induced DNA damage, clearly localized within the nuclear compartment. The line profile highlights a robust fluorescent signal generated by the direct conjugate, and widefield microscopy effectively facilitates the analysis of DNA damage responses in both cell populations and individual nuclei.

However, it's worth noting that measurements taken with the caliper tool indicate distances of nearly 0.90 microns (900nm). This suggests that the observed foci likely represent an aggregate signal, as demonstrated by super-resolution data. Therefore, it may be beneficial to reassess claims regarding single foci in widefield microscopy. Such reflections could enhance the understanding and interpretation of our findings in this area.

Image Correction

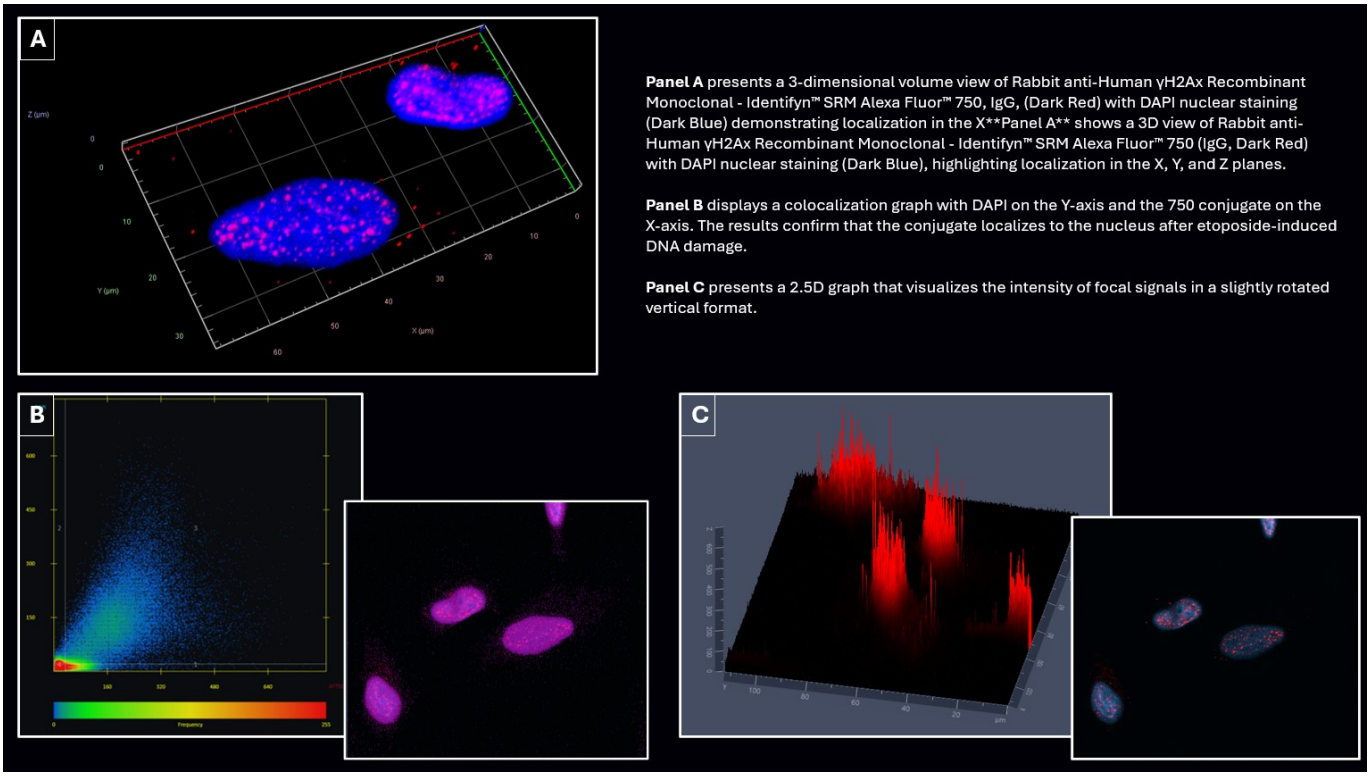
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	DC-000031
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	32
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A75BFA8AD1000AC26959391A7AB63A9FD595077AE5977FD2428012D08BF61603
Method PDF SHA-256	C4A51D39046DA3D84D5027C5EE301F000A2FA4B0DCBE9BA0292A428F1E9E3393

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	48

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 705, 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	69 Slices (6.80µm)
Channels	2
Scaling (Per Pixel)	0.110µm X 0.110µm X 0.100µm
Image Size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	14 Bit
Image Center Position	X: 104.68mm, Y: 54.17mm
Stage Position	X: 0.00mm, Y: 0.00mm
ROI Center Offset	X: 0.00, Y: 0.00µm (Manual Stage)
Reflector	AF750-490007 | 49 DAPI
Beam Splitter	760 | 395
Filter Excitation Wavelength	672 - 748 | 335-383
Filter Emission Wavelength	765 - 855 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @18% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms | 18ms
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.25
Imaging Device	Axiocam 705 | Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.51µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% (750), Threshold 13% (DAPI), Ramp 50% both channels, Maximum 100% both channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Render Mode	Surface

FIELD	SOURCE-DOCUMENTED VALUE
Grid distance	1
Angle X	56
Angle Y	-102
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

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™

Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate
Cat ID# - 000031

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 specifically 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 - Identifyn® SRM Alexa Fluor™ 750- Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 69 Slices (6.80μm)

Channels = 2
Scaling (Per Pixel) = 0.110μm X 0.110μm X 0.100μm
Image Size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18μm X 112.59μm
Bit Depth = 14 Bit
Image Center Position = X: 104.68mm, Y: 54.17mm
Stage Position = X: 0.00mm, Y: 0.00mm
ROI Center Offset = X: 0.00, Y: 0.00μm (Manual Stage)

750 -

Reflector = AF750-490007
Beam Splitter = 760
Filter Excitation Wavelength = 672 - 748
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @18%
Exposure Time = 200ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.25
Imaging Device = Axiocam 705
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 = 18ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing - Panel A

3D Volume Projection = Precise
Appearance = Threshold 13% (750), Threshold 13% (DAPI), Ramp 50% both channels, Maximum 100% both channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Colocalization View - Panel B

Horizontal Axis - 750, Threshold 31, Range Auto
Vertical Axis - DAPI, Threshold 22, Range Auto

Region 3 - Colocalization Selected (Violet mask) shows the areas where the 750 and DAPI channels are localized in the same space.

2.5D Image Processing - Panel C

2.5D Display

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

2.5D Display Options

Angle X = 56
Angle Y = -102
Z Scaling = 1.0
Ambient =50
Specular = 20
Shininess = 50
Light Height = 2.0

Summary

The Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG effectively responds to DNA damage by localizing in the nucleus and forming aggregates known as foci. Although widefield images provide valuable information, the resolution improved through Widefield SIM still limits the ability to link individual foci to specific events. Super-resolution techniques reveal that these foci are clusters of signals resulting from resolution limitations in the Y and Z planes. The data demonstrate the target specificity and exceptional brightness of the 750 direct conjugate, making it suitable for use in all microscopy techniques.

Image Corrections

None

Image by E.F. Simon

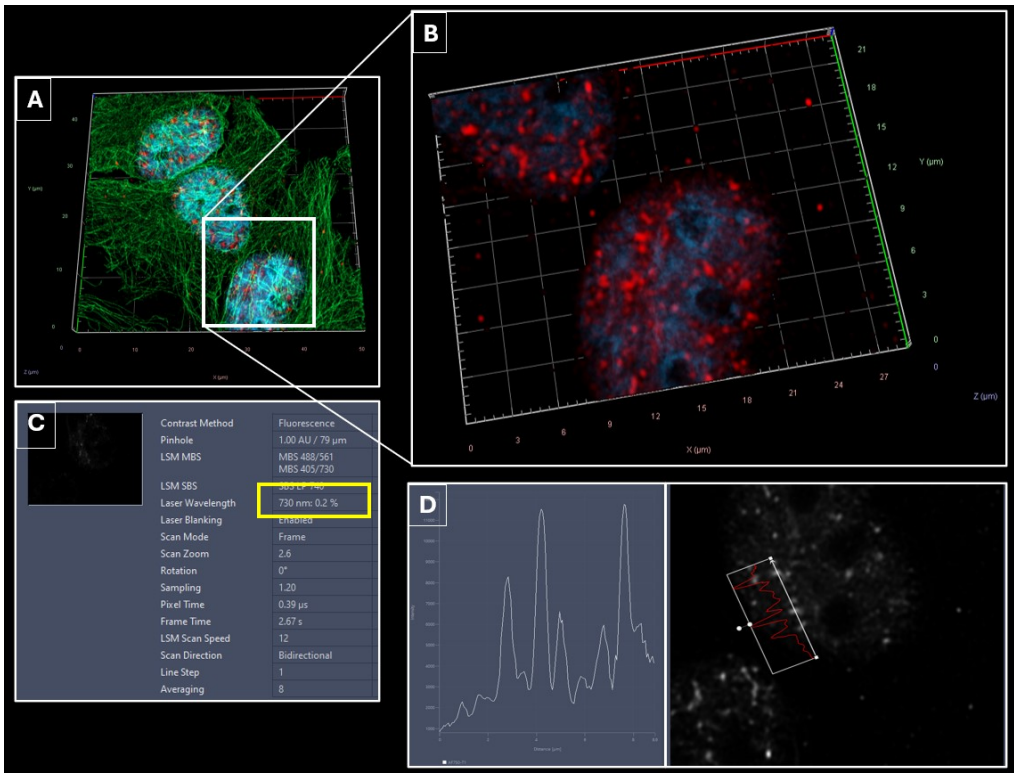
SOURCE PROVENANCE

Catalog	DC-000031
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 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	48
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A0768061802B928B279B16A1F4BDD125A509EEECCA026093B93E30795D049EB2
Method PDF SHA-256	901B14E3D42D7558CC5712C641B1AF99587A1AB77A7450DDAD0DDB0722405292

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A presents a three-dimensional maximum projection view of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750, IgG dark red), alongside DAPI nuclear staining (blue) and alpha-tubulin (green).

Panel B utilizes a Region of Interest (ROI) from the image in Panel A. Here, the alpha-tubulin has been removed to enhance the visibility of the 750 signal's specificity and to illustrate the formation of aggregates or foci within the nuclear compartment.

Panel C showcases data directly from the image acquisition information tab of LSM 980. It reveals that the Rabbit anti-Human γH2Ax Recombinant Monoclonal **- Identifyn™ SRM Alexa Fluor™ 750, IgG required only 0.2% laser input to achieve optimal image acquisition**, demonstrating that the conjugated primary antibody is exceptionally bright.

Panel D employs a line profile tool to further illustrate the specific formation of foci within the nucleus and the robustness of the signal.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 750
METADATA VALUES	60

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	109 Slices (3.24µm) - Main Image Acquisition
Channels	3
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.030µm
Image Size (Pixels)	857 X 802
Image Size (Scaled)	50.39µm X 47.16µm
Bit Depth	16 Bit
Image Center Position	X: 101.32mm, Y: 50.80mm
Stage Position	X: 101.32mm, Y: 50.80mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 79µm | 1.00AU / 49µm | 1.00AU / 42µm
LMS MBS	MBS 488/561 & 405/730
LMS SBS	SBS LP 740 | Mirror
Laser Wavelength	730nm @0.2% | 488nm @0.07% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.6
Rotation	0°
Sampling	1.20
Pixel Time	0.39µs
Frame Time	2.67s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	752 | 493 | 353
Emission Wavelength	779 | 517 | 465
Effective NA	1.4
Detection Wavelength	755 - 900 | 471 - 530 | 410 - 471
Imaging Device	NIR Channel 2 | GaAsP-Pmt3 | GaAsp-Pmt3
Detector Type	GaAs-Pmt | GaAsP-Pmt | GaAsp-Pmt

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	600 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 6.8 (3D, Auto) | Filter: 6.5 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (750), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 1% (750), 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)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 8.9), Y (Min 797 and Max 12343)

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 - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate
Cat ID# - 000031

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000012

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 specifically 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 - Identifyn® SRM Alexa Fluor™ 750 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then 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 1:100 . Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

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 = 109 Slices (3.24µm) - Main Image Acquisition

Channels = 3
Scaling (Per Pixel) = 0.059µm X 0.059µm X 0.030µm
Image Size (Pixels) = 857 X 802
Image Size (Scaled) = 50.39µm X 47.16µm
Bit Depth = 16 Bit
Image Center Position = X: 101.32mm, Y: 50.80mm
Stage Position = X: 101.32mm, Y: 50.80mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

750-

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 79µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = SBS LP 740
Laser Wavelength = 730nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.39µs
Frame Time = 2.67s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
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 = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

488-

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 49µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror
Laser Wavelength = 488nm @0.07%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.39µs
Frame Time = 2.67s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 471 - 530
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-Pmt
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.5 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 42µm
LMS MBS = MBS 488/561 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.6
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.39µs
Frame Time = 2.67s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
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: 6.5 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (750), Threshold 1% (488), 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)

3D Image Processing - Panel B

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

LSM 980 Zen Acquisition Info Tab - Panel C

Shown is the acquisition of the 750 channel using the 730nm laser at 0.2%

Profile View Display - Panel B

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 8.9), Y (Min 797 and Max 12343)

Summary

Panel A presents a three-dimensional maximum projection view of the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG (shown in dark red), alongside DAPI nuclear staining (in dark blue) and alpha-tubulin (in green).

Panel B utilizes a Region of Interest (ROI) from the image in Panel A. Here, the alpha-tubulin has been removed to enhance the visibility of the 750 signal's specificity and to illustrate the formation of aggregates or foci within the nuclear compartment.

Panel C showcases data directly from the image acquisition information tab of the LSM 980. It reveals that the Rabbit anti-Human γH2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG required only 0.2% laser input to achieve optimal image acquisition, demonstrating that the conjugated primary antibody is exceptionally bright.

Panel D employs a line profile tool to illustrate further the specific formation of foci within the nucleus and the robustness of the signal.

Image Corrections

None

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	DC-000031
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	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	ADD2DA60BB83C4C85EE11B510CCD3A4E1467079802D41D208265C89581220356
Method PDF SHA-256	6A0D90529E751A227BF58BCB026850018BFF9DC9AE851A56627061DEDA8EEE2E