BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

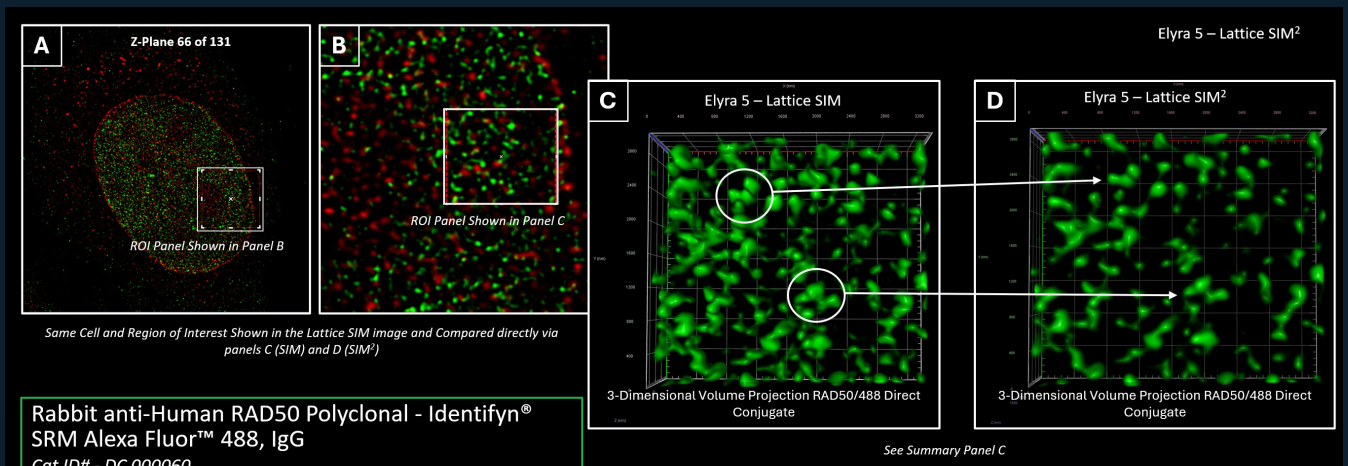
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488

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 of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate, using the same raw image, region of interest (ROI), and acquisition parameters as the corresponding lattice SIM dataset. The white-boxed region denotes the ROI used for comparative analysis in Panel B.

Panel B presents a higher-magnification two-dimensional view of the same ROI shown in Panel A, processed using SIM² reconstruction while maintaining identical acquisition and processing parameters used for SIM. This ensures that observed differences reflect resolution-dependent effects rather than changes in imaging conditions or post-processing.

Panel C displays the lattice SIM reconstruction of the ROI from prior analysis, included here for direct comparison. Two white circular annotations highlight representative RAD50 signal domains that appear bulbous and unresolved at SIM resolution, consistent with composite repair assemblies observed at this gate.

Panel D shows the corresponding SIM² reconstruction of the same ROI, with arrows indicating the identical spatial locations highlighted in Panel C. At SIM² resolution, these RAD50 signal domains exhibit subtle narrowing and increased spatial refinement relative to SIM. This reflects a measurable gain in resolution; however, the overall organization remains globular and unresolved, and discrete subunit-level architecture is not revealed.

Together, these panels demonstrate that while SIM² provides an incremental improvement in spatial resolution relative to SIM—manifested as narrowing of RAD50 signal domains toward patterns later observed by single-molecule localization microscopy—this gain does not constitute biological ground truth. Definitive resolution of RAD50 subunit organization and repair complex architecture requires progression to STORM, which uniquely resolves the molecular-scale features underlying the composite structures observed at SIM and SIM². RAD50 localization and recruitment behavior remain 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 conjugation.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000060 | 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-000060 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-000060 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	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 38 eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 38 eGFP; 50 Cy 5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Confocal	405/DAPI; 488; 647	3; None; 488nm @0.2%; 639nm @0.2%; 405nm @0.2%; 493; 653; 353
Airyscan	405/DAPI; 488; 647	3; None; 488nm @0.3%; 639nm @0.1%; 405nm @0.2%; 493; 653; 353
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 -SR; T3-Axiocam 820 #2-SR; 488
SIM²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820-SR; T1-Axiocam 820 -SR; T3-Axiocam 820 #2-SR; 488

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 488.

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: 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: 1.4. 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; 647.

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: 40 Slices (3.9 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 551 X 510; 183 X 192; Image Size (Pixels): 551 X 510; 183 X 192; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 200 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 49.

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; 647.

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: 121 Slices (3.60 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 914 X 894; Image Size (Pixels): 914 X 894; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.32µs; Frame Time: 3.52s. Illumination: Laser Wavelength: 488nm @0.2%; 639nm @0.2%. Optical/scan: Pinhole: 1.00AU / 51µm; 1.00AU / 65µm; Scan Zoom: 2.1; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 7.4 (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; 647.

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: 111 Slices (3.30 µm); Channels: 3; Scaling (Per Pixel): 35.00 nm X 35.00 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 247 X 216; Image Size (Pixels): 1566 X 1566; 247 X 216; 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: 488nm @0.3%; 639nm @0.1%. Optical/scan: Pinhole: 5.00AU / 250µm; 5.00AU / 328µm; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: 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: 59.

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; 647.

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: 131 Slices (3.9 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 2412 X 2225; 420 X 357; Image Size (Pixels): 2412 X 2225; 420 X 357; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 488 nm @2.50%; 640 nm @2.0%; Illumination Wavelength: 488; 640. 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 80%, 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; 647.

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: 131 Slices (3.9 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 2132 X 2114; 423 X 421; Image Size (Pixels): 2132 X 2114; 423 X 421; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 488 nm @2.50%; 640 nm @2.0%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 66.

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; 647.

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-000060 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

Airyscan / Scaling (Per Pixel): 35.00 nm X 35.00 nm X 30.00 nm -> 0.03500 µm X 0.03500 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)=247 X 216; Image Size (Scaled)=8.72 µm X 7.62 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.86%.

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)=2412 X 2225; Image Size (Scaled)=50.92 µm X 46.98 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

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)=2132 X 2114; Image Size (Scaled)=45.01 µm X 44.63 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

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

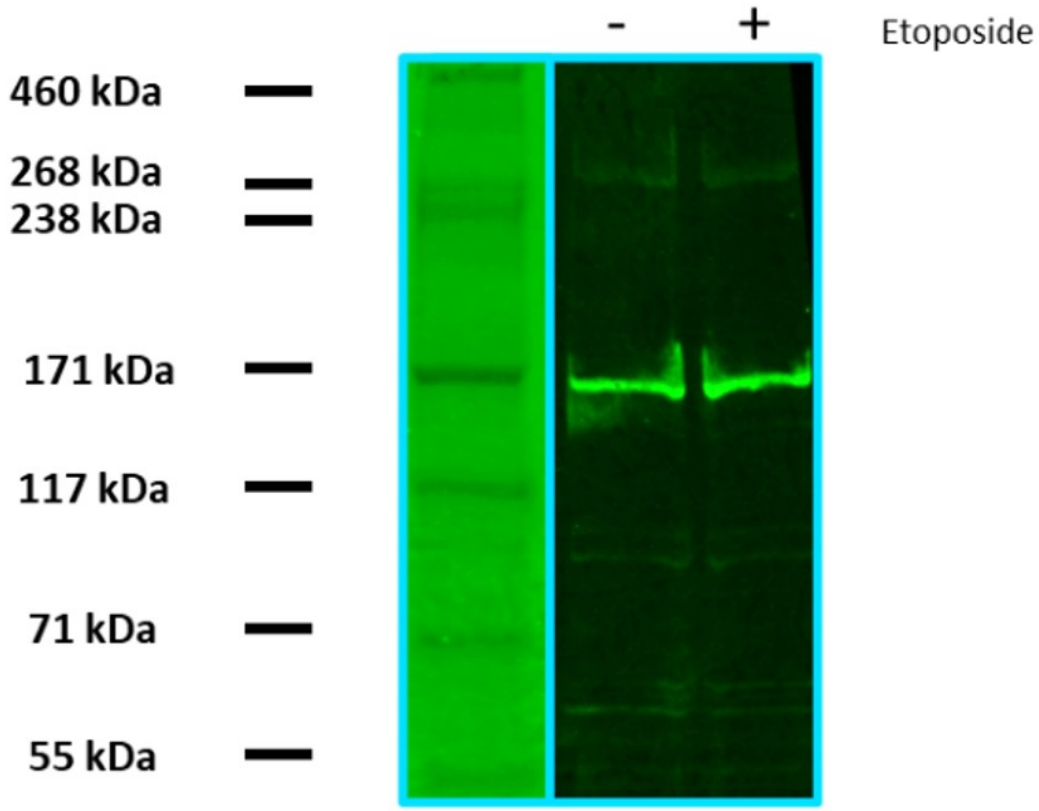
This dossier preserves the complete available evidence progression for DC-000060. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	488
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	488nm
Emission Filter	513±17nm
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™ 488, IgG
Cat ID# - DC 000060

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™ 488, 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 = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

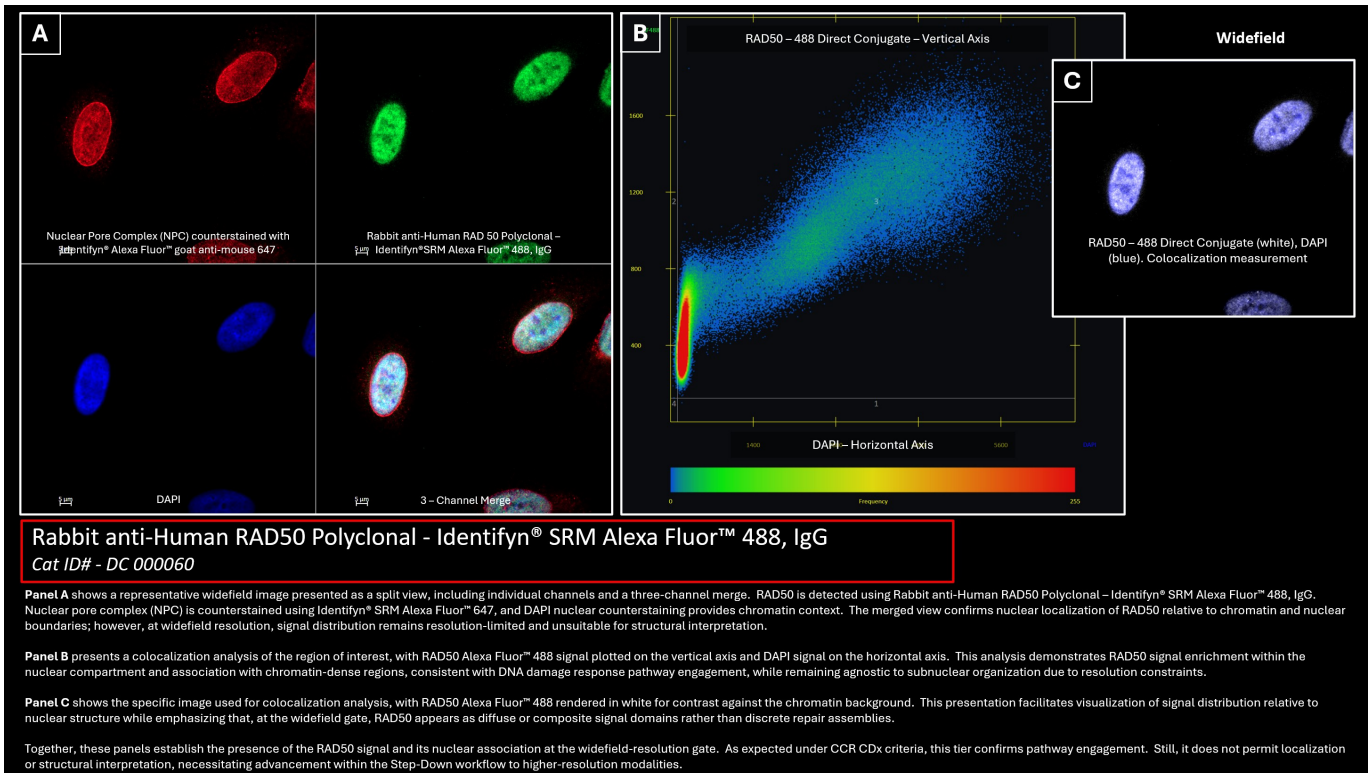
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000060
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	990223122D5102874B8D2D8BF0E1CBFBF3188F57048A94529B3F482D112E8048
Method PDF SHA-256	32CCAF1CDE5A265262D1AFAD8E6A9253D2B111BAA51BF9782B7050236016D6CD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG

Cat ID# - DC 000060

Panel A shows a representative widefield image presented as a split view, including individual channels and a three-channel merge. RAD50 is detected using Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 488, IgG. Nuclear pore complex (NPC) is counterstained using Identifyn® SRM Alexa Fluor™ 647, and DAPI nuclear counterstaining provides chromatin context. The merged view confirms nuclear localization of RAD50 relative to chromatin and nuclear boundaries; 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™ 488 signal plotted on the vertical axis and DAPI signal on the horizontal axis. This analysis demonstrates RAD50 signal enrichment within the nuclear compartment and association with chromatin-dense regions, consistent with DNA damage response pathway engagement, while remaining agnostic to subnuclear organization due to resolution constraints.

Panel C shows the specific image used for colocalization analysis, with RAD50 Alexa Fluor™ 488 rendered in white for contrast against the chromatin background. This presentation facilitates visualization of signal distribution relative to nuclear structure while emphasizing that, at the widefield gate, RAD50 appears as diffuse or composite signal domains rather than discrete repair assemblies.

Together, these panels establish the presence of the RAD50 signal and its nuclear association at the widefield-resolution gate. As expected under CCR CDx criteria, this tier confirms pathway engagement. Still, it does not permit localization or structural interpretation, necessitating advancement within the Step-Down workflow to higher-resolution modalities.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
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 @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	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.80 µm | 1.03 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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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 @25.00%
Exposure Time = 250 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

647 -

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 @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 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 NPC monoclonal antibody, visualized using Identifyn® SRM Alexa Fluor™ 647 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™ 488 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 117, Range Auto
Vertical Axis - AF488, Threshold 123, 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 Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG. Nuclear pore complex (NPC) is counterstained using Identifyn® SRM Alexa Fluor™ 647, and DAPI nuclear counterstaining provides chromatin context. The merged view confirms nuclear localization of RAD50 relative to chromatin and nuclear boundaries; 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™ 488 signal plotted on the vertical axis and DAPI signal on the horizontal axis. This analysis demonstrates RAD50 signal enrichment within the nuclear compartment and association with chromatin-dense regions, consistent with DNA damage response pathway engagement, while remaining agnostic to subnuclear organization due to resolution constraints.

Panel C shows the specific image used for colocalization analysis, with RAD50 Alexa Fluor™ 488 rendered in white for contrast against the chromatin background. This presentation facilitates visualization of signal distribution relative to nuclear structure while emphasizing that, at the widefield gate, RAD50 appears as diffuse or composite signal domains rather than discrete repair assemblies.

Together, these panels establish the presence of the RAD50 signal and its nuclear association at the widefield-resolution gate. As expected under CCR CDx criteria, this tier confirms pathway engagement. Still, it does not permit localization or structural interpretation, necessitating advancement within the Step-Down workflow to higher-resolution modalities.

Image Correction

None

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

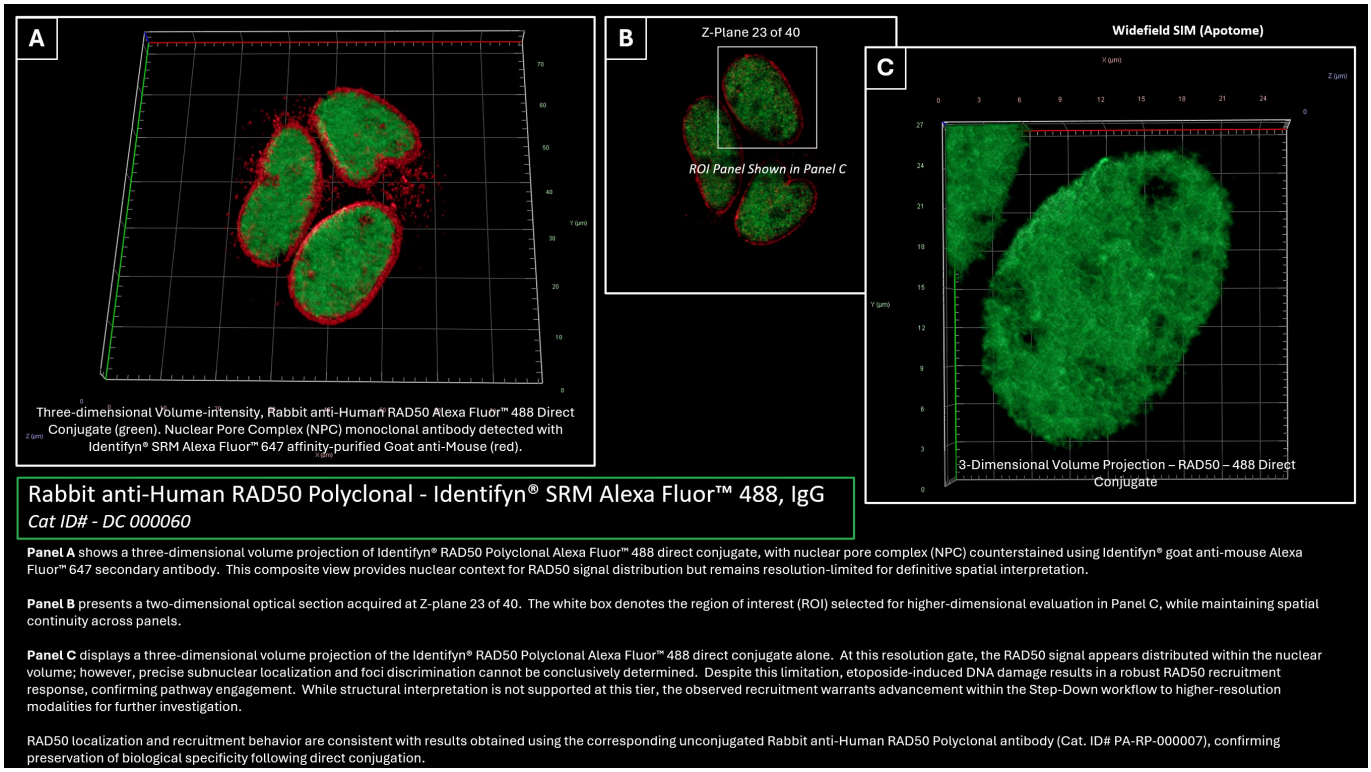
SOURCE PROVENANCE

Catalog	DC-000060
Stage	Widefield
Instrument	ZEISS Axio Imager.M1

SOURCE PROVENANCE

Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6F277E87B09BE51E2665587D851FDA74CC69FA4D01E6D691D9FD8F5B8E2FDB33
Method PDF SHA-256	1E5BE29DB403FDDF06B2E4B1592FE32DD45AE9F14DE6C00D755A5E88D64DAA36

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	49

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	40 Slices (3.9 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	551 X 510 | 183 X 192
Image Size (Scaled)	78.71 µm X 72.86 µm | 26.14 µm X 27.43 µm
Bit Depth	14 Bit
Image Center Position	X: 64.33 µm, Y: 50.76 µm
Stage Position	X: 64.33 µm, Y: 50.76 µm
ROI Center Offset	X: 1.14 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 @20.00% | X-Cite Xylis Lamp Intensity @15.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	200 ms | 150 ms | 25 ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.00 µm | 1.30 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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) - Panel A, 3 Volume Projection, Panel B, Z-plane 23 of 40

Z-Stack = 40 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 551 X 510
Image Size (Scaled) = 78.71 µm X 72.86 µm
Bit Depth = 14 Bit
Image Center Position = X: 64.33 µm, Y: 50.76 µm
Stage Position = X: 64.33 µm, Y: 50.76 µm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

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

Z-Stack = 40 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 183 X 192
Image Size (Scaled) = 26.14 µm X 27.43 µm
Bit Depth = 14 Bit
Image Center Position = X: 64.33 µm, Y: 50.76 µm
Stage Position = X: 64.33 µm, Y: 50.76 µm
ROI Center Offset = X: 1.14 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 @20.00%
Exposure Time = 200 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

647 -

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 @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

DAPI - Not Shown in Data Analysis

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 = 1.25
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 - Panel A

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

2- Dimensional View - Panel C

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific is shown in red.

Image from Z-plane 23 of 40.

3D Image Processing - Panel C

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

Summary

Panel A shows a three-dimensional volume projection of Identifyn® RAD50 Polyclonal Alexa Fluor™ 488 direct conjugate, with nuclear pore complex (NPC) counterstained using Identifyn® goat anti-mouse Alexa Fluor™ 647 secondary antibody. This composite view provides nuclear context for RAD50 signal distribution but remains resolution-limited for definitive spatial interpretation.

Panel B presents a two-dimensional optical section acquired at Z-plane 23 of 40. The white box denotes the region of interest (ROI) selected for higher-dimensional evaluation in Panel C, while maintaining spatial continuity across panels.

Panel C displays a three-dimensional volume projection of the Identifyn® RAD50 Polyclonal Alexa Fluor™ 488 direct conjugate alone. At this resolution gate, the RAD50 signal appears distributed within the nuclear volume; however, precise subnuclear localization and foci discrimination cannot be conclusively determined. Despite this limitation, etoposide-induced DNA damage results in a robust RAD50 recruitment response, confirming pathway engagement. While structural interpretation is not supported at this tier, the observed recruitment warrants advancement within the Step-Down workflow to higher-resolution modalities for further investigation.

RAD50 localization and recruitment behavior 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

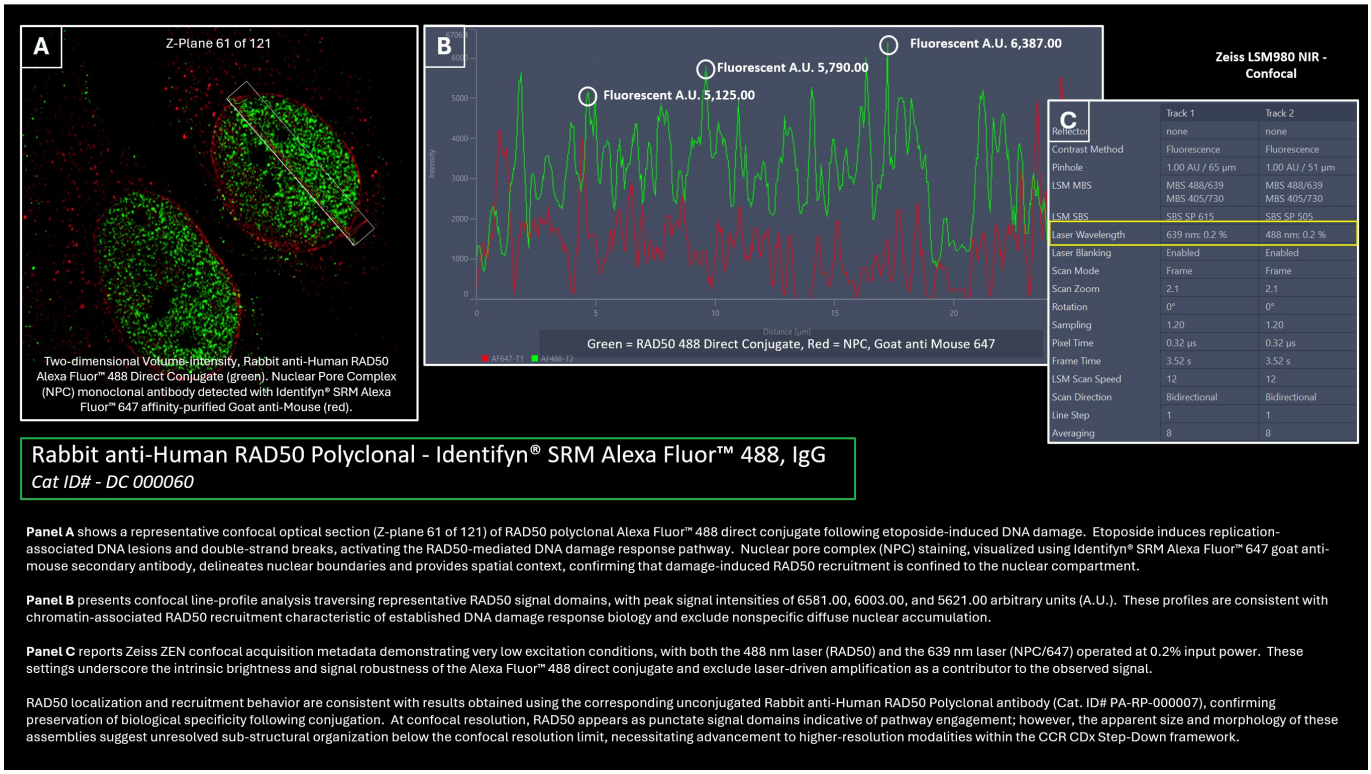
SOURCE PROVENANCE

Catalog	DC-000060
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	81185E8D06C6C5BA8ADF569ED69722F442FCD53E72ABCE357783F2E3A35E8D0D
Method PDF SHA-256	E7B1848F154EA671C3D2573F31539B365EB2A0ED58131269A467E99D7F500D58

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG
Cat ID# - DC 000060

Panel A shows a representative confocal optical section (Z-plane 61 of 121) of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage. Etoposide induces replication-associated DNA lesions and double-strand breaks, activating the RAD50-mediated DNA damage response pathway. Nuclear pore complex (NPC) staining, visualized using Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody, delineates nuclear boundaries and provides spatial context, confirming that damage-induced RAD50 recruitment is confined to the nuclear compartment.

Panel B presents confocal line-profile analysis traversing representative RAD50 signal domains, with peak signal intensities of 6581.00, 6003.00, and 5621.00 arbitrary units (A.U.). These profiles are consistent with chromatin-associated RAD50 recruitment characteristic of established DNA damage response biology and exclude nonspecific diffuse nuclear accumulation.

Panel C reports Zeiss ZEN confocal acquisition metadata demonstrating very low excitation conditions, with both the 488 nm laser (RAD50) and the 639 nm laser (NPC/647) operated at 0.2% input power. These settings underscore the intrinsic brightness and signal robustness of the Alexa Fluor™ 488 direct conjugate and exclude laser-driven amplification as a contributor to the observed signal.

RAD50 localization and recruitment behavior 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 conjugation. At confocal resolution, RAD50 appears as punctate signal domains indicative of pathway engagement; however, the apparent size and morphology of these assemblies suggest unresolved sub-structural organization below the confocal resolution limit, necessitating advancement to higher-resolution modalities within the CCR CDx Step-Down framework.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
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	121 Slices (3.60 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	914 X 894
Image Size (Scaled)	53.78 µm X 52.60 µm
Bit Depth	16 Bit
Image Center Position	X: 85.53 µm, Y: 52.63 µm
Stage Position	X: 85.53 µm, Y: 52.63 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 51µm | 1.00AU / 65µm | 1.00AU / 43µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505 | SBS SP 615 | Mirror
Laser Wavelength	488nm @0.2% | 639nm @0.2% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	1.20
Pixel Time	0.32µs
Frame Time	3.52s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	511 - 550 | 640 - 732 | 435 - 475
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) | Filter: 7.4 (3D, Auto) | Filter: 6.3 (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 26.0), Y (Min 0 and Max 6706)

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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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, 2-Dimensional view at Z-plane 61 of 121, Line Profile Panel B

Z-Stack = 121 Slices (3.60 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 914 X 894
Image Size (Scaled) = 53.78 µm X 52.60 µm
Bit Depth = 16 Bit
Image Center Position = X: 85.53 µm, Y: 52.63 µm
Stage Position = X: 85.53 µm, Y: 52.63 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32µs
Frame Time = 3.52s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.2 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32µs
Frame Time = 3.52s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.4 (3D, Auto)

DAPI - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 43µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32µs
Frame Time = 3.52s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435 - 475
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.3 (3D, Auto)

2- Dimensional View - Panel A

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific is shown in red.

Image from Z-plane 61 of 121 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 26.0), Y (Min 0 and Max 6706)

This panel also measures NPC counterstained with the 647relative fluorescence and defines the nuclear compartment in 2 dimensions.

Zen Acquisition Info Tab -

Shown is the acquisition of the 488 channel (RAD50 488 direct conjugate) using the 488 nm laser at 0.20%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A shows a representative confocal optical section (Z-plane 61 of 121) of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage. Etoposide induces replication-associated DNA lesions and double-strand breaks, activating the RAD50-mediated DNA damage response pathway. Nuclear pore complex (NPC) staining, visualized using Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody, delineates nuclear boundaries and provides spatial context, confirming that damage-induced RAD50 recruitment is confined to the nuclear compartment.

Panel B presents confocal line-profile analysis traversing representative RAD50 signal domains, with peak signal intensities of 6581.00, 6003.00, and 5621.00 arbitrary units (A.U.). These profiles are consistent with chromatin-associated RAD50 recruitment characteristic of established DNA damage response biology and exclude nonspecific diffuse nuclear accumulation.

Panel C reports Zeiss ZEN confocal acquisition metadata demonstrating very low excitation conditions, with both the 488 nm laser (RAD50) and the 639 nm laser (NPC/647) operated at 0.2% input power. These settings underscore the intrinsic brightness and signal robustness of the Alexa Fluor™ 488 direct conjugate and exclude laser-driven amplification as a contributor to the observed signal.

RAD50 localization and recruitment behavior 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 conjugation. At confocal resolution, RAD50 appears as punctate signal domains indicative of pathway engagement; however, the apparent size and morphology of these assemblies suggest unresolved sub-structural organization below the confocal resolution limit, necessitating advancement to higher-resolution modalities within the CCR CDx Step-Down framework.

Image Correction

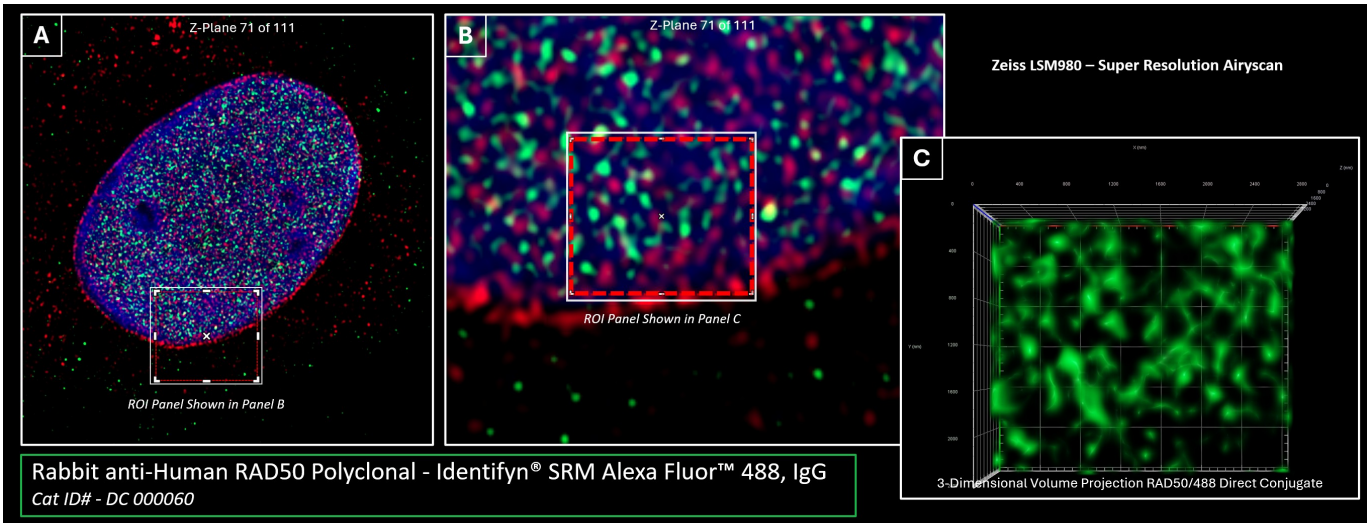
NONE

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

SOURCE PROVENANCE

Catalog	DC-000060
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	1C01A2FED7C1A668BFE16CB2B855BF50E3F8986418563ED3AB27D578025047CD
Method PDF SHA-256	AE1936FD7185EA2B4BE2929DDE6F6521F91FA8D944774D710B2C0D63877ECD44

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A shows super-resolution Airyscan imaging of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage, displayed as a representative two-dimensional optical section. Nuclear pore complex (NPC) staining, visualized using an Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody, together with DAPI nuclear counterstaining, delineates nuclear boundaries and chromatin architecture, confirming strict nuclear confinement of the RAD50 signal. Damage-induced intranuclear foci are clearly observed, consistent with canonical RAD50 recruitment as part of the MRN complex to sites of replication stress and DNA double-strand breaks. Localization and recruitment behavior are concordant with results obtained using the corresponding unconjugated Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological response following conjugation.

Panel B presents a two-dimensional Airyscan projection of the region of interest (ROI) indicated in Panel A. At this resolution gate, Airyscan provides improved lateral discrimination relative to confocal imaging, enabling partial separation of closely spaced RAD50 signal domains that appear confluent at the confocal tier. The ROI defined here is advanced for further structural assessment in Panel C.

Panel C shows a three-dimensional volume projection of the ROI from Panel B, demonstrating that RAD50 foci observed at the confocal gate now begin to exhibit emerging structural features in three dimensions. While Airyscan reveals early organization beyond punctate signal, the degree of separation and structural definition remains limited, and individual sub-units or higher-order architectural features cannot be reliably resolved. Consistent with the CCR CDx Step-Down framework, these findings warrant continued progression to higher-resolution modalities, including SIM, SIM^2, and STORM, for definitive structural interpretation of RAD50 organization.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	59

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	111 Slices (3.30 µm)
Channels	3
Scaling (Per Pixel)	0.03500 µm X 0.03500 µm X 0.03000 µm
Image size (Pixels)	1566 X 1566 | 247 X 216 | 81 X 69
Image Size (Scaled)	53.78 µm X 52.60 µm | 8.72 µm X 7.62 µm | 2.86 µm X 2.44 µm
Bit Depth	16 Bit
Image Center Position	X: 86.04 µm, Y: 50.04 µm
Stage Position	X: 86.04 µm, Y: 50.04 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 250µm | 5.00AU / 328µm | 5.00AU / 214µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505 | SBS LP 640
Laser Wavelength	488nm @0.3% | 639nm @0.1% | 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	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.4
Detection Wavelength	499 - 548 | 643 - 735 | 422 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	8.7 (3D, Auto) | 9.1 (3D, Auto) | 7.7 (3D, 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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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, 2-Dimensional view at Z-plane 71 of 111

Z-Stack = 111 Slices (3.30 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 1566 X 1566
Image Size (Scaled) = 53.78 µm X 52.60 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.04 µm, Y: 50.04 µm
Stage Position = X: 86.04 µm, Y: 50.04 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 2-Dimensional view at Z-plane 71 of 111

Z-Stack = 111 Slices (3.30 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 247 X 216
Image Size (Scaled) = 8.72 µm X 7.62 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.04 µm, Y: 50.04 µm
Stage Position = X: 86.04 µm, Y: 50.04 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 111 Slices (3.30 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 81 X 69
Image Size (Scaled) = 2.86 µm X 2.44 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.04 µm, Y: 50.04 µm
Stage Position = X: 86.04 µm, Y: 50.04 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.3%
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 = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 8.7 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @0.1%
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 = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.1 (3D, Auto)

DAPI -

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 = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 7.7 (3D, Auto)

2- Dimensional View - Panels A and B

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific is shown in red.

Image from Z-plane 71 of 111, and the white box denotes the region of interest.

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 super-resolution Airyscan imaging of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage, displayed as a representative two-dimensional optical section. Nuclear pore complex (NPC) staining, visualized using an Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody, together with DAPI nuclear counterstaining, delineates nuclear boundaries and chromatin architecture, confirming strict nuclear confinement of the RAD50 signal. Damage-induced intranuclear foci are clearly observed, consistent with canonical RAD50 recruitment as part of the MRN complex to sites of replication stress and DNA double-strand breaks. Localization and recruitment behavior are concordant with results obtained using the corresponding unconjugated Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological response following conjugation.

Panel B presents a two-dimensional Airyscan projection of the region of interest (ROI) indicated in Panel A. At this resolution gate, Airyscan provides improved lateral discrimination relative to confocal imaging, enabling partial separation of closely spaced RAD50 signal domains that appear confluent at the confocal tier. The ROI defined here is advanced for further structural assessment in Panel C.

Panel C shows a three-dimensional volume projection of the ROI from Panel B, demonstrating that RAD50 foci observed at the confocal gate now begin to exhibit emerging structural features in three dimensions. While Airyscan reveals early organization beyond punctate signal, the degree of separation and structural definition remains limited, and individual sub-units or higher-order architectural features cannot be reliably resolved. Consistent with the CCR CDx Step-Down framework, these findings warrant continued progression to higher-resolution modalities, including SIM, SIM², and STORM, for definitive structural interpretation of RAD50 organization.

Image Correction

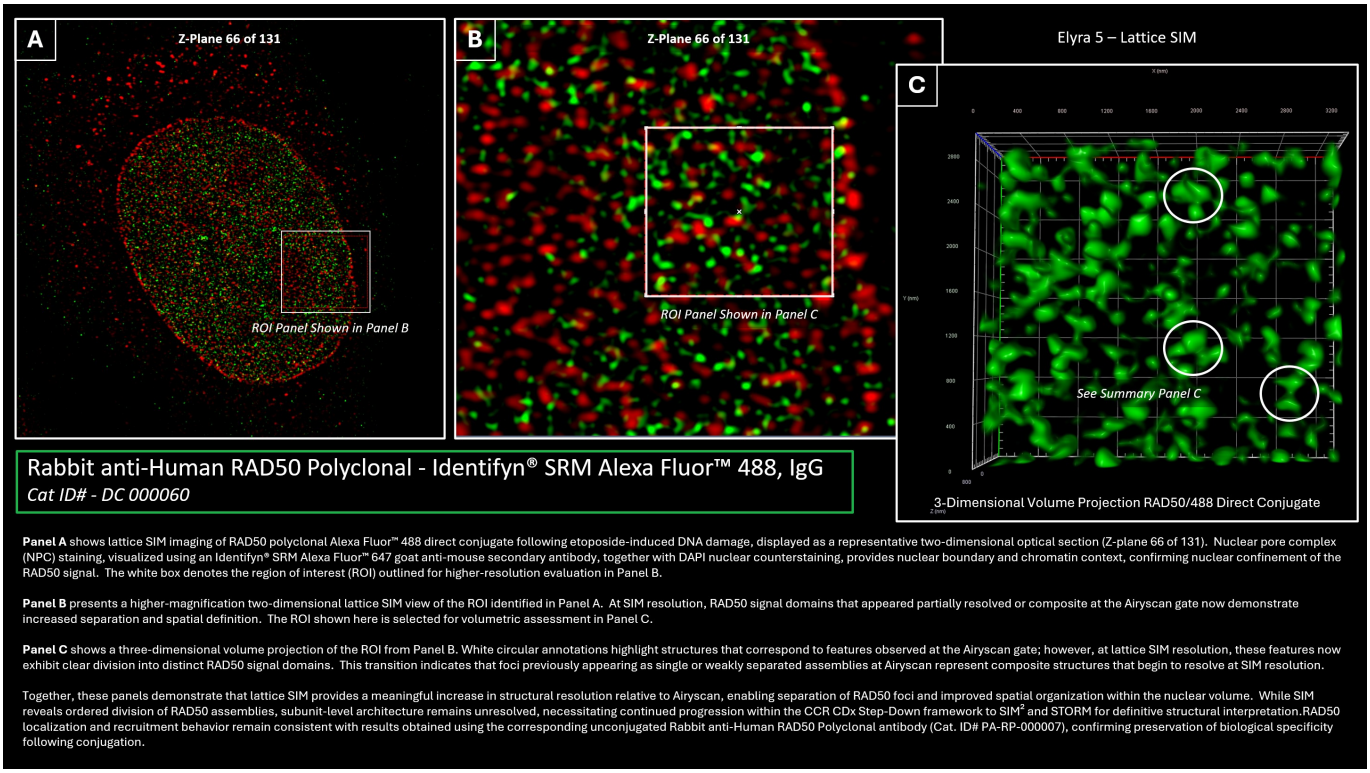
NONE

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

SOURCE PROVENANCE

Catalog	DC-000060
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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4F2D7511B337F12E6876B351CBEA999C0F32CBAC9804459FACE3EBFE38A128FF
Method PDF SHA-256	8432CB7F6E6B2C5AE3DCF2E8047EF9CDD493653FEB7FF9AEBA7C00FCEEFC7C59

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
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	131 Slices (3.9 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	2412 X 2225 | 420 X 357 | 160 X 144
Image Size (Scaled)	50.92 µm X 46.98 µm | 8.87 µm X 7.54 µm | 3.38 µm X 3.04 µm
Bit Depth	16 Bit
Image Center Position	X: 62.45 mm, Y: 40.60 mm
Stage Position	X: 62.45 mm, Y: 40.60 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	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 -SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 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	120 ms | 100 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @2.50% | 640 nm @2.0% | 405 nm @2.0%
Frame Time	1.59 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	11.1659 (488), 10.3463 (647), 9.9408 (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 80%, 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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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 66 of 131

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 2412 X 2225
Image Size (Scaled) = 50.92 µm X 46.98 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.60 mm
Stage Position = X: 62.45 mm, Y: 40.60 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI, Z-plane 66 of 131

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 420 X 357
Image Size (Scaled) = 8.87 µm X 7.54 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.60 mm
Stage Position = X: 62.45 mm, Y: 40.60 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 160 X 144
Image Size (Scaled) = 3.38 µm X 3.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.60 mm
Stage Position = X: 62.45 mm, Y: 40.60 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

647 -

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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @2.0%
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 = 11.1659 (488), 10.3463 (647), 9.9408 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2- Dimensional View - Panels A and B

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific is shown in red.

Image from Z-plane 66 of 131, and the white box denotes the region of interest.

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 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3 White circles denote areas of interest to be discussed in the summary.

Summary

Panel A shows lattice SIM imaging of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate following etoposide-induced DNA damage, displayed as a representative two-dimensional optical section (Z-plane 66 of 131). Nuclear pore complex (NPC) staining, visualized using an Identifyn® SRM Alexa Fluor™ 647 goat anti-mouse secondary antibody, together with DAPI nuclear counterstaining, provides nuclear boundary and chromatin context, confirming nuclear confinement of the RAD50 signal. The white box 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 identified in Panel A. At SIM resolution, RAD50 signal domains that appeared partially resolved or composite at the Airyscan gate now demonstrate increased separation and spatial definition. The ROI shown here is selected for volumetric assessment in Panel C.

Panel C shows a three-dimensional volume projection of the ROI from Panel B. White circular annotations highlight structures that correspond to features observed at the Airyscan gate; however, at lattice SIM resolution, these features now exhibit clear division into distinct RAD50 signal domains. This transition indicates that foci previously appearing as single or weakly separated assemblies at Airyscan represent composite structures that begin to resolve at SIM resolution.

Together, these panels demonstrate that lattice SIM provides a meaningful increase in structural resolution relative to Airyscan, enabling separation of RAD50 foci and improved spatial organization within the nuclear volume. While SIM reveals ordered division of RAD50 assemblies, subunit-level architecture remains unresolved, necessitating continued progression within the CCR CDx Step-Down framework to SIM² and STORM for definitive structural interpretation.

RAD50 localization and recruitment behavior remain 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 conjugation.

Image Correction

NONE

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

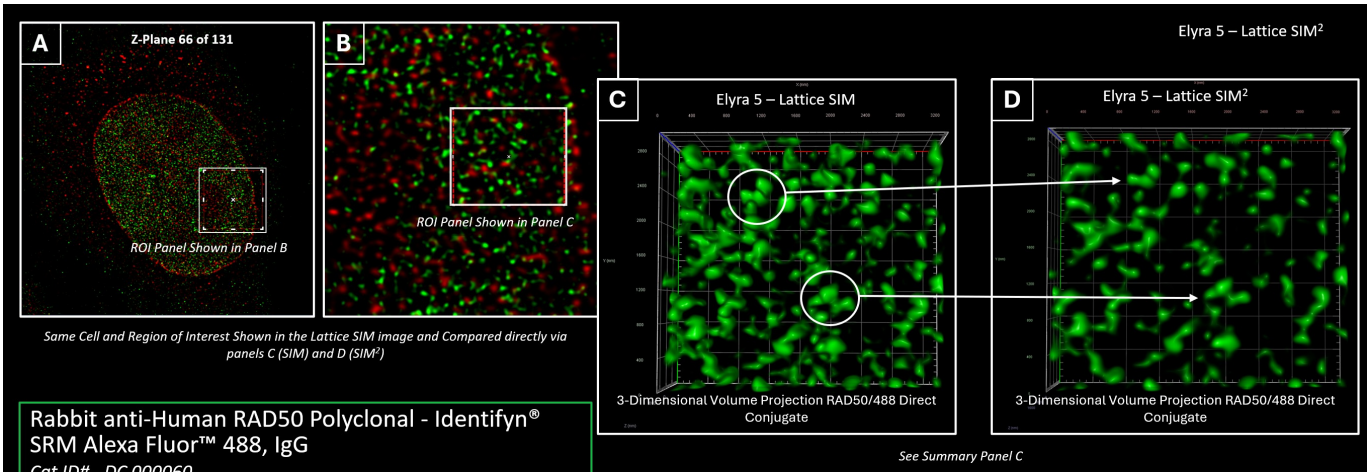
SOURCE PROVENANCE

Catalog	DC-000060
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

SOURCE PROVENANCE

Image SHA-256	97486BC46996C6704D48F8484551230A0D2745763C8344B1B7F028786C7C172F
Method PDF SHA-256	DB897DA1F88DED77EF85863E7068AA8FD9D52634369ABC40AE87ADF88EC66729

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A shows a representative two-dimensional lattice SIM² optical section of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate, using the same raw image, region of interest (ROI), and acquisition parameters as the corresponding lattice SIM dataset. The white-boxed region denotes the ROI used for comparative analysis in Panel B.

Panel B presents a higher-magnification two-dimensional view of the same ROI shown in Panel A, processed using SIM² reconstruction while maintaining identical acquisition and processing parameters used for SIM. This ensures that observed differences reflect resolution-dependent effects rather than changes in imaging conditions or post-processing.

Panel C displays the lattice SIM reconstruction of the ROI from prior analysis, included here for direct comparison. Two white circular annotations highlight representative RAD50 signal domains that appear bulbous and unresolved at SIM resolution, consistent with composite repair assemblies observed at this gate.

Panel D shows the corresponding SIM² reconstruction of the same ROI, with arrows indicating the identical spatial locations highlighted in Panel C. At SIM² resolution, these RAD50 signal domains exhibit subtle narrowing and increased spatial refinement relative to SIM. This reflects a measurable gain in resolution; however, the overall organization remains globular and unresolved, and discrete subunit-level architecture is not revealed.

Together, these panels demonstrate that while SIM² provides an incremental improvement in spatial resolution relative to SIM—manifested as narrowing of RAD50 signal domains toward patterns later observed by single-molecule localization microscopy—this gain does not constitute biological ground truth. Definitive resolution of RAD50 subunit organization and repair complex architecture requires progression to STORM, which uniquely resolves the molecular-scale features underlying the composite structures observed at SIM and SIM².RAD50 localization and recruitment behavior remain 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 conjugation.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	66

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	131 Slices (3.9 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	2132 X 2114 | 423 X 421 | 160 X 139
Image Size (Scaled)	45.01 µm X 44.63 µm | 8.93 µm X 8.89 µm | 3.38 µm X 2.93 µm
Bit Depth	16 Bit
Image Center Position	X: 62.45 mm, Y: 40.61 mm | X: 62.45 mm, Y: 40.60 mm
Stage Position	X: 62.45 mm, Y: 40.61 mm | X: 62.45 mm, Y: 40.60 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	488 | 640 | 405
Channel Name	T2-Axiocam 820-SR | T1-Axiocam 820 -SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 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	120 ms | 100 ms | 50 ms
Depth of Focus	0.81 µm | 1.13 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	488 nm @2.50% | 640 nm @2.0% | 405 nm @2.0%
Frame Time	1.59 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 80%, 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™ 488, IgG
Cat ID# - DC 000060

Identifyn® Secondary Antibodies

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

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™ 488, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 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 66 of 131

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 2132 X 2114
Image Size (Scaled) = 45.01 µm X 44.63 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.61 mm
Stage Position = X: 62.45 mm, Y: 40.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI, Z-plane 66 of 131

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 423 X 421
Image Size (Scaled) = 8.93 µm X 8.89 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.61 mm
Stage Position = X: 62.45 mm, Y: 40.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 160 X 139
Image Size (Scaled) = 3.38 µm X 2.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.45 mm, Y: 40.60 mm
Stage Position = X: 62.45 mm, Y: 40.60 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

647 -

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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @2.0%
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 - 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 - Panels A and B

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 488, IgG is shown in green, Nuclear Pore Complex (NPC) monoclonal antibody detected with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific is shown in red.

Image from Z-plane 66 of 131, and the white box denotes the region of interest.

3D Image Processing - Panel C, Image from the SIM Data Set

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 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

2 White circles denote areas of interest, followed by arrows pointing to the exact location in the SIM2 image for comparison.

3D Image Processing - Panel D, SIM2 Image

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 80%, 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 SIM² optical section of RAD50 polyclonal Alexa Fluor™ 488 direct conjugate, using the same raw image, region of interest (ROI), and acquisition parameters as the corresponding lattice SIM dataset. The white-boxed region denotes the ROI used for comparative analysis in Panel B.

Panel B presents a higher-magnification two-dimensional view of the same ROI shown in Panel A, processed using SIM² reconstruction while maintaining identical acquisition and processing parameters used for SIM. This ensures that observed differences reflect resolution-dependent effects rather than changes in imaging conditions or post-processing.

Panel C displays the lattice SIM reconstruction of the ROI from prior analysis, included here for direct comparison. Two white circular annotations highlight representative RAD50 signal domains that appear bulbous and unresolved at SIM resolution, consistent with composite repair assemblies observed at this gate.

Panel D shows the corresponding SIM² reconstruction of the same ROI, with arrows indicating the identical spatial locations highlighted in Panel C. At SIM² resolution, these RAD50 signal domains exhibit subtle narrowing and increased spatial refinement relative to SIM. This reflects a measurable gain in resolution; however, the overall organization remains globular and unresolved, and discrete subunit-level architecture is not revealed.

Together, these panels demonstrate that while SIM² provides an incremental improvement in spatial resolution relative to SIM-manifested as narrowing of RAD50 signal domains toward patterns later observed by single-molecule localization microscopy-this gain does not constitute biological ground truth. Definitive resolution of RAD50 subunit organization and repair complex architecture requires progression to STORM, which uniquely resolves the molecular-scale features underlying the composite structures observed at SIM and SIM².

RAD50 localization and recruitment behavior remain 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 conjugation.

Image Correction

NONE

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

SOURCE PROVENANCE

Catalog	DC-000060
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	66
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	85F568523D403357966D501924EEE52FBFB3E1A77646B7EED10146A302D9E8BA
Method PDF SHA-256	A28C5D911727A15DDFF532472823B6A61BAD24705386F16117CFFE5CB074ED31