BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

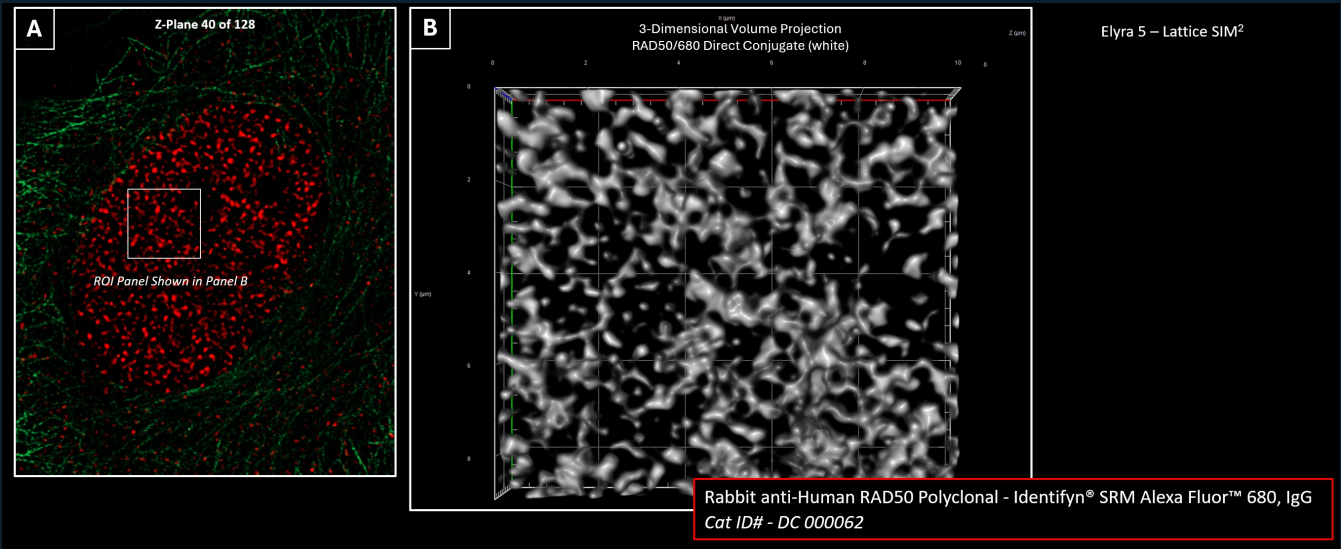
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680

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

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A shows a representative two-dimensional lattice SIM² optical section (Z-plane 40 of 128) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context consistent with the corresponding lattice SIM dataset. A white-boxed region denotes the region of interest (ROI) delineated for volumetric assessment in Panel B.

Panel B presents a three-dimensional volume projection of a larger intranuclear field acquired at lattice SIM² resolution. In three dimensions, RAD50 assemblies exhibit greater spatial refinement and heterogeneous organization across the nuclear volume than lattice SIM. SIM² reconstruction enhances the identification of chromatin-associated structural features and reveals RAD50 organization variability that is not apparent at lower-resolution tiers.

Together, these panels demonstrate that lattice SIM² provides a meaningful gain in resolution and chromatin-contextual organization over lattice SIM, enabling improved identification of RAD50-associated structural features within the nucleus. However, despite this improvement, discrete subunit-level architecture remains unresolved. In accordance with the CCR CDx Step-Down framework, definitive resolution of the underlying molecular subunits requires progression to single-molecule localization microscopy (STORM).

RAD50 localization and recruitment behavior remain consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000062 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for RAD50, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM²

BENNETT ASSESSMENT

The preserved DC-000062 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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 / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000062 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 7 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

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	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 680	3; 38 eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 680	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 680	3; None; 639nm @1.80%; 488nm @0.07%; 405nm @0.2%; 679; 493; 353
Airyscan	405/DAPI; 488; 680	3; None; 639nm @2.60%; 488nm @0.08%; 405nm @0.2%; 679; 493; 353
SIM	405/DAPI; 488; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640

MODALITY ASSESSMENT / PART 1 OF 7

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 680.

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 7

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: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 300 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @30.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

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; 488; 680.

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 7

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: 50 Slices (4.9 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 1084 X 893; Image Size (Pixels): 1084 X 893; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 250 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.25; 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 58.

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; 680.

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 7

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: 181 Slices (5.4 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 972 X 972; Image Size (Pixels): 972 X 972; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.35µs; Frame Time: 3.22s. Illumination: Laser Wavelength: 639nm @1.80%; 488nm @0.07%. Optical/scan: Pinhole: 1.00AU / 68µm; 1.00AU / 49µm; Scan Zoom: 2.3; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.3 (3D, Auto); Filter: 8.9 (3D, Auto). Structured source metadata values: 55.

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; 680.

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 7

Airyscan / 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: 141 Slices (4.2 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1566 X 1566; 521 X 607; Image Size (Pixels): 1566 X 1566; 521 X 607; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.66µs; Frame Time: 7.82s. Illumination: Laser Wavelength: 639nm @2.60%; 488nm @0.08%. Optical/scan: Pinhole: 5.00AU / 331µm; 5.00AU / 250µm; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 7

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 132 Slices (3.93 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 3894 X 4099; 616 X 497; Image Size (Pixels): 3894 X 4099; 616 X 497; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 125 ms; 100 ms; Frame Time: 1.65 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @6.0%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 7

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 128 Slices (3.81 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1403 X 1705; 479 X 423; Image Size (Pixels): 1403 X 1705; 479 X 423; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 125 ms; 100 ms; Frame Time: 1.65 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @5.0%; 488 nm @1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved DC-000062 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3894 X 4099; Image Size (Scaled)=82.21 µm X 86.54 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1403 X 1705; Image Size (Scaled)=29.62 µm X 36.00 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM -> 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.

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

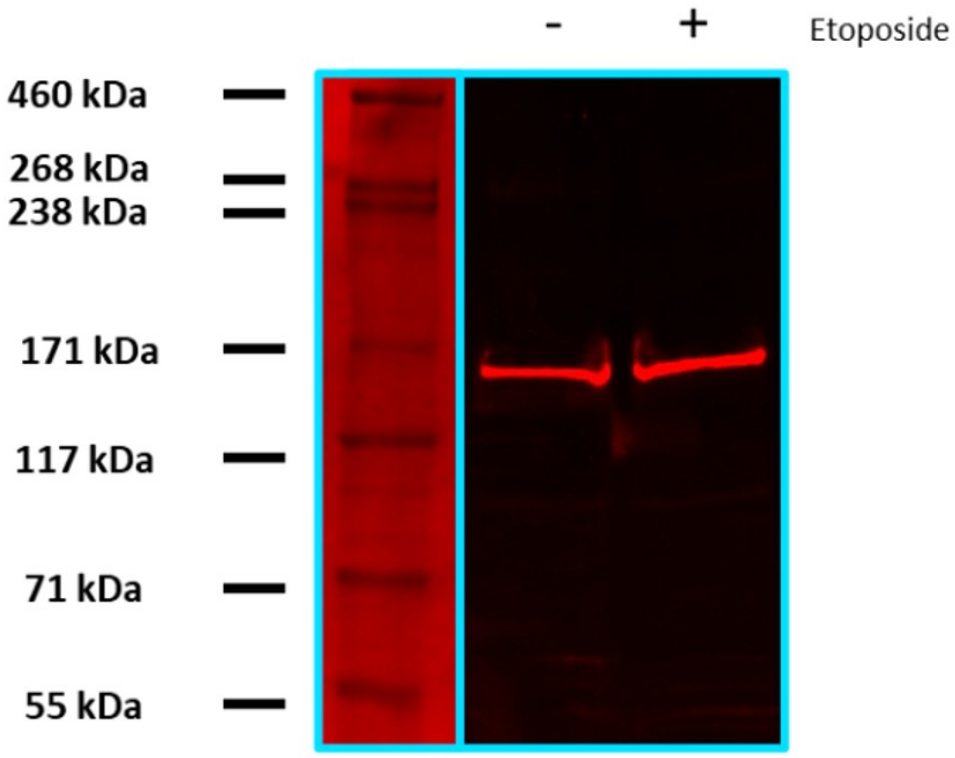
This dossier preserves the complete available evidence progression for DC-000062. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	680
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	685nm
Emission Filter	720±24nm
Detector	PMT
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. 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™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

Expected MW: 153 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. The Thermo Fisher's HiMark™ Pre-stained Protein Standard 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, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG for 20 hours, followed by five washes in 1X 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 = 685nm
Emission Filter = 720±24nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

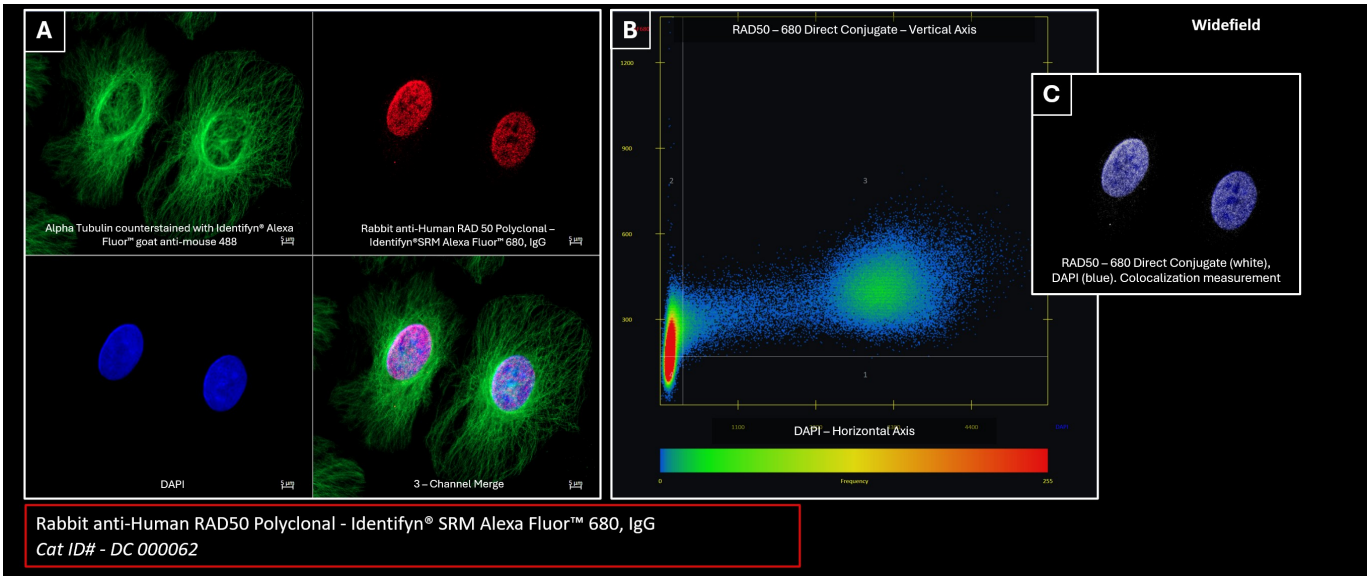
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000062
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	15749A4DB43B88A7FAB25B57F81BC00CEB9BC5AB79085C57796A4CFE7E5E5CEE
Method PDF SHA-256	2BD54EA2F962E40776B981B5234BDE24962E6072B23CDBB55B28065F2FE8BF2B

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A shows a representative widefield image presented as a split view, including individual channels and a three-channel merge. RAD50 is detected using a RAD50 Alexa Fluor™ 680 direct conjugate. α-Tubulin is visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody and serves as an exclusionary structural marker defining cytoplasmic architecture, while DAPI nuclear counterstaining provides chromatin context. The merged view confirms nuclear localization of RAD50 relative to cytoplasmic boundaries and chromatin; however, at widefield resolution, signal distribution remains resolution-limited and unsuitable for structural interpretation.

Panel B presents a colocalization analysis of the region of interest, with RAD50 Alexa Fluor™ 680 signal plotted on the vertical axis and DAPI signal on the horizontal axis. This analysis demonstrates enrichment of the RAD50 signal within the nuclear compartment and its association with chromatin-dense regions, consistent with engagement of the DNA damage response pathway, while remaining constrained by the widefield resolution.

Panel C shows the specific image used for the colocalization analysis, with RAD50 Alexa Fluor™ 680 rendered in white for contrast against the chromatin background. This representation facilitates visualization of RAD50 distribution relative to nuclear structure while emphasizing that, at the widefield gate, RAD50 appears as a diffuse or composite signal rather than discrete repair assemblies.

RAD50 localization and nuclear association are consistent with results obtained using the corresponding unconjugated Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	39

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	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	38 eGFP | 50 Cy 5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 655 | 335 - 383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @30.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 300 ms | 25 ms
Excitation Wavelength	493 | 679 | 353
Emission Wavelength	517 | 702 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.80 µm | 1.09 µm | 0.72 µm
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 RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

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 = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18 µm X 112.59 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @30.00%
Exposure Time = 300 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09 µm
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 @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Alpha Tubulin, visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L) (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The top-right panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680 IgG. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panel B, and Image Panel C, measurement was the entire image acquisition.

Horizontal Axis - DAPI, Threshold 328, Range Auto
Vertical Axis - AF488, Threshold 169, Range Auto

Summary

Panel A shows a representative widefield image presented as a split view, including individual channels and a three-channel merge. RAD50 is detected using a RAD50 Alexa Fluor™ 680 direct conjugate. α-Tubulin is visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody and serves as an exclusionary structural marker defining cytoplasmic architecture, while DAPI nuclear counterstaining provides chromatin context. The merged view confirms nuclear localization of RAD50 relative to cytoplasmic boundaries and chromatin; however, at widefield resolution, signal distribution remains resolution-limited and unsuitable for structural interpretation.

Panel B presents a colocalization analysis of the region of interest, with RAD50 Alexa Fluor™ 680 signal plotted on the vertical axis and DAPI signal on the horizontal axis. This analysis demonstrates enrichment of the RAD50 signal within the nuclear compartment and its association with chromatin-dense regions, consistent with engagement of the DNA damage response pathway, while remaining constrained by the widefield resolution.

Panel C shows the specific image used for the colocalization analysis, with RAD50 Alexa Fluor™ 680 rendered in white for contrast against the chromatin background. This representation facilitates visualization of RAD50 distribution relative to nuclear structure while emphasizing that, at the widefield gate, RAD50 appears as a diffuse or composite signal rather than discrete repair assemblies.

RAD50 localization and nuclear association are consistent with results obtained using the corresponding unconjugated Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

Image Correction

None

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

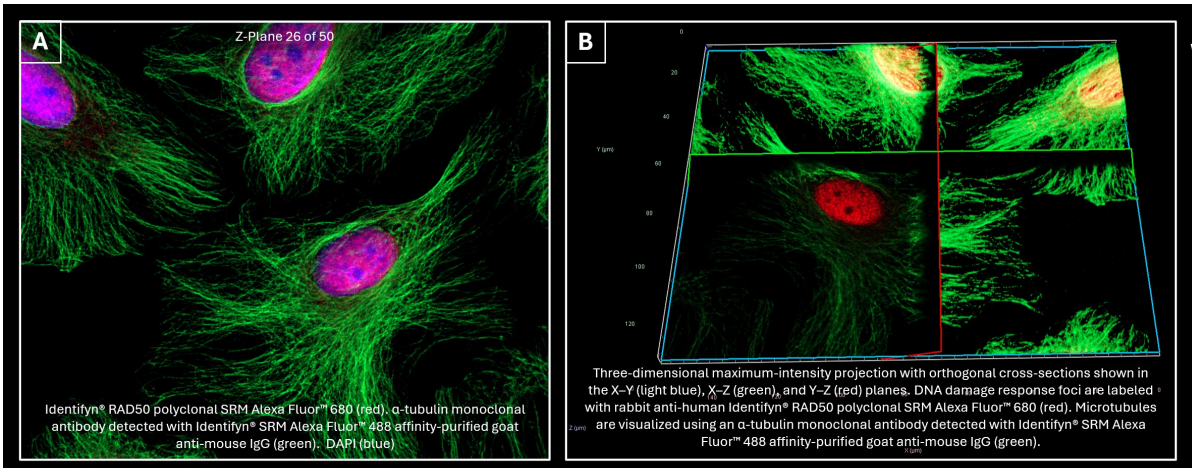
SOURCE PROVENANCE

Catalog	DC-000062
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:

SOURCE PROVENANCE

Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AD77827C6161D65478999F226F19AB251556DE8918749BBE0A87D05DB61B02B2
Method PDF SHA-256	A8C0A01DF6BE252FCF39389C9C6705B30CD20446888045A847291968D6551842

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

Panel A shows a representative two-dimensional widefield-SIM (Apotome) optical section (Z-plane 26 of 50) following etoposide-induced DNA damage. Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, and DAPI nuclear counterstaining. α-Tubulin serves as an exclusionary structural marker defining cytoplasmic architecture, while DAPI provides chromatin context and confirms nuclear confinement of the RAD50 signal. At this resolution gate, the RAD50 signal is present throughout the nuclear volume, consistent with damage-associated recruitment, but remains diffuse and unresolved.

Panel B presents an orthogonal wedge view derived from a three-dimensional maximum-intensity projection of the same dataset, displayed for RAD50 Alexa Fluor™ 680 and α-tubulin Alexa Fluor™ 488. Orthogonal slicing confirms that the RAD50 signal resides within the nuclear volume and is not associated with cytoplasmic structures; however, spatial localization and foci discrimination remain resolution-limited at the Apotome tier.

Together, these panels establish RAD50 signal presence and nuclear recruitment at the widefield-SIM gate while demonstrating that this resolution tier does not support reliable localization or structural interpretation. In accordance with the CCR CDx Step-Down framework, these findings necessitate advancement to higher-resolution modalities to resolve RAD50 organization and damage-associated assemblies.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	58

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	50 Slices (4.9 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	1084 X 893
Image Size (Scaled)	154.86 µm X 127.57 µm
Bit Depth	14 Bit
Image Center Position	X: 72.15 µm, Y: 55.91 µm
Stage Position	X: 72.15 µm, Y: 55.91 µm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00% | X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	250 ms | 150 ms | 25 ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.25 | 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.36 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, 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)

FIELD	SOURCE-DOCUMENTED VALUE
X-Y	Activate was selected, textured opaque was chosen, and a light blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-20% | -5% | 0%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	75° | -75°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

Control Data - Primary Unconjugated Antibody

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) - Panels A and B

Z-Stack = 50 Slices (4.9 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 1084 X 893
Image Size (Scaled) = 154.86 µm X 127.57 µm
Bit Depth = 14 Bit
Image Center Position = X: 72.15 µm, Y: 55.91 µm
Stage Position = X: 72.15 µm, Y: 55.91 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25.00%
Exposure Time = 250 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.36 µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
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 @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used. 3D Image Processing

2 Dimensional View- Panel A

Shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG (red), Alpha Tubulin counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific (green) and DAPI (blue).

Z-plane 26 of 50

3D Image Processing - Panel B

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

Clipping Image Process - Panel X

Clipping planes are introduced in Panel B to highlight the RAD50 680 direct conjugate within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = Activate was selected, textured opaque was chosen, and a light blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -20%
Clip X = 0°
Clip Z = 0°

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -5%
Clip Y = 75°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%
Clip Y = -75°
Clip Z = 0°

Summary

Panel A shows a representative two-dimensional widefield-SIM (Apotome) optical section (Z-plane 26 of 50) following etoposide-induced DNA damage. Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, and DAPI nuclear counterstaining. α-Tubulin serves as an exclusionary structural marker defining cytoplasmic architecture, while DAPI provides chromatin context and confirms nuclear confinement of the RAD50 signal. At this resolution gate, the RAD50 signal is present throughout the nuclear volume, consistent with damage-associated recruitment, but remains diffuse and unresolved.

Panel B presents an orthogonal wedge view derived from a three-dimensional maximum-intensity projection of the same dataset, displayed for RAD50 Alexa Fluor™ 680 and α-tubulin Alexa Fluor™ 488. Orthogonal slicing confirms that the RAD50 signal resides within the nuclear volume and is not associated with cytoplasmic structures; however, spatial localization and foci discrimination remain resolution-limited at the Apotome tier.

Together, these panels establish RAD50 signal presence and nuclear recruitment at the widefield-SIM gate while demonstrating that this resolution tier does not support reliable localization or structural interpretation. In accordance with the CCR CDx Step-Down framework, these findings necessitate advancement to higher-resolution modalities to resolve RAD50 organization and damage-associated assemblies.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

Image Correction

NONE

Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

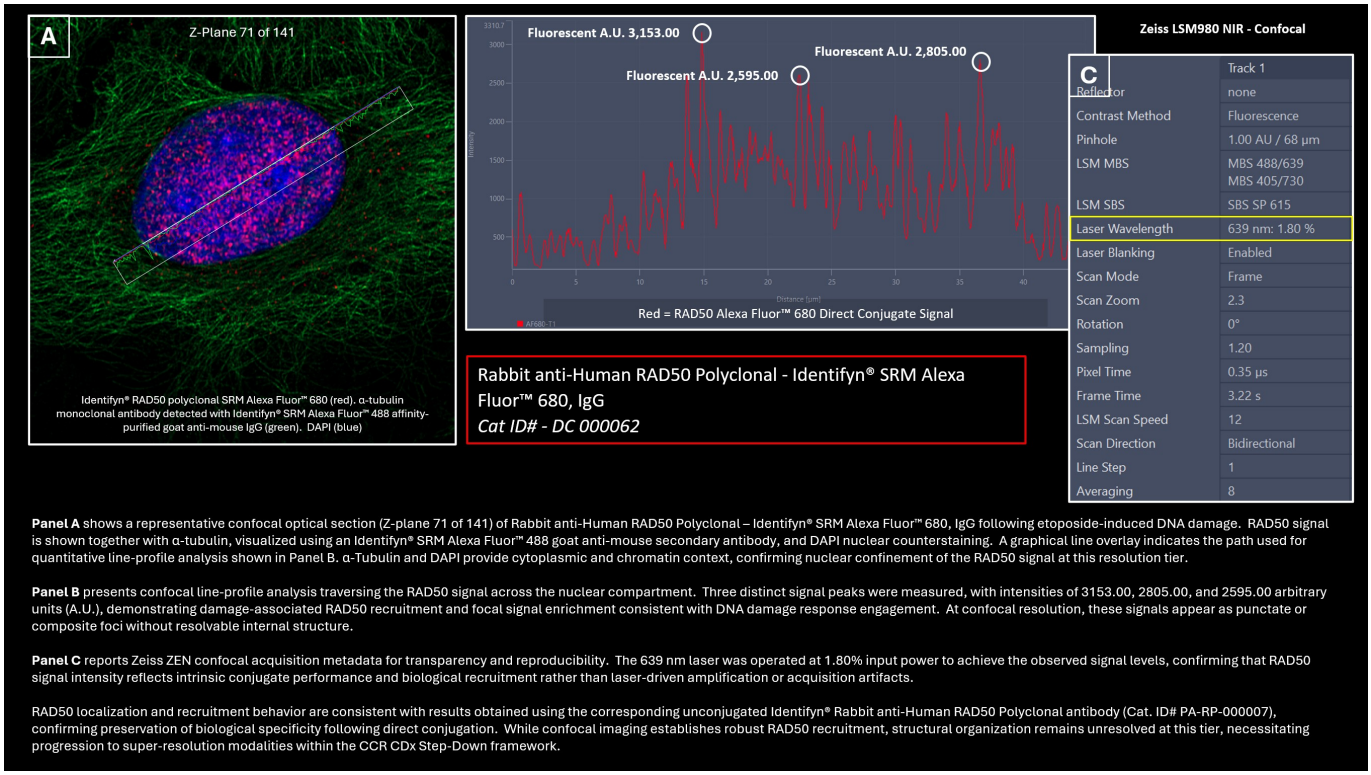
SOURCE PROVENANCE

Catalog	DC-000062
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome

SOURCE PROVENANCE

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	58
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DE899CB618E9E663CED80B6386A3279168C4D4ACAC50F37A0E192515BDFE75C6
Method PDF SHA-256	FA16295C52A888318502ECC80728893FA8348FA159BCF70B84F2114DA5F9F310

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A shows a representative confocal optical section (Z-plane 71 of 141) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 signal is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, and DAPI nuclear counterstaining. A graphical line overlay indicates the path used for quantitative line-profile analysis shown in Panel B. α-Tubulin and DAPI provide cytoplasmic and chromatin context, confirming nuclear confinement of the RAD50 signal at this resolution tier.

Panel B presents confocal line-profile analysis traversing the RAD50 signal across the nuclear compartment. Three distinct signal peaks were measured, with intensities of 3153.00, 2805.00, and 2595.00 arbitrary units (A.U.), demonstrating damage-associated RAD50 recruitment and focal signal enrichment consistent with DNA damage response engagement. At confocal resolution, these signals appear as punctate or composite foci without resolvable internal structure.

Panel C reports Zeiss ZEN confocal acquisition metadata for transparency and reproducibility. The 639 nm laser was operated at 1.80% input power to achieve the observed signal levels, confirming that RAD50 signal intensity reflects intrinsic conjugate performance and biological recruitment rather than laser-driven amplification or acquisition artifacts.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation. While confocal imaging establishes robust RAD50 recruitment, structural organization remains unresolved at this tier, necessitating progression to super-resolution modalities within the CCR CDx Step-Down framework.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	55

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	181 Slices (5.4 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	972 X 972
Image Size (Scaled)	57.15 µm X 57.15 µm
Bit Depth	16 Bit
Image Center Position	X: 84.67 µm, Y: 50.59 µm
Stage Position	X: 84.67 µm, Y: 50.59 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 68µm | 1.00AU / 49µm | 1.00AU / 44µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615 | Mirror
Laser Wavelength	639nm @1.80% | 488nm @0.07% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.3
Rotation	0°
Sampling	1.20
Pixel Time	0.35µs
Frame Time	3.22s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	674 - 745 | 479 - 540 | 438 - 480
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	650 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.3 (3D, Auto) | Filter: 8.9 (3D, Auto) | Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 44.8), Y (Min 86 and Max 3311)

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 RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

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) - Panel A, Z-plane 71 of 141, Line Profile Measurement

Z-Stack =181 Slices (5.4 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 972 X 972
Image Size (Scaled) = 57.15 µm X 57.15 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.67 µm, Y: 50.59 µm
Stage Position = X: 84.67 µm, Y: 50.59 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 68µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @1.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.35µs
Frame Time = 3.22s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 674 - 745
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.3 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 49µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 488nm @0.07%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.35µs
Frame Time = 3.22s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 479 - 540
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 44µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.35µs
Frame Time = 3.22s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 438 - 480
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

2- Dimensional View - Panel A

Panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG. Alpha Tubulin, with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, and DAPI,

Image from Z-plane 64 of 181 and the Line Profile graphic showing the location where the measurement was taken.

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 44.8), Y (Min 86 and Max 3311)

Shown are 3 different measurements of foci intensity.

Zen Acquisition Info Tab -

Shown is the acquisition of the 680 channel (RAD50 680 direct conjugate) using the 639 nm laser at 1.80%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A shows a representative confocal optical section (Z-plane 71 of 141) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 signal is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, and DAPI nuclear counterstaining. A graphical line overlay indicates the path used for quantitative line-profile analysis shown in Panel B. α-Tubulin and DAPI provide cytoplasmic and chromatin context, confirming nuclear confinement of the RAD50 signal at this resolution tier.

Panel B presents confocal line-profile analysis traversing the RAD50 signal across the nuclear compartment. Three distinct signal peaks were measured, with intensities of 3153.00, 2805.00, and 2595.00 arbitrary units (A.U.), demonstrating damage-associated RAD50 recruitment and focal signal enrichment consistent with DNA damage response engagement. At confocal resolution, these signals appear as punctate or composite foci without resolvable internal structure.

Panel C reports Zeiss ZEN confocal acquisition metadata for transparency and reproducibility. The 639 nm laser was operated at 1.80% input power to achieve the observed signal levels, confirming that RAD50 signal intensity reflects intrinsic conjugate performance and biological recruitment rather than laser-driven amplification or acquisition artifacts.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation. While confocal imaging establishes robust RAD50 recruitment, structural organization remains unresolved at this tier, necessitating progression to super-resolution modalities within the CCR CDx Step-Down framework.

Image Correction

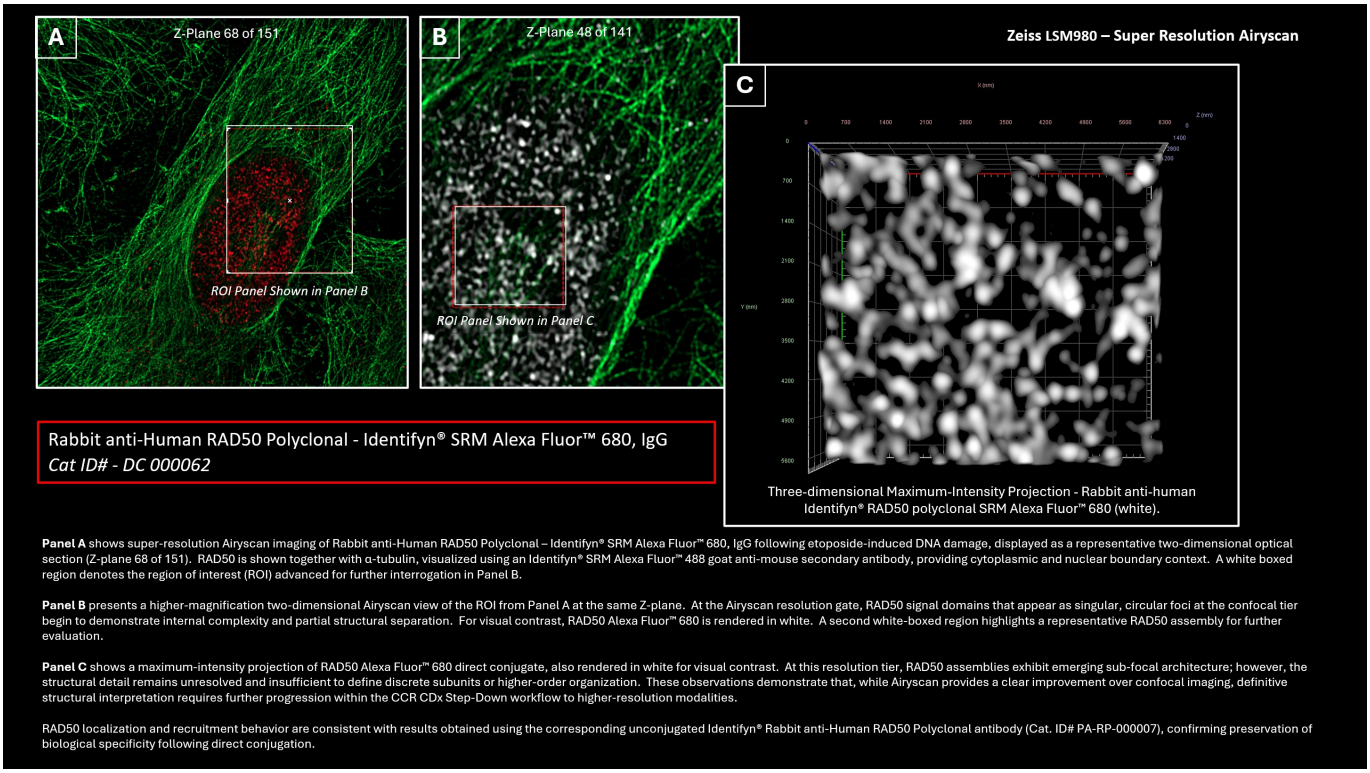
NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000062
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	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CD0847D69F46B481CED006B2F4F6508FF8F0C4EEBD160CC2A98871476176BF74
Method PDF SHA-256	A293FDC4825F4A569B1BB0A7565B5D8F23F8AD681224316B2DD86674D99718A5

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	62

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 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	141 Slices (4.2 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	1566 X 1566 | 521 X 607 | 181 X 166
Image Size (Scaled)	55.27 µm X 55.27 µm | 18.39 µm X 21.42 µm | 6.39 µm X 5.86 µm
Bit Depth	16 Bit
Image Center Position	X: 84.54 µm, Y: 49.71 µm
Stage Position	X: 84.54 µm, Y: 49.71 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 331µm | 5.00AU / 250µm | 5.00AU / 214µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 550 | SBS SP 505
Laser Wavelength	639nm @2.60% | 488nm @0.08% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66µs
Frame Time	7.82s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.4
Detection Wavelength	655 - 735 | 499 - 548 | 422 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	850 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	9.1 (3D, Auto) | 8.7 (3D, Auto) | 7.8(3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2%, Ramp 0%, Maximum 100%
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 75%, 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 RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

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) - Panel A, Z-plane 68 of 141

Z-Stack =141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 1566 X 1566
Image Size (Scaled) = 55.27 µm X 55.27 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.54 µm, Y: 49.71 µm
Stage Position = X: 84.54 µm, Y: 49.71 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, Z-plane 68 of 141

Z-Stack =141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 521 X 607
Image Size (Scaled) = 18.39 µm X 21.42 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.54 µm, Y: 49.71 µm
Stage Position = X: 84.54 µm, Y: 49.71 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C, 3-Dimensional Maximum Projection

Z-Stack =141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 181 X 166
Image Size (Scaled) = 6.39 µm X 5.86 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.54 µm, Y: 49.71 µm
Stage Position = X: 84.54 µm, Y: 49.71 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 331µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @2.60%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 655 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution= 9.1 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.08%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution= 8.7 (3D, Auto)

DAPI - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66µs
Frame Time = 7.82s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution= 7.8(3D, Auto)

2- Dimensional View - Panel A and B

Panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG. Alpha Tubulin, with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific.

Image from Z-plane 68 of 141.

3D Image Processing - Panel C

3D Maximum Projection = Precise
Appearance = Threshold 2%, Ramp 0%, Maximum 100%
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows super-resolution Airyscan imaging of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage, displayed as a representative two-dimensional optical section (Z-plane 68 of 151). RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context. A white boxed region denotes the region of interest (ROI) advanced for further interrogation in Panel B.

Panel B presents a higher-magnification two-dimensional Airyscan view of the ROI from Panel A at the same Z-plane. At the Airyscan resolution gate, RAD50 signal domains that appear as singular, circular foci at the confocal tier begin to demonstrate internal complexity and partial structural separation. For visual contrast, RAD50 Alexa Fluor™ 680 is rendered in white. A second white-boxed region highlights a representative RAD50 assembly for further evaluation.

Panel C shows a maximum-intensity projection of RAD50 Alexa Fluor™ 680 direct conjugate, also rendered in white for visual contrast. At this resolution tier, RAD50 assemblies exhibit emerging sub-focal architecture; however, the structural detail remains unresolved and insufficient to define discrete subunits or higher-order organization. These observations demonstrate that, while Airyscan provides a clear improvement over confocal imaging, definitive structural interpretation requires further progression within the CCR CDx Step-Down workflow to higher-resolution modalities.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

Image Correction

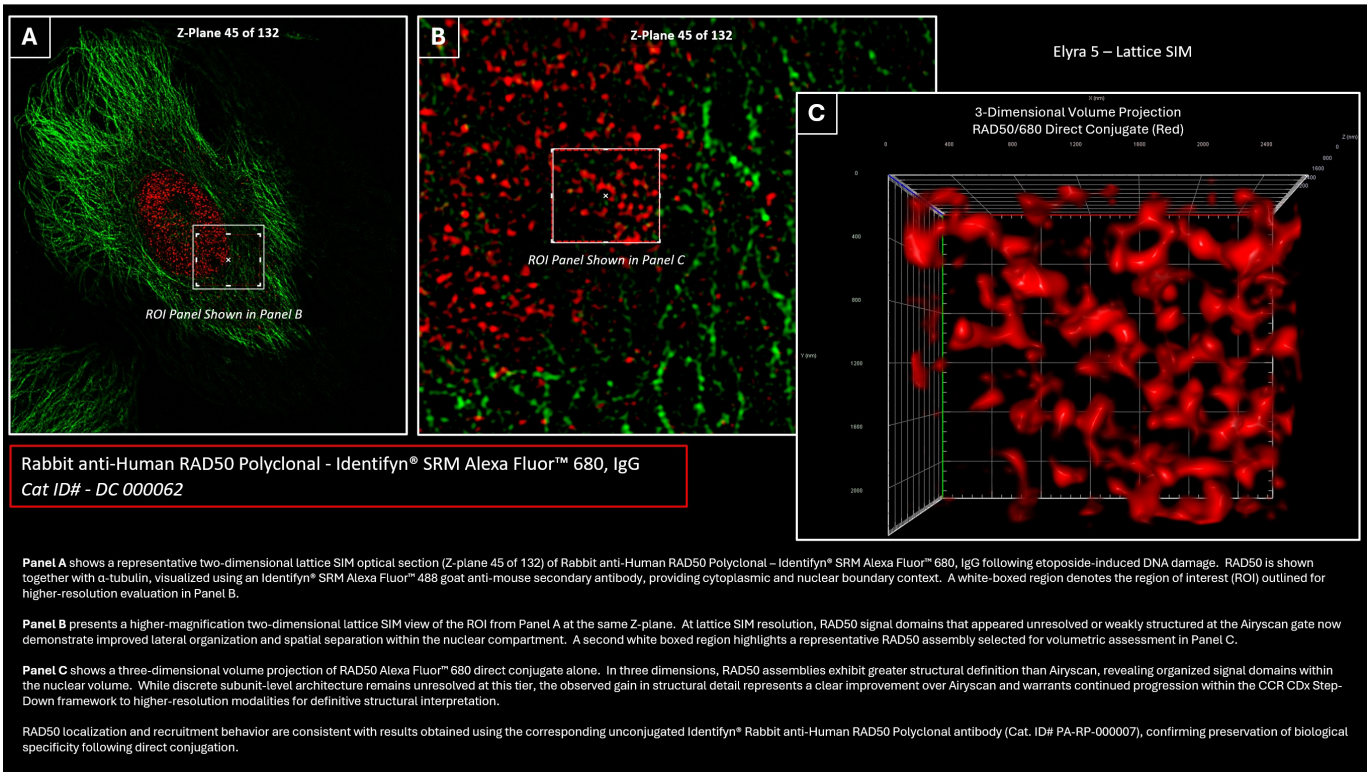
NONE

Image by J.K. Bennett
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000062
Stage	Airyscan
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	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	72CB90A47CD55E6F60015AC1599775E29C6BCE93EE454F35D125CA44056573CB
Method PDF SHA-256	567697138A05C1B15B1F2D3FDEA80BE8811825D58BD2DF27DE14A1F375F0E8AB

ORIGINAL IMAGE EVIDENCE / SIM



Panel A shows a representative two-dimensional lattice SIM optical section (Z-plane 45 of 132) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context. A white-boxed region denotes the region of interest (ROI) outlined for higher-resolution evaluation in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM view of the ROI from Panel A at the same Z-plane. At lattice SIM resolution, RAD50 signal domains that appeared unresolved or weakly structured at the Airyscan gate now demonstrate improved lateral organization and spatial separation within the nuclear compartment. A second white boxed region highlights a representative RAD50 assembly selected for volumetric assessment in Panel C.

Panel C shows a three-dimensional volume projection of RAD50 Alexa Fluor™ 680 direct conjugate alone. In three dimensions, RAD50 assemblies exhibit greater structural definition than Airyscan, revealing organized signal domains within the nuclear volume. While discrete subunit-level architecture remains unresolved at this tier, the observed gain in structural detail represents a clear improvement over Airyscan and warrants continued progression within the CCR CDx Step-Down framework to higher-resolution modalities for definitive structural interpretation.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	132 Slices (3.93 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	3894 X 4099 | 616 X 497 | 127 X 109
Image Size (Scaled)	82.21 µm X 86.54 µm | 13.01 µm X 10.49 µm | 2.68 µm X 2.30 µm
Bit Depth	16 Bit
Image Center Position	X: 58.85 mm, Y: 37.50 mm
Stage Position	X: 58.85 mm, Y: 37.50 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	125 ms | 100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @6.0% | 488 nm @1.50% | 405 nm @2.0%
Frame Time	1.65 s | 1.33 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.1452 (680), 11.1611 (488), 10.1214 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, 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 RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Z-plane 45 of 132

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 3894 X 4099
Image Size (Scaled) = 82.21 µm X 86.54 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.85 mm, Y: 37.50 mm
Stage Position = X: 58.85 mm, Y: 37.50 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI, Z-plane 45 of 132

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 616 X 497
Image Size (Scaled) = 13.01 µm X 10.49 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.85 mm, Y: 37.50 mm
Stage Position = X: 58.85 mm, Y: 37.50 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C, 3-Dimensional Volume Projection

Z-Stack = 132 Slices (3.93 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 127 X 109
Image Size (Scaled) = 2.68 µm X 2.30 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.85 mm, Y: 37.50 mm
Stage Position = X: 58.85 mm, Y: 37.50 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 125 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @6.0%
Frame Time = 1.65 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI - Not Shown in Data Analysis

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @2.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.1452 (680), 11.1611 (488), 10.1214 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2- Dimensional View - Panels A and B

Panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG. Alpha Tubulin, with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific.

Image from Z-plane 45 of 132

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows a representative two-dimensional lattice SIM optical section (Z-plane 45 of 132) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context. A white-boxed region denotes the region of interest (ROI) outlined for higher-resolution evaluation in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM view of the ROI from Panel A at the same Z-plane. At lattice SIM resolution, RAD50 signal domains that appeared unresolved or weakly structured at the Airyscan gate now demonstrate improved lateral organization and spatial separation within the nuclear compartment. A second white boxed region highlights a representative RAD50 assembly selected for volumetric assessment in Panel C.

Panel C shows a three-dimensional volume projection of RAD50 Alexa Fluor™ 680 direct conjugate alone. In three dimensions, RAD50 assemblies exhibit greater structural definition than Airyscan, revealing organized signal domains within the nuclear volume. While discrete subunit-level architecture remains unresolved at this tier, the observed gain in structural detail represents a clear improvement over Airyscan and warrants continued progression within the CCR CDx Step-Down framework to higher-resolution modalities for definitive structural interpretation.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

Image Correction

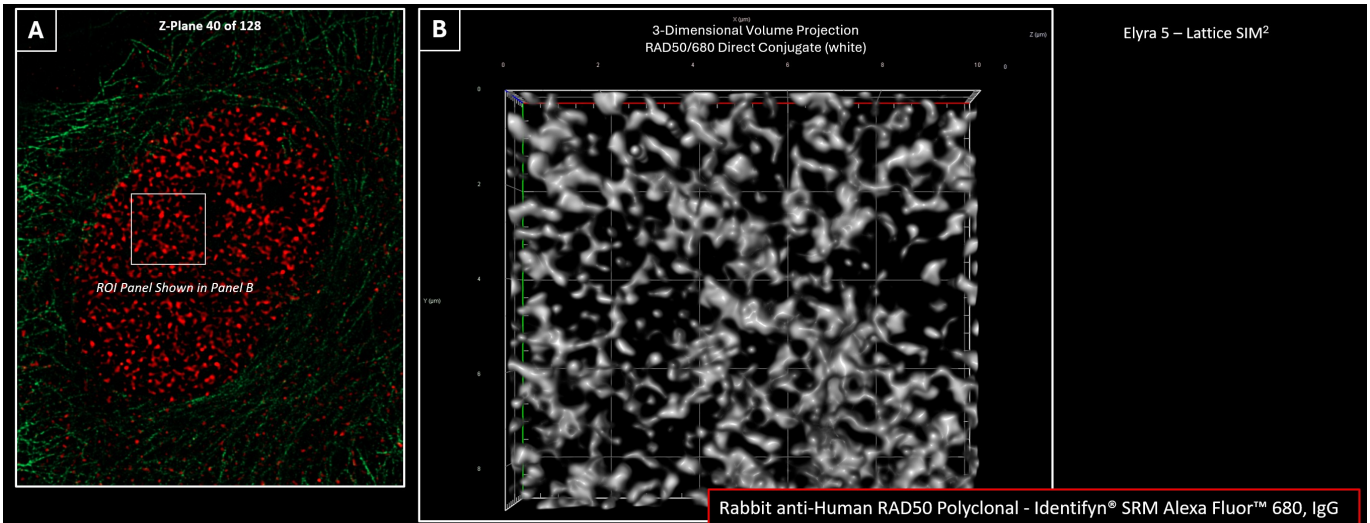
NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000062
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5CBBE98D0945690DB25867E40A8279EBDEFB9B13A2F57B843F508B4E5B1C5CD3
Method PDF SHA-256	F25E9B60EC07FD6D7E03B3A05CD3C737D002B4243E0BAEB84BA87F6EDFB2C5D9

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A shows a representative two-dimensional lattice SIM² optical section (Z-plane 40 of 128) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context consistent with the corresponding lattice SIM dataset. A white-boxed region denotes the region of interest (ROI) delineated for volumetric assessment in Panel B.

Panel B presents a three-dimensional volume projection of a larger intranuclear field acquired at lattice SIM² resolution. In three dimensions, RAD50 assemblies exhibit greater spatial refinement and heterogeneous organization across the nuclear volume than lattice SIM. SIM² reconstruction enhances the identification of chromatin-associated structural features and reveals RAD50 organization variability that is not apparent at lower-resolution tiers.

Together, these panels demonstrate that lattice SIM² provides a meaningful gain in resolution and chromatin-contextual organization over lattice SIM, enabling improved identification of RAD50-associated structural features within the nucleus. However, despite this improvement, discrete subunit-level architecture remains unresolved. In accordance with the CCR CDx Step-Down framework, definitive resolution of the underlying molecular subunits requires progression to single-molecule localization microscopy (STORM).

RAD50 localization and recruitment behavior remain consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	62

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	128 Slices (3.81 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1403 X 1705 | 479 X 423
Image Size (Scaled)	29.62 µm X 36.00 µm | 10.11 µm X 8.93 µm
Bit Depth	16 Bit
Image Center Position	X: 61.58 mm, Y: 39.45 mm
Stage Position	X: 61.58 mm, Y: 39.45 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	125 ms | 100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @5.0% | 488 nm @1.00% | 405 nm @3.0%
Frame Time	1.65 s | 1.33 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, 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 RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG
Cat ID# - DC 000062

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

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Z-plane 40 of 128

Z-Stack = 128 Slices (3.81 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1403 X 1705
Image Size (Scaled) = 29.62 µm X 36.00 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.58 mm, Y: 39.45 mm
Stage Position = X: 61.58 mm, Y: 39.45 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 3-Dimensional Volume Projection

Z-Stack = 128 Slices (3.81 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 479 X 423
Image Size (Scaled) = 10.11 µm X 8.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.58 mm, Y: 39.45 mm
Stage Position = X: 61.58 mm, Y: 39.45 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 -SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 125 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @5.0%
Frame Time = 1.65 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI - Not Shown in Data Analysis

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @3.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

2- Dimensional View - Panel A

Panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG. Alpha Tubulin, with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific.

Image from Z-plane 40 of 128

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows a representative two-dimensional lattice SIM optical section (Z-plane 45 of 132) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 680, IgG following etoposide-induced DNA damage. RAD50 is shown together with α-tubulin, visualized using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, providing cytoplasmic and nuclear boundary context. A white-boxed region denotes the region of interest (ROI) outlined for higher-resolution evaluation in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM view of the ROI from Panel A at the same Z-plane. At lattice SIM resolution, RAD50 signal domains that appeared unresolved or weakly structured at the Airyscan gate now demonstrate improved lateral organization and spatial separation within the nuclear compartment. A second white boxed region highlights a representative RAD50 assembly selected for volumetric assessment in Panel C.

Panel C shows a three-dimensional volume projection of RAD50 Alexa Fluor™ 680 direct conjugate alone. In three dimensions, RAD50 assemblies exhibit greater structural definition than Airyscan, revealing organized signal domains within the nuclear volume. While discrete subunit-level architecture remains unresolved at this tier, the observed gain in structural detail represents a clear improvement over Airyscan and warrants continued progression within the CCR CDx Step-Down framework to higher-resolution modalities for definitive structural interpretation.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

Image Correction

NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000062
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	ED1C318F24ECE232EF55BE291BC74A553A3CC4ED4D806347C908E27FCF5A33A6
Method PDF SHA-256	50F58FB9C70CE2374025BB7549DEFE8E5760A905A50DC298A8367369820E5659