

IDENTIFYN RESOLUTION STEPDOWN

RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Recombinant Monoclonal

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8

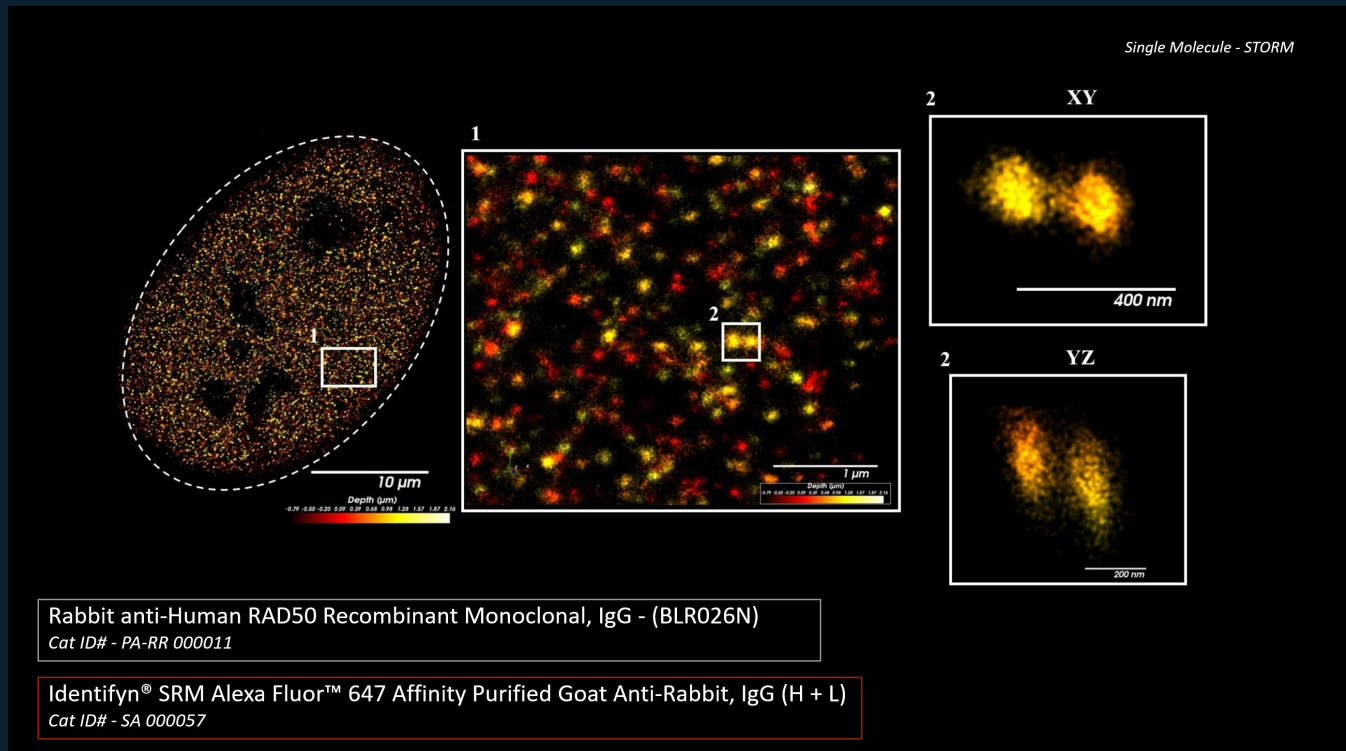
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000011 | 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.

- Sample preparation and immunofluorescence staining
- Antibody/reagent performance documentation
- Etoposide treatment / DNA-damage model
- Western blot
- Widefield
- Widefield SIM — Apotome
- Confocal
- Airyscan
- SIM
- SIM²
- Single-molecule STORM
- 3D / Z-stack acquisition
- Multi-channel microscopy
- Spatial / localization analysis
- Intensity analysis
- Control experiment
- BENNETT governed evidence assessment

SCIENTIFIC QUESTION

How does RAD50-attributed nuclear signal appear across the available Stepdown modalities after etoposide treatment, without treating spatial overlap as direct molecular interaction?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Single-molecule STORM
-> Control

BENNETT ASSESSMENT

The record demonstrates full cross-resolution CRO execution through STORM. RAD50-attributed nuclear signal is repeatedly represented, while overlap with H2AX-containing signal remains spatial evidence rather than proof of direct RAD50-H2AX interaction, break count or molecular architecture.

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 / DAMAGE-ASSOCIATED RAD50 REDISTRIBUTION / INTERACTION NOT ESTABLISHED

Across the preserved presentations, RAD50-attributed signal is nuclear and becomes increasingly focal and spatially segmented after etoposide exposure. The control reports a higher treated mean in selected regions. These observations are consistent with RAD50 participation in the MRN double-strand-break response, including processing of etoposide-induced lesions, but do not establish direct RAD50-H2AX interaction, one-focus/one-break identity or nanoscale complex architecture.

SETTINGS ASSESSMENT

The source manifest preserves 8 ordered evidence stage(s). BENNETT now receives the source-documented microscope configuration and structured acquisition metadata for every represented stage, including sampling, detector/camera, illumination, timing, scan and processing fields where present. The full source method remains intact behind each image.

BIOLOGICAL SUPPORT

BENNETT can correlate the nuclear focal redistribution with established MRN biology. It cannot infer direct binding to H2AX, count DNA breaks, identify individual MRN complexes or assign paired SIM2 shapes to tethered DNA ends.

CONTROL ASSESSMENT

The source reports means of 4,406.031 treated and 430.889 untreated; BENNETT arithmetic gives a ratio of 10.225. This is a source-selected comparison without the replication and statistics needed for a population effect claim.

CLAIM CEILING

The record supports etoposide-associated redistribution of RAD50-attributed signal from a lower-intensity diffuse nuclear pattern to stronger focal organization in the displayed source regions, with progressively finer displayed segmentation across modalities. It does not independently validate specificity, abundance, direct interaction, break number or molecular architecture.

PROCESSING DISCLOSURE

Retrospective BENNETT evaluation of preserved Identifyn presentation images, source-reported results, documented settings and independently verified primary literature. BENNETT did not reprocess native image files or repeat the historical measurements. Reconciled metadata values are derivative QA determinations from internal record consistency, not new image measurements.

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	647	NOT APPLICABLE - scanner detection record
Widefield	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 3 channel(s); Reflector: 50 Cy 5 38 eGFP 49 DAPI
Widefield SIM — Apotome	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 2 channel(s); Reflector: 50 Cy 5 38 HE eGFP
Confocal	405/DAPI; 488; 555; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 4 channel(s); Laser Wavelength: 488 nm @ 0.05% 639 nm @ 0.2% 561 nm @ 0.4% 405 nm @ 0.3%
Airyscan	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 2 channel(s); Laser Wavelength: 639 nm @ 0.50% 488 nm @ 0.05%
SIM	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 2 channel(s); Laser Wavelength: 640 nm @ 2.0% 488 nm @ 1.50%
SIM²	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 2 channel(s); Laser Wavelength: 640 nm @ 2.0% 488 nm @ 1.50%
Single-molecule STORM	647	PASS - ACQUISITION ENTRIES CONSISTENT; 1 channel(s); Color Channel: Both Channels
Control	405/DAPI; 488; 647	PASS - ACQUISITION ENTRIES CONSISTENT; 1 channel(s); Laser Wavelength: 639 nm @ 0.2%

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

Platform/configuration: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.. Key documented settings: Detection mode: Fluorescence. Laser: 638 nm. Emission Filter: 676 ± 37 nm. Detector: PMT. Pixel size: 100 μm . Scan Speed: High. Focus: Membrane (0 μm in Z-axis). Detected channels/dyes: 647. Structured source metadata values: 11.

VISIBLE OBSERVATION

The preserved blot shows untreated and etoposide-treated lanes with a source-marked band near the expected RAD50 high-molecular-weight region.

SOURCE-REPORTED RESULT

The source gives an expected RAD50 molecular weight of 154 kDa and documents Azure Sapphire FL detection with no post-processing.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Band position and presentation intensity do not independently establish antibody specificity, target identity, linearity or quantitative treatment effect.

CLAIM CEILING

The blot is consistent with a source-attributed RAD50-sized species; it does not independently establish band identity, specificity or a quantitative treatment effect.

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Channels: 3. Scaling (Per Pixel): 0.110 μm X 0.110 μm . Image size (Pixels): 836 X 836. Bit Depth: 14 Bit. Imaging Device: Axiocam 705. Exposure Time: 250 ms; 150 ms. Effective NA: 1.4. Light source: X-Cite Xylis Lamp Intensity @ 25.00%; X-Cite Xylis Lamp Intensity @ 15.00%. Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 40.

VISIBLE OBSERVATION

RAD50-attributed red puncta are distributed within green H2AX-containing nuclei, with multiple regions of yellow/orange spatial overlap in the merge.

SOURCE-REPORTED RESULT

The source reports strong nuclear co-occupancy in a selected ROI and explicitly distinguishes chromatin co-distribution from direct interaction.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Diffraction-limited overlap can merge nearby structures. Co-occurrence does not establish binding, direct interaction or molecular-scale colocalization.

CLAIM CEILING

The image supports nuclear RAD50/H2AX co-occurrence at widefield scale, not direct RAD50-H2AX binding or damage-specific targeting of H2AX.

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM - Apotome / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Z-Stack: 90 Slices (9.0 μm). Channels: 2. Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm . Bit Depth: 14 Bit. Imaging Device: Axiocam 807 Mono. Exposure Time: 200 ms; 150 ms. Effective NA: 1.25. Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected). Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 50.

VISIBLE OBSERVATION

The 3D orthogonal presentation places RAD50-attributed signal within H2AX-containing nuclear volumes across the displayed axes.

SOURCE-REPORTED RESULT

The source interprets the orthogonal views as three-dimensional co-distribution while stopping short of direct molecular interaction.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Optical sectioning improves background rejection and axial presentation but is not reconstructed super-resolution and does not prove molecular association.

CLAIM CEILING

The record supports three-dimensional nuclear co-distribution at optical-sectioning scale, not molecular association.

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Z-Stack: 111 Slices (3.3 μm). Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm . Pinhole: 1.00 AU / 50 μm ; 1.00 AU / 65 μm . Laser Wavelength: 488 nm @ 0.05%; 639 nm @ 0.2%. Imaging Device: GaAsP-Pmt3. Detector Type: GaAsP-PMT. Scan Zoom: 1.6. LSM Scan Speed: 12. Averaging: 8. Detected channels/dyes: 405/DAPI; 488; 555; 647. Structured source metadata values: 61.

VISIBLE OBSERVATION

The confocal presentation shows a source-selected nuclear RAD50 distribution and an accompanying line profile with localized intensity peaks.

SOURCE-REPORTED RESULT

The source reports a selected optical plane and line-profile analysis intended to characterize focal nuclear organization.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

The displayed section or projection is source selected. Without native arrays, BENNETT cannot repeat segmentation, intensity analysis or three-dimensional quantification.

CLAIM CEILING

The record supports source-selected focal nuclear distribution; BENNETT does not recompute the profile or infer population statistics.

MODALITY ASSESSMENT / PART 5 OF 9

Airyscan / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

Platform/configuration: Zeiss LSM 980 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dye:. Key documented settings: Z-Stack: 171 Slices (6.0 μm); 121 Slices (3.6 μm). Scaling (Per Pixel): 0.035 μm X 0.035 μm X 0.030 μm . Pinhole: 5.00 AU / 328 μm ; 5.00 AU / 250 μm . Laser Wavelength: 639 nm @ 0.50%; 488 nm @ 0.05%. Imaging Device: Airyscan. Scan Zoom: 2.1. LSM Scan Speed: 6. Averaging: 4. AiryScan Mode: Super Resolution: 10.0 (3D, Auto); Super Resolution: 9.5 (3D, Auto). Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 51.

VISIBLE OBSERVATION

The Airyscan views separate the RAD50-attributed nuclear signal into more distinct puncta and irregular assemblies than the lower-resolution presentations.

SOURCE-REPORTED RESULT

The source compares the displayed Airyscan morphology with a same-product STORM reference.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Native detector data, calibration and processing QC are unavailable; apparent fine morphology cannot establish numerical resolution or molecular structure.

CLAIM CEILING

The record supports increased displayed feature separation, not the claim that each punctum is a repair complex or one DNA break.

MODALITY ASSESSMENT / PART 6 OF 9

SIM / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Z-Stack: 142 Slices (4.23 μm). Scaling (Per Pixel): 0.02111 μm X 0.02111 μm X 0.02979 μm . Imaging Device: AxioCam 820. Exposure Time: 120 ms. Laser Wavelength: 640 nm @ 2.0%; 488 nm @ 1.50%. Algorithm: SIM. Result Sampling: 4. Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 51.

VISIBLE OBSERVATION

The SIM presentation resolves some lower-resolution foci into multiple adjacent or elongated red features.

SOURCE-REPORTED RESULT

The source describes increased heterogeneity and explicitly notes that feature dimensions exceed molecular scale because of resolution and labeling geometry.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Raw patterned frames, modulation assessment and reconstruction-artifact checks are unavailable; fine forms may be biological, reconstruction dependent or both.

CLAIM CEILING

The record supports partial separation of RAD50-attributed assemblies, not true molecular dimensions or architectural equivalence to STORM.

MODALITY ASSESSMENT / PART 7 OF 9

SIM2 / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Z-Stack: 142 Slices (4.23 μm); 40 Slices (1.17 μm) - Gallery was reduced to the Z-Planes that have RAD50 Signal. Scaling (Per Pixel): 0.02111 μm X 0.02111 μm X 0.02979 μm . Imaging Device: AxioCam 820. Exposure Time: 120 ms. Laser Wavelength: 640 nm @ 2.0%; 488 nm @ 1.50%. Algorithm: SIM2. Process Sampling: 4. Result Sampling: 4. Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 53.

VISIBLE OBSERVATION

The SIM2 presentation includes paired, elongated and irregular RAD50-attributed forms within the selected nuclear volume.

SOURCE-REPORTED RESULT

The source labels some forms as apparent doublets while acknowledging that they may arise from limited separation, point-spread effects or labeling geometry.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

The iterative reconstruction is not independently reproducible from the presentation; fine forms cannot be assigned molecular identity, dimensions or mechanism.

CLAIM CEILING

The record supports paired-looking displayed features; it does not identify DNA ends, tethered structures or RAD50 dimers.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

Platform/configuration: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:. Key documented settings: Camera: Both Cameras. Widefield Camera Exposure Time: 100 ms; 20 ms. Super Resolution Camera Exposure Time: 20 ms. Autofocus Drift Correction: On. Photoactivation/Imaging Laser: 0% Selected. Exposure Time: 20 ms Selected. Detected channels/dyes: 647. Structured source metadata values: 81.

VISIBLE OBSERVATION

The localization presentation resolves dense nuclear RAD50-attributed signal into smaller punctate and irregular substructures within selected regions.

SOURCE-REPORTED RESULT

The source documents drift correction, precision filters and DBSCAN settings used in its localization workflow.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

Localization tables, uncertainty distributions, blinking correction, density controls and independent cluster analysis are unavailable. Apparent clusters are not molecule counts or binding complexes.

CLAIM CEILING

The record supports displayed nanoscale substructure, not molecule count, cluster truth, repair-complex identity or exact localization precision.

MODALITY ASSESSMENT / PART 9 OF 9

Control / BOUNDED CORRELATION / SCIENTIST REVIEW REQUIRED

DOCUMENTED SETTINGS

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:. Key documented settings: Channels: 1. Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm . Bit Depth: 16 Bit. Imaging Device: GaAsP-Pmt3. Detected channels/dyes: 405/DAPI; 488; 647. Structured source metadata values: 52.

VISIBLE OBSERVATION

The treated presentation is more punctate and visibly stronger than the lower-intensity diffuse untreated presentation.

SOURCE-REPORTED RESULT

The source reports arithmetic mean intensities of 4,406.031 treated and 430.889 untreated, a treated/untreated ratio of 10.225 by BENNETT arithmetic, and describes an approximately tenfold source result.

LITERATURE CORRELATION

The pattern is compatible with RAD50's established role in the MRN complex during double-strand-break recognition and processing, including repair of etoposide-induced topoisomerase-II DNA adducts.

INTERPRETIVE LIMIT

A source-selected control presentation without replication, variance, blinding and inferential statistics cannot establish a population effect or analytical specificity by itself.

CLAIM CEILING

For the displayed source-selected regions, treated RAD50-attributed signal is higher and more focal. No population effect, significance, abundance or causation claim is authorized.

CROSS-MODALITY SYNTHESIS

The recurring signal pattern progresses from blended nuclear foci to increasingly separated substructure while remaining nuclear. That continuity is consistent with RAD50 damage-response localization, but each modality retains distinct acquisition and processing limits.

The modalities are not treated as a quantitative intensity ladder. Literature establishes consistency and boundaries; it does not independently validate the Identifyn record.

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.

- The MRN complex, including RAD50, participates in detection and processing of double-strand breaks and in removal/resection of etoposide-induced topoisomerase-II DNA adducts.
- H2AX-containing chromatin is not equivalent to gamma-H2AX damage foci, and spatial overlap does not prove direct RAD50-H2AX interaction.
- SIM and localization microscopy require native reconstruction/localization QC before fine forms are assigned molecular dimensions or identities.

REFERENCES

1. Aparicio T, et al. MRN, CtIP, and BRCA1 mediate repair of topoisomerase II-DNA adducts. *J Cell Biol.* 2016;212:399-408. doi:10.1083/jcb.201504005. <https://pubmed.ncbi.nlm.nih.gov/26880199/>
2. Myler LR, et al. Single-molecule imaging reveals how Mre11-Rad50-Nbs1 initiates DNA break repair. *Mol Cell.* 2017;67:891-898.e4. doi:10.1016/j.molcel.2017.08.002. <https://pubmed.ncbi.nlm.nih.gov/28867292/>
3. Moser B, et al. Fluorescence colocalization microscopy analysis can be improved by combining object-recognition with pixel-intensity-correlation. *Biotechnol J.* 2017;12:1600332. doi:10.1002/biot.201600332. <https://pubmed.ncbi.nlm.nih.gov/27420480/>
4. 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. <https://www.nature.com/articles/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 - AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02111 μ m X 0.02111 μ m X 0.02979 μ m. 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)=2462 X 2204; Image Size (Scaled)=51.98 μ m X 46.53 μ m; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02111 μ m X 0.02111 μ m X 0.02979 μ m. 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)=1374 X 1593; Image Size (Scaled)=29.01 μ m X 33.63 μ m; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED WITH SCIENTIST-SUPPLIED HISTORICAL ERROR AUTHORITY

Western blot / etoposide duration: 20 hours -> 24 hours. The broader conventional imaging/control record supports 24 hours, and the scientist responsible for the historical record identified this class of etoposide discrepancy as a historical source-entry/copy-paste error. Majority repetition alone is not the authority for this correction. Supporting evidence: Widefield through STORM and Control state 2 μ M etoposide for 24 hours. Authority: Scientist-supplied historical-record correction context - Dr. Brian T. Bennett. Preservation: The original Western 20-hour source value remains unchanged in preserved provenance.

UNRESOLVED - PRESERVED AS CONFLICT

Airyscan / Z-stack depth: 171 slices; 0.030 μ m Z spacing; 6.0 μ m depth -> Preserved as conflicting source values. The values do not reconcile within rounding tolerance and the record does not uniquely identify which entry is wrong. Supporting evidence: A second Airyscan stack (121 slices, 3.6 μ m) is consistent with 0.030 μ m spacing, but it does not resolve the first stack.

HIGH CONFIDENCE - AUTO-RECONCILED

Control / Alexa Fluor 647 anti-rabbit secondary reagent: Header: Goat Anti-Rabbit, SA-000057; Plus/Minus Damage body: Donkey Anti-Rabbit, 1:1000 -> Donkey Anti-Rabbit, SA-000060, 1:1000 for the conventional Control workflow. The control method body agrees with the repeated conventional Widefield-through-SIM2 workflow; the isolated control header matches the distinct STORM reagent and is a cross-stage copy/paste conflict. Supporting evidence: Widefield, Apotome, Confocal, Airyscan, SIM and SIM2 repeatedly identify AF647 Donkey Anti-Rabbit SA-000060 at 1:1000; STORM separately identifies AF647 Goat Anti-Rabbit SA-000057 at 1:200.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked
Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

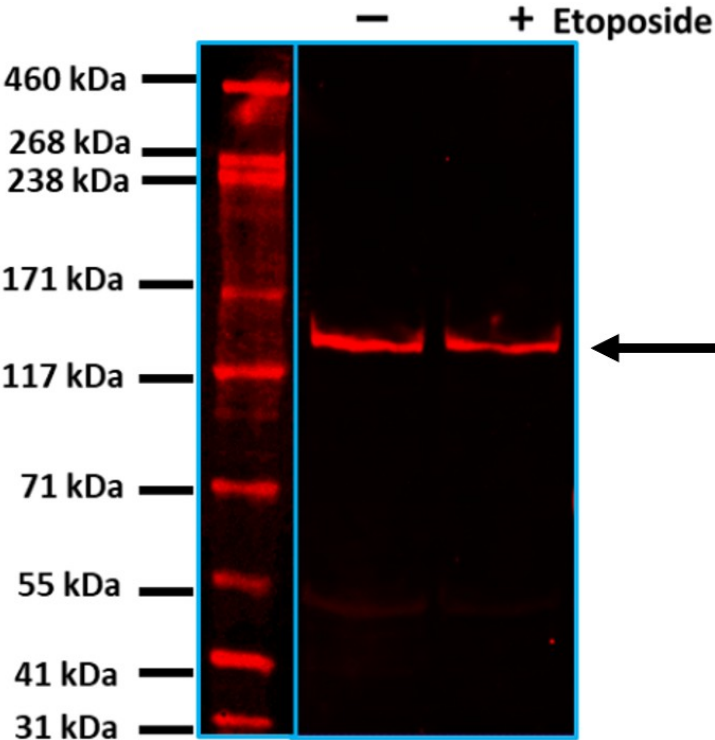
This dossier preserves the complete available evidence progression for PA-RR-000011. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	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	647
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 Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	S. Khanal

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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Expected MW: 154 kDa

HeLa cells were treated with 2 μ M etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 μ g) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis on NuPAGE™ Tris-Acetate Mini Protein Gels (3-8%, 1.0 mm) in a Mini Gel Tank. Thermo Fisher™ 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 1X PBS. The blocked membrane was incubated with Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) for 20 hours, and then washed 5 times in 1X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated for 1 hour, followed by 5 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 Imaging.

Scan Settings

Detection mode = Fluorescence

Laser = 638 nm

Emission Filter = 676 $\pm$ 37 nm

Detector = PMT

Intensity = L3

Pixel size = 100 μ m

Scan Speed = High

Quality = High

Focus = Membrane (0 μ m in Z-axis)

Post Processing

NONE

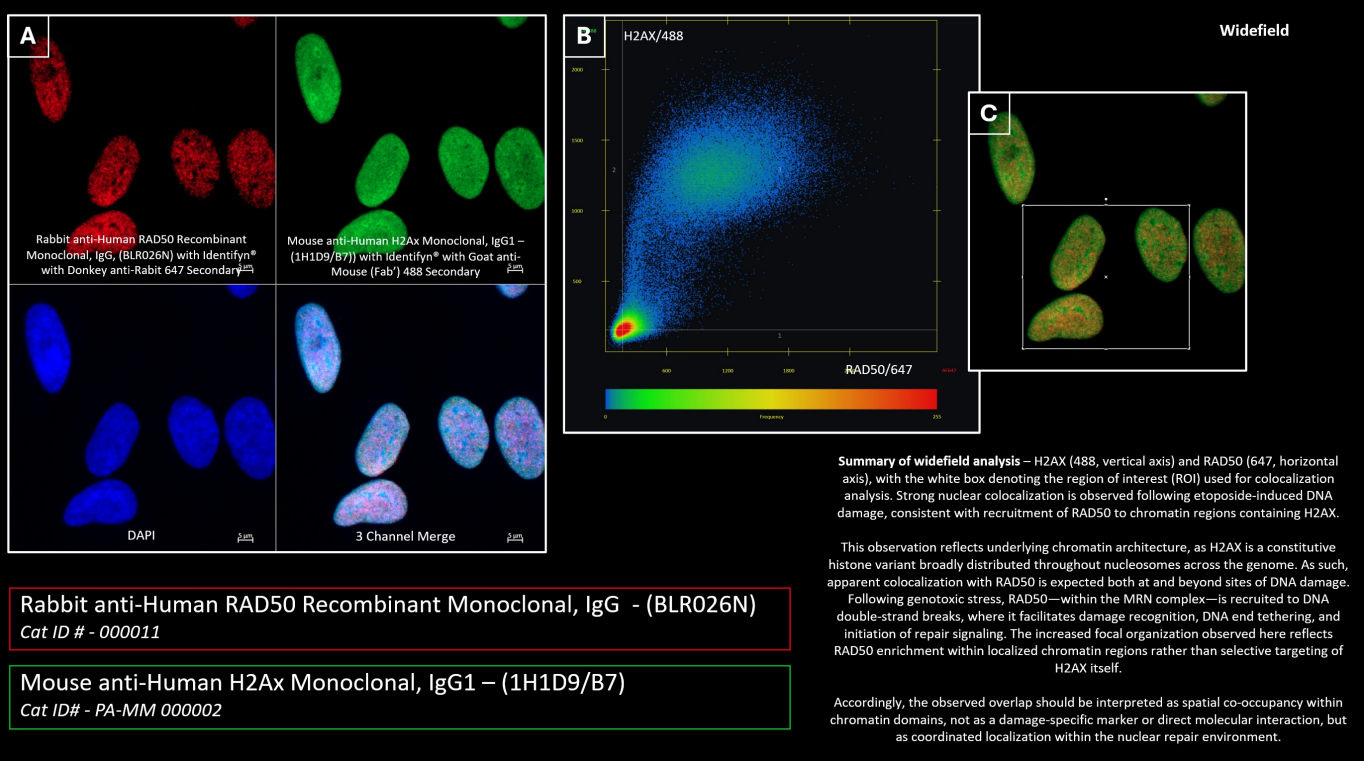
Image: S. Khanal

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	PA-RR-000011
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	42835AA7740D79D06FFE96C2AF6E72D0380FC8054B2FEBB71010F4EDF96CE3FD
Method PDF SHA-256	1C7ED3F9708A9E12EA99334598419701711E7C837097261834640EC3876D8437

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	40

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)	836 X 836
Image Size (Scaled)	91.56 μm X 91.56 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	50 Cy 5 38 eGFP 49 DAPI
Beam Splitter	660 495 395
Filter Excitation Wavelength	625 - 655 450 - 490 335 - 383
Filter Emission Wavelength	665 - 715 500 - 550 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 25.00% X-Cite Xylis Lamp Intensity @ 15.00% X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	250 ms 150 ms 25 ms
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 μm 0.80 μm 0.72 μm
Binning Mode	2,2
Image Measurement	Region of Interest, panel C, measuring the three nuclei shown.

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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, 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) = 836 X 836

Image Size (Scaled) = 91.56 μm X 91.56 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

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 @ 25.00%

Exposure Time = 250 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

488 -

Reflector = 38 eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 - 550

Contrast Method = Fluorescence

Light Source = X-Cite Xylis Lamp Intensity @ 15.00%

Exposure Time = 150 ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 0.80 μm

Binning Mode = 2,2

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 μ m
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L). The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panels B and C

Vertical Axis - AF488, Threshold 196, Range Auto
 Horizontal Axis - AF647, Threshold 156, Range Auto

Image Measurement = Region of Interest, panel C, measuring the three nuclei shown.

Summary of Widefield Analysis -

H2Ax (488, vertical axis) and RAD50 (647, horizontal axis), with the white box denoting the region of interest (ROI) used for colocalization analysis. Strong nuclear colocalization is observed following etoposide-induced DNA damage, consistent with RAD50 recruitment to chromatin regions containing H2Ax.

This observation reflects the underlying chromatin architecture, as H2Ax is a constitutive histone variant broadly distributed across nucleosomes throughout the genome. As such, apparent colocalization with RAD50 is expected both at and beyond sites of DNA damage. Following genotoxic stress, RAD50-within the MRN complex-is recruited to DNA double-strand breaks, where it facilitates damage recognition, DNA end tethering, and initiation of repair signaling. The increased focal organization observed here reflects RAD50 enrichment within localized chromatin regions rather than selective targeting of H2Ax itself.

Accordingly, the observed overlap should be interpreted as spatial co-occupancy within chromatin domains, not as a damage-specific marker or direct molecular interaction, but as coordinated localization within the nuclear repair environment.

Image Correction

NONE

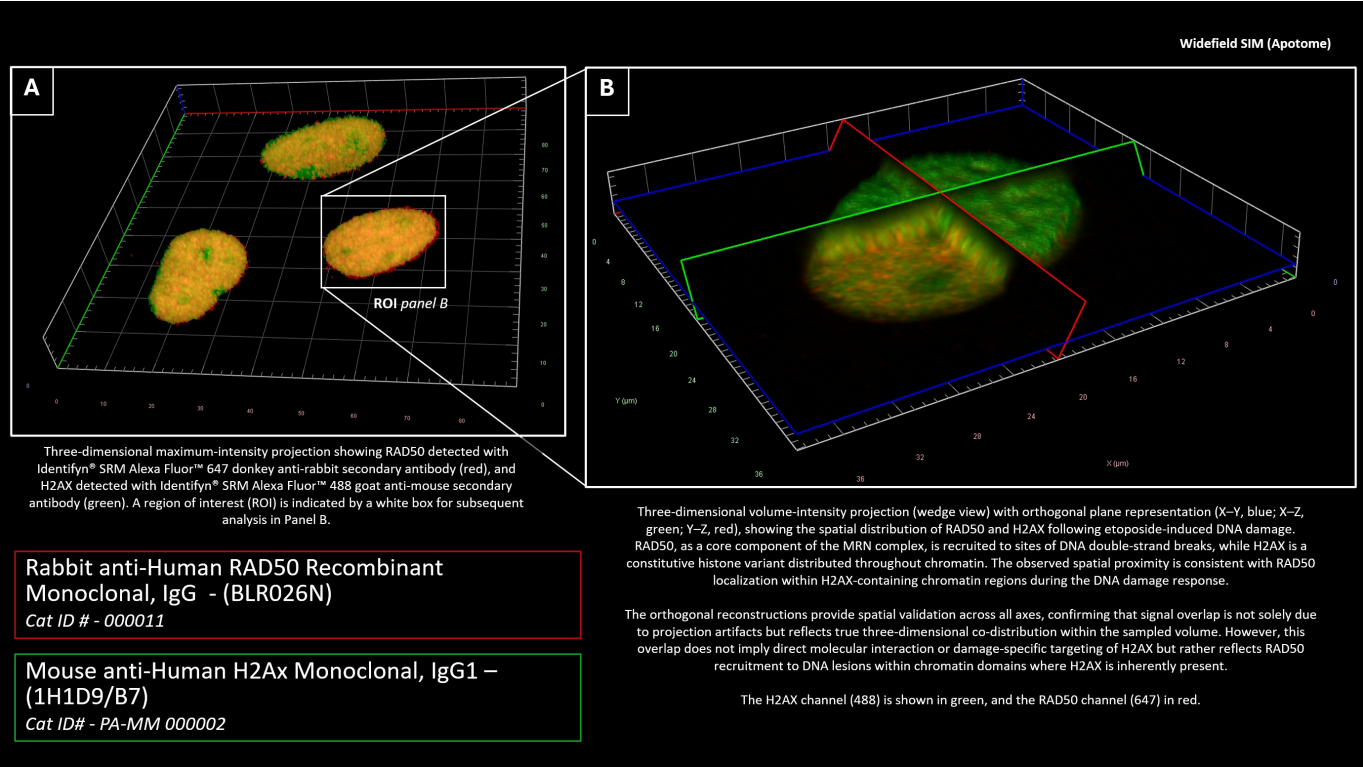
Image by E.F.Simon
Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	PA-RR-000011
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D9298DA1CEBBC143A6A96E48D4680D309A543149283844F0A9951FD6076CE3FA
Method PDF SHA-256	211AB91302804BE1895A8BCFE145B513ECCD647AF480FB0EFE3FF17976899EED

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	50

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	90 Slices (9.0 μm)
Channels	2
Scaling (Per Pixel)	0.143 μm X 0.143 μm X 0.100 μm
Image size (Pixels)	629 X 625 273 X 264
Image Size (Scaled)	89.86 μm X 89.29 μm 39.00 μm X 37.71 μm
Bit Depth	14 Bit
Image Center Position	X: 59.32 mm, Y: 52.40 mm
Stage Position	X: 59.32 mm, Y: 52.40 mm
ROI Center Offset	X: 1.14 μm , Y: 0.00 μm
Reflector	50 Cy 5 38 HE eGFP
Beam Splitter	660 495
Filter Excitation Wavelength	625 - 655 450 - 490
Filter Emission Wavelength	665 - 715 500 - 550
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 20.00% X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time	200 ms 150 ms
Excitation Wavelength	653 493
Emission Wavelength	668 517
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.30 μm 1.00 μm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

FIELD	SOURCE-DOCUMENTED VALUE
X-Y	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-41% 0%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	30° -30°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 90 Slices (9.0 µm)

Channels = 2

Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm

Image size (Pixels) = 629 X 625

Image Size (Scaled) = 89.86 µm X 89.29 µm

Bit Depth = 14 Bit

Image Center Position = X: 59.32 mm, Y: 52.40 mm

Stage Position = X: 59.32 mm, Y: 52.40 mm

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

Z Stack - (3D) - Panel B, ROI

Z-Stack = 90 Slices (9.0 µm)

Channels = 2

Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm

Image size (Pixels) = 273 X 264

Image Size (Scaled) = 39.00 µm X 37.71 µm

Bit Depth = 14 Bit

Image Center Position = X: 59.32 mm, Y: 52.40 mm

Stage Position = X: 59.32 mm, Y: 52.40 mm

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

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 @ 20.00%

Exposure Time = 200 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

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light Source = X-Cite Xylis Lamp Intensity @ 15.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.25
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 1.00 μ m
 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 Maximum Projection = Precise
 Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

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

Clipping Image Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) and the Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = -41%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = 30°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = -30°

Clip Z = 0°

Summary

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation (X-Y, blue; X-Z, green; Y-Z, red), showing the spatial distribution of RAD50 and H2Ax following etoposide-induced DNA damage. RAD50, as a core component of the MRN complex, is recruited to sites of DNA double-strand breaks, while H2Ax is a constitutive histone variant distributed throughout chromatin. The observed spatial proximity is consistent with RAD50 localization within H2Ax-containing chromatin regions during the DNA damage response.

The orthogonal reconstructions provide spatial validation across all axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this overlap does not imply direct molecular interaction or damage-specific targeting of H2Ax; rather, it reflects RAD50 recruitment to DNA lesions within chromatin domains where H2Ax is inherently present.

The H2Ax channel (488) is shown in green, and the RAD50 channel (647) in red.

Image Correction

NONE

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

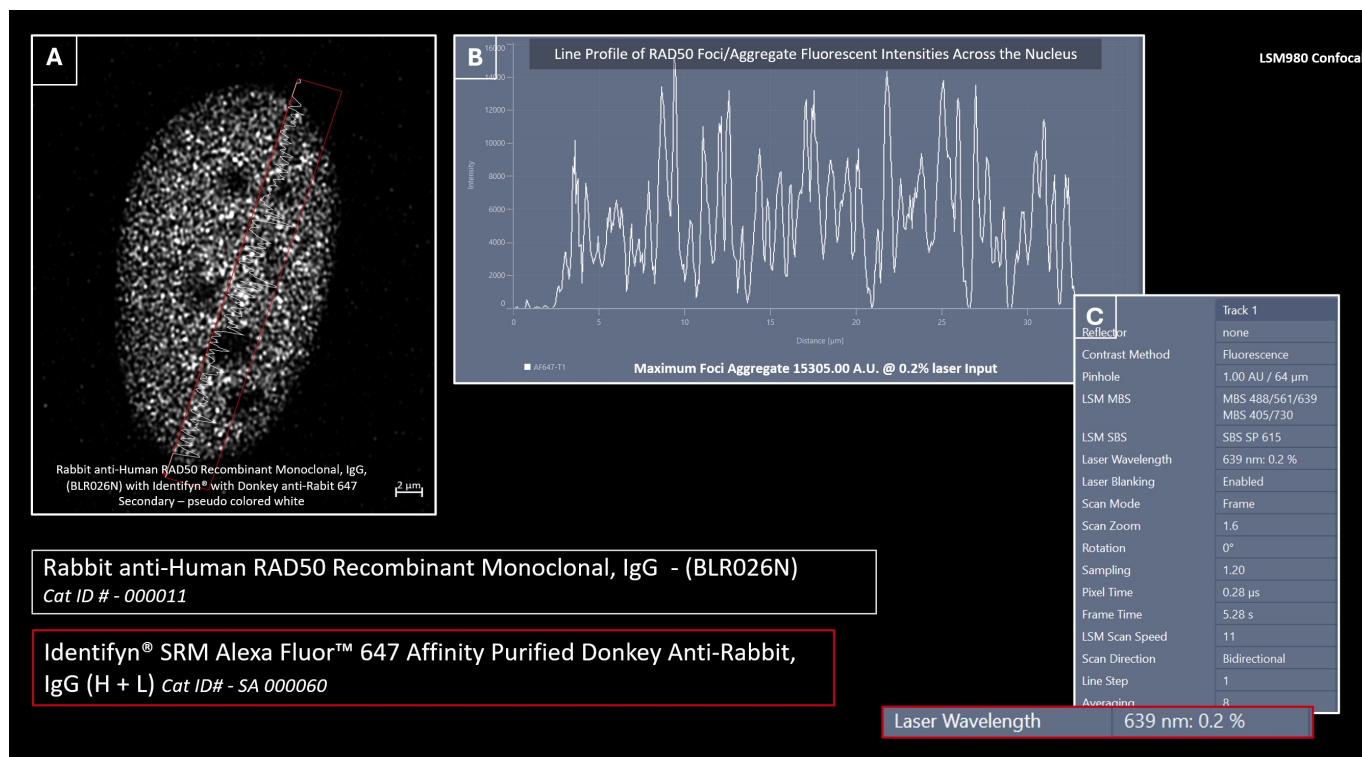
SOURCE PROVENANCE

Catalog	PA-RR-000011
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

SOURCE PROVENANCE

Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	60B97BBE5F7D9E5EB214478F8A62434FD0B8A0DBDEF7421A725AA9C696960422
Method PDF SHA-256	8F3B42D15E2C97C3F6476B885828FD10D8D4C82B5A9F97570040E45E6E40AA3B

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	61

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	111 Slices (3.3 μm)
Channels	4
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image size (Pixels)	528 X 673
Image Size (Scaled)	31.06 μm X 39.59 μm
Bit Depth	16 Bit
Image Center Position	X: 72.53 mm, Y: 52.26 mm
Stage Position	X: 72.53 mm, Y: 52.26 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 50 μm 1.00 AU / 65 μm 1.00 AU / 56 μm 1.00 AU / 44 μm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS SP 505 SBS SP 615 SBS SP 550 Mirror
Laser Wavelength	488 nm @ 0.05% 639 nm @ 0.2% 561 nm @ 0.4% 405 nm @ 0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.20
Pixel Time	0.28 μs
Frame Time	5.28 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 653 553 353
Emission Wavelength	517 668 568 465
Effective NA	1.4
Detection Wavelength	511 - 545 645 - 700 560 - 600 435 - 480
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V 550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.4 (3D, Auto) Filter: 8.0 (3D, Auto) Filter: 7.7 (3D, Auto) Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 35.8), Y (Min 0 and Max 16070)
Maximum Peak	15,305.00 A.U.

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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A, Z-Plane 68 of 111

Z-Stack = 111 Slices (3.3 µm)

Channels = 4

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image size (Pixels) = 528 X 673

Image Size (Scaled) = 31.06 µm X 39.59 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.53 mm, Y: 52.26 mm

Stage Position = X: 72.53 mm, Y: 52.26 mm

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

488 - Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00 AU / 50 µm

LMS MBS = MBS 488/561/639 & 405/730

LMS SBS = SBS SP 505

Laser Wavelength = 488 nm @ 0.05%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.6

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.28 µs

Frame Time = 5.28 s

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 - 545

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)

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 65 µm
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS SP 615
 Laser Wavelength = 639 nm @ 0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 µs
 Frame Time = 5.28 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 645 - 700
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 8.0 (3D, Auto)

555 - Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 56 µm
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS SP 550
 Laser Wavelength = 561 nm @ 0.4%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 µs
 Frame Time = 5.28 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553

Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 560 - 600
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.7 (3D, Auto)

DAPI - Collected but not shown in Confocal Data Analysis (See Multiplex for all Channels)

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 44 μ m
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405 nm @ 0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 μ s
 Frame Time = 5.28 s
 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 - 480
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.8 (3D, Auto)

2D View - Panel A

Panel A shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The image corresponds to Z-plane 68 of 111, with the line profile indicated and measured in Panel B.

Profile View Display - Panels B & C

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 35.8), Y (Min 0 and Max 16070)

This panel quantifies RAD50 foci, or resolution-limited aggregates, following etoposide-induced DNA damage. Line profile analysis reveals well-defined intensity peaks and spatial confinement, supporting localized enrichment of RAD50 at damage-associated regions.

Maximum Peak = 15,305.00 A.U.

Zen Acquisition Info Tab - Panel D

Shown are the acquisition parameters for the 647 channel (RAD50), collected using the 639 nm laser at 0.2% power. Scan zoom, scan speed, and line-averaging settings are provided to ensure transparency and reproducibility of line-profile measurements.

Summary

This dataset presents high-resolution confocal images of RAD50 in HeLa cells following etoposide-induced DNA damage, acquired under tightly controlled, reproducible conditions. Using a Zeiss LSM 980 system with 63x/1.40 NA optics, a 3D z-stack (111 slices, 3.3 μm total depth) was collected with consistent acquisition parameters, including low laser power (647 channel at 0.2%), 16-bit depth, and line averaging (8 $\times$), ensuring high signal fidelity with minimal photobleaching or saturation.

RAD50, detected in the 647 channel, exhibits discrete nuclear foci consistent with its recruitment to DNA double-strand breaks as part of the MRN complex. Line profile analysis across selected regions demonstrates well-defined intensity peaks (maximum $\sim 15,305$ A.U.), confirming localized enrichment and spatial confinement of RAD50 within damage-associated regions.

Although additional channels (H2Ax, NPC, DAPI) were acquired as part of a multiplex dataset, the present analysis focuses on RAD50 to provide quantitative clarity and to avoid conflating signal interpretation. Pseudo-coloring of the 647 channel was applied to enhance contrast for measurement without altering underlying signal characteristics.

Biologically, these observations align with established RAD50 function in DNA damage recognition, end tethering, and initiation of repair signaling. The presence of resolution-limited foci reflects accumulation at sites of DNA damage rather than discrete molecular structures, consistent with the limits of confocal imaging and antibody-based labeling.

Overall, this dataset demonstrates a high-quality, reproducible confocal acquisition capturing RAD50 recruitment dynamics, suitable for quantitative analysis and downstream integration into multiplex imaging workflows.

Image Correction

The RAD50 channel (647) was pseudocolored white to enhance visual contrast for measurements.

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

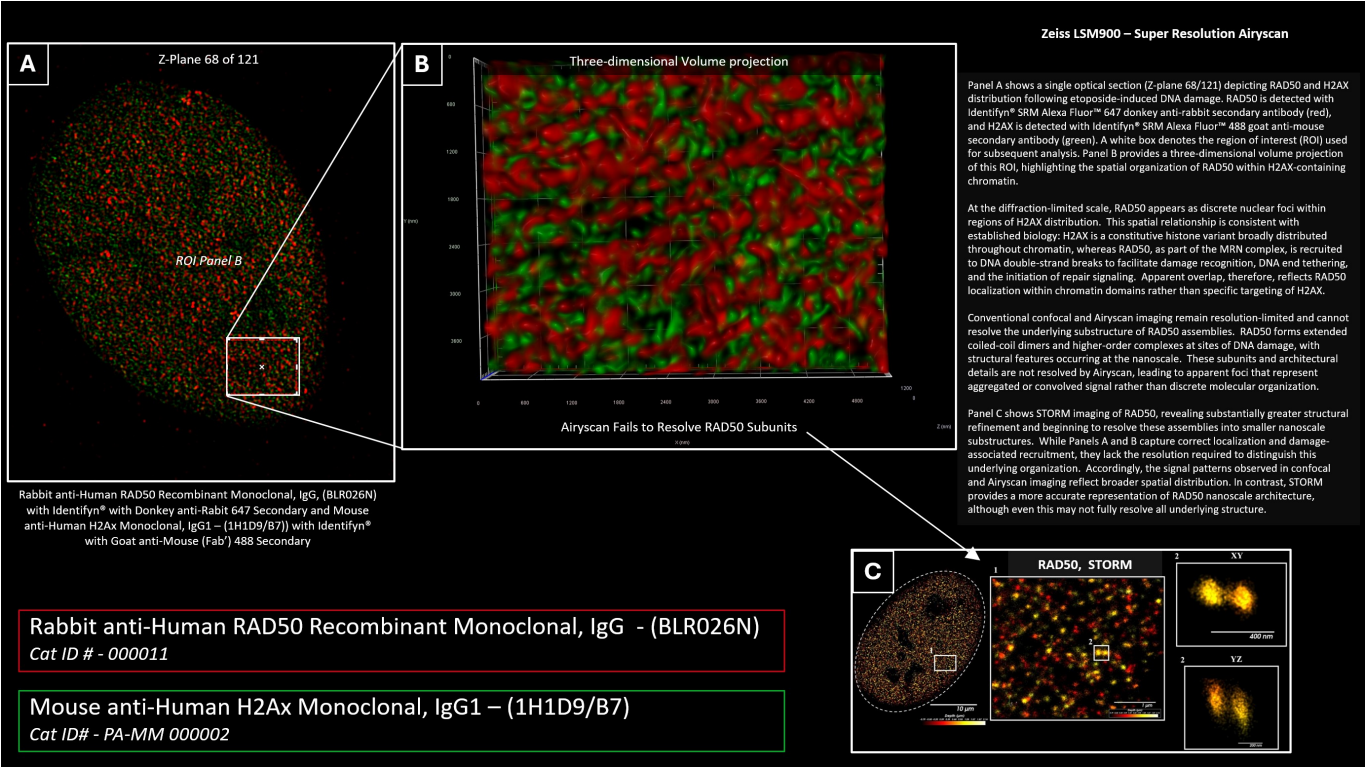
SOURCE PROVENANCE

Catalog	PA-RR-000011
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 paramet

SOURCE PROVENANCE

Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E0A402C8AF49969255D7230B273E4B224C9C4CFF38816C9C260C863FC19A10DD
Method PDF SHA-256	2210987D1D27F249D007E6BD577A37DD20F7A62D3398D05AF761122BEA42FF2E

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	51

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, Axio Observer.Z1/7, using with a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	171 Slices (6.0 µm) 121 Slices (3.6 µm)
Channels	2
Scaling (Per Pixel)	0.035 µm X 0.035 µm X 0.030 µm
Image size (Pixels)	856 X 958 149 X 117
Image Size (Scaled)	30.22 µm X 33.85 µm 5.26 µm X 4.13 µm
Bit Depth	16 Bit
Image Center Position	X: 72.54 mm, Y: 53.04 mm
Stage Position	X: 72.54 mm, Y: 53.04 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm 5.00 AU / 250 µm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS LP 640 SBS SP 550
Laser Wavelength	639 nm @ 0.50% 488 nm @ 0.05%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	2.00
Pixel Time	1.15 µs
Frame Time	17.86 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 493
Emission Wavelength	668 517
Effective NA	1.4
Detection Wavelength	643 - 745 499 - 548
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	Super Resolution: 10.0 (3D, Auto) Super Resolution: 9.5 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 4% all channels, Ramp 96% 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 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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 the identification and processing of 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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

Zeiss LSM 980 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 68 of 121, Panel A

Z-Stack = 171 Slices (6.0 µm)

Channels = 2

Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm

Image size (Pixels) = 856 X 958

Image Size (Scaled) = 30.22 µm X 33.85 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.54 mm, Y: 53.04 mm

Stage Position = X: 72.54 mm, Y: 53.04 mm

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

Z Stack - (3D) - Panel B

Z-Stack = 121 Slices (3.6 µm)

Channels = 2

Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm

Image size (Pixels) = 149 X 117

Image Size (Scaled) = 5.26 µm X 4.13 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.54 mm, Y: 53.04 mm

Stage Position = X: 72.54 mm, Y: 53.04 mm

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

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 328 μ m
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS LP 640
 Laser Wavelength = 639 nm @ 0.50%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.1
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.15 μ s
 Frame Time = 17.86 s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 643 - 745
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 250 μ m
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS SP 550
 Laser Wavelength = 488 nm @ 0.05%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.1
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.15 μ s
 Frame Time = 17.86 s
 LSM Scan Speed = 6
 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
 Airyscan Mode = Super Resolution: 9.5 (3D, Auto)

2-Dimensional View- Panel A

Shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L). A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 4% all channels, Ramp 96% 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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset, illustrating the resolution limitations of Airyscan imaging. While Airyscan improves localization precision and structural detail compared with conventional confocal microscopy, it still cannot fully resolve the nanoscale organization of RAD50 assemblies revealed by STORM.

Summary

Panel A shows a single optical section (Z-plane 68/121) depicting RAD50 and H2Ax distribution following etoposide-induced DNA damage. RAD50 is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and H2Ax is detected with Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody (green). A white box denotes the region of interest (ROI) used for subsequent analysis. Panel B provides a three-dimensional volume projection of this ROI, highlighting the spatial organization of RAD50 within H2Ax-containing chromatin.

At the diffraction-limited scale, RAD50 appears as discrete nuclear foci within regions of H2Ax distribution. This spatial relationship is consistent with established biology: H2Ax is a constitutive histone variant broadly distributed throughout chromatin, whereas RAD50, as part of the MRN complex, is recruited to DNA double-strand breaks to facilitate damage recognition, DNA end tethering, and the initiation of repair signaling. Apparent overlap, therefore, reflects RAD50 localization within chromatin domains rather than specific targeting of H2Ax.

Conventional confocal and Airyscan imaging remain resolution-limited and cannot resolve the underlying substructure of RAD50 assemblies. RAD50 forms extended coiled-coil dimers and higher-order complexes at sites of DNA damage, with structural features occurring at the nanoscale. These subunits and architectural details are not resolved by Airyscan, leading to apparent foci that represent aggregated or convolved signal rather than discrete molecular organization.

Panel C shows STORM imaging of RAD50, revealing substantially greater structural refinement and the beginning of resolution of these assemblies into smaller nanoscale substructures. While Panels A and B capture correct localization and damage-associated recruitment, they lack the resolution required to distinguish this underlying organization. Accordingly, the signal patterns observed in confocal and Airyscan imaging reflect broader spatial distribution. In contrast, STORM provides a more accurate representation of RAD50 nanoscale architecture, although even this may not fully resolve all underlying structure.

Image Correction

NONE

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

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

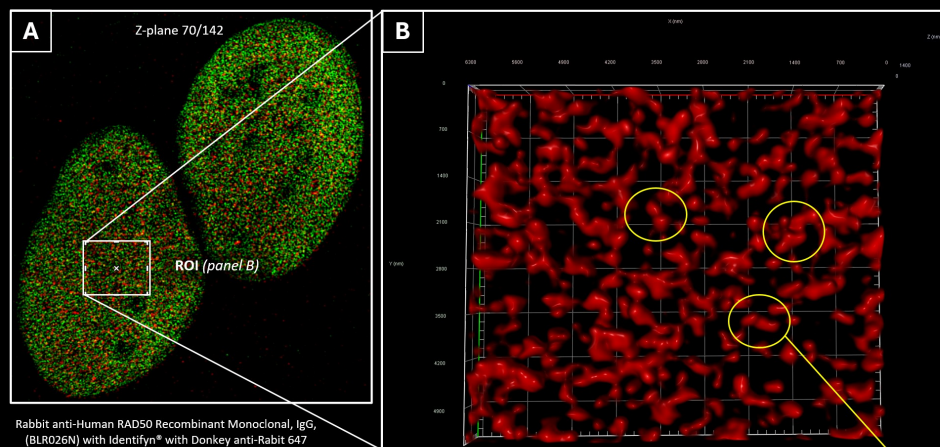
Catalog	PA-RR-000011
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dy
Structured source metadata vales	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	BEB024B54A8A7D7D1E88B6FB374A7A8254BA905011FA574BFC96DB5745679F25
Method PDF SHA-256	E8B647E9813C823A938A14938CB694D2C6FF5C11CC173870C5033491ED49232B

ORIGINAL IMAGE EVIDENCE / SIM

Elyra 5 – Lattice SIM

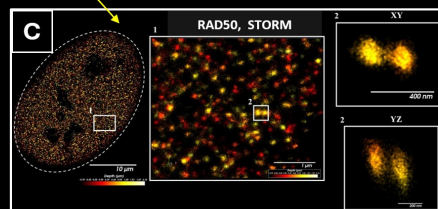


Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG, (BLR026N) with Identifyn® with Donkey anti-Rabbit 647 Secondary and Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7) with Identifyn® with Goat anti-Mouse (Fab') 488 Secondary

Three-dimensional volume projection, ROI Panel A, Omitting H2AX/488 for Visual Clarity of RAD50

Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N)
Cat ID # - 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 – (1H1D9/B7)
Cat ID# - PA-MM 000002



Panels A–C: RAD50 (647) and H2AX (488) following etoposide-induced DNA damage. Panel A shows a single optical section (Z-plane 70/142) with both channels. Panel B presents a 3D volume projection of the ROI, with H2AX omitted to enhance visualization of RAD50 organization. Lattice SIM resolves structures that appear as single foci under confocal and Airyscan into more spatially distinct features; however, these remain resolution-limited.

Panel C shows STORM imaging of RAD50, revealing nanoscale substructure not resolved by SIM. While SIM exhibits some apparent similarities to STORM, these are qualitative and likely influenced by resolution-driven conflation and interpretive bias, and do not confirm structural equivalence. Given RAD50's extended coiled-coil architecture, SIM-resolved features exceed true molecular dimensions due to point spread function and labeling geometry. Thus, SIM suggests underlying organization but does not fully resolve nanoscale structure, which is more accurately captured by STORM.

See methods for Full Summary

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	51

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	142 Slices (4.23 μm)
Channels	2
Scaling (Per Pixel)	0.02111 μm X 0.02111 μm X 0.02979 μm
Image size (Pixels)	2462 X 2204 302 X 258
Image Size (Scaled)	51.98 μm X 46.53 μm 6.38 μm X 5.45 μm
Bit Depth	16 Bit
Image Center Position	X: 66.49 mm, Y: 35.66 mm
Stage Position	X: 66.49 mm, Y: 35.66 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 488
Channel Name	T1-Axiocam 820 -SR T2-Axiocam 820-SR
Excitation Wavelength	640 488
Emission Wavelength	729 525
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	1.13 μm 0.81 μm
Binning Mode	2,2
Laser Wavelength	640 nm @ 2.0% 488 nm @ 1.50%
Frame Time	1.59 s
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	10.5107 (647), 11.1617 (488)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 4% all channels, Ramp 96% 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 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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 the identification and processing of 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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

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) - 2D at Z-Plane 70 of 142, Panel A

Z-Stack = 142 Slices (4.23 µm)

Channels = 2

Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm

Image size (Pixels) = 2462 X 2204

Image Size (Scaled) = 51.98 µm X 46.53 µm

Bit Depth = 16 Bit

Image Center Position = X: 66.49 mm, Y: 35.66 mm

Stage Position = X: 66.49 mm, Y: 35.66 mm

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

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

Z-Stack = 142 Slices (4.23 µm)

Channels = 2

Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm

Image size (Pixels) = 302 X 258

Image Size (Scaled) = 6.38 µm X 5.45 µm

Bit Depth = 16 Bit

Image Center Position = X: 66.49 mm, Y: 35.66 mm

Stage Position = X: 66.49 mm, Y: 35.66 mm

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

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 = 120 ms

Depth of Focus = 1.13 µm

Binning Mode = 2,2

Laser Wavelength = 640 nm @ 2.0%

Frame Time = 1.59 s

Scan Direction = Unidirectional

Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μ m
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @ 1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 2
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 10.5107 (647), 11.1617 (488)
 Fitting = Fast Fit
 Normalization = Auto

2-Dimensional View - Panel A

Shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7) visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L). A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 4% all channels, Ramp 96% 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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset to demonstrate the resolution limitations of Lattice SIM; however, Lattice SIM provides improved localization precision and structural detail relative to Airyscan imaging. Moreover, the signal pattern is consistent with the Airyscan data and is more closely resolution-limited than that of a single-molecule STORM image.

Summary

Using lattice structured illumination microscopy (SIM), Panel A shows a single optical section (Z-plane 70/142) depicting RAD50 (647) and H2Ax (488) following etoposide-induced DNA damage. RAD50 is visualized with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody, and H2Ax with Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody. Panel B presents a three-dimensional volume projection of the region of interest, with the H2Ax channel omitted to enhance visualization of RAD50 structural organization.

Structures that appear as single foci under confocal and Airyscan imaging are further resolved by SIM into spatially distinct features, revealing increased heterogeneity within RAD50-associated assemblies. However, despite this improvement, the observed structural detail remains constrained by SIM's effective resolution.

In Panel C, single-molecule STORM imaging of RAD50 provides substantially greater structural refinement, resolving these assemblies into smaller, discrete nanoscale substructures. While lattice SIM begins to reveal patterns that show some similarity to STORM-resolved organization, these correspondences remain qualitative and should be interpreted with caution. The apparent similarities may reflect an underlying structural organization, but they are prone to resolution-driven conflation and may also reflect interpretive bias rather than true architectural equivalence.

Importantly, RAD50 (~153 kDa) forms extended coiled-coil dimers and higher-order assemblies, yet the apparent dimensions of SIM-resolved features substantially exceed the expected molecular scale. This reflects the combined effects of the point spread function and labeling geometry (including antibody size and fluorophore displacement), indicating that these structures do not represent true molecular dimensions. As such, SIM captures partially resolved yet resolution-limited aggregates, whereas STORM begins to resolve the underlying nanoscale organization.

Collectively, these data demonstrate that while lattice SIM improves upon confocal and Airyscan and begins to suggest underlying RAD50 organization, it remains insufficient to resolve the nanoscale architecture fully. Observed similarities to STORM may indicate a partially resolved structure, but definitive interpretation requires higher-resolution imaging, as provided by single-molecule approaches.

Image Correction

NONE

Image by J. H. Cheong

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

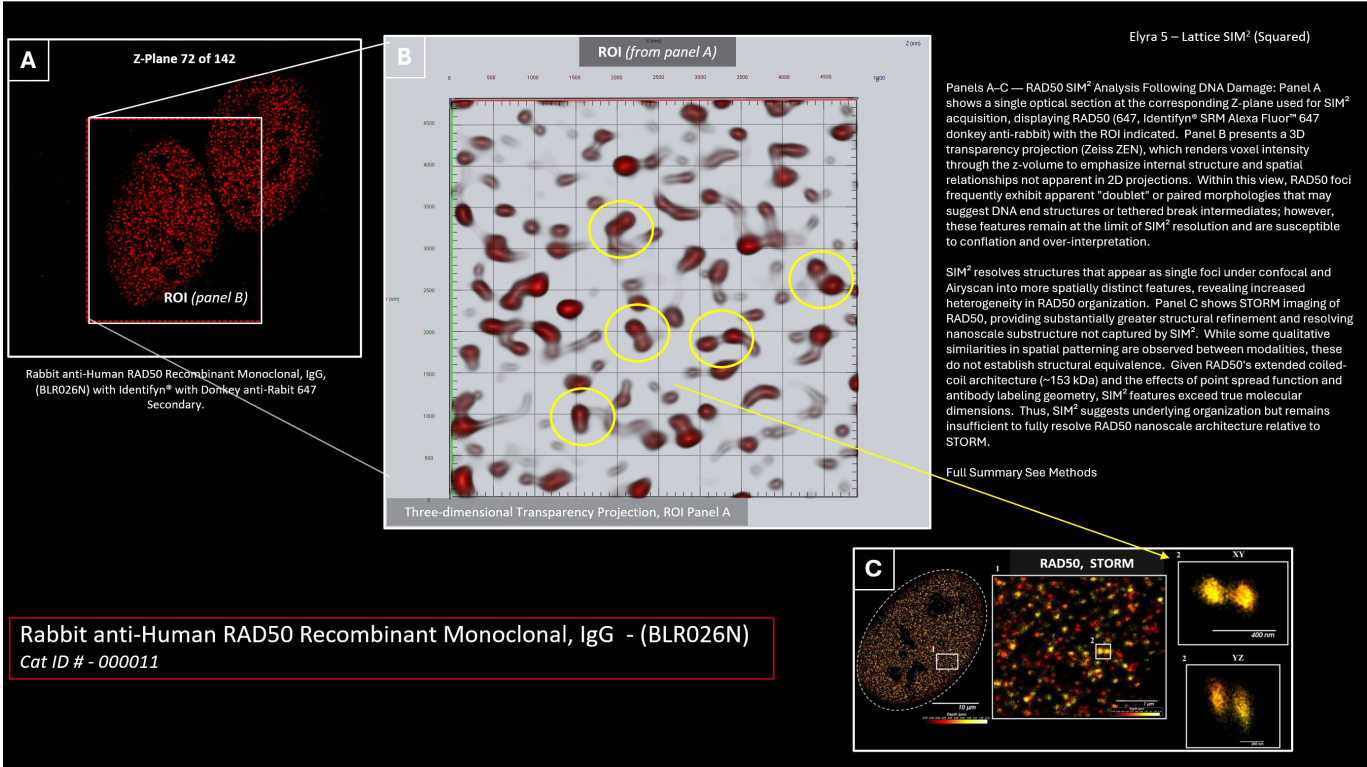
SOURCE PROVENANCE

Catalog	PA-RR-000011
Stage	SIM
Instrument	ZEISS Elyra

SOURCE PROVENANCE

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 f
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E60EFF4A72BF1361F78836DE32607ACBA882A6CF56D0F08CEEFB2C4F7A41862E
Method PDF SHA-256	DD38C89B751CDF4254B8601E496DA5236FC280DDF0E1A2340542E2BC7242F37E

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	53

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	142 Slices (4.23 µm) 40 Slices (1.17 µm) - Gallery was reduced to the Z-Planes that have RAD50 Signal
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.02979 µm
Image size (Pixels)	1374 X 1593 232 X 227
Image Size (Scaled)	29.01 µm X 33.63 µm 4.90 µm X 4.75 µm
Bit Depth	16 Bit
Image Center Position	X: 66.49 mm, Y: 35.66 mm
Stage Position	X: 66.49 mm, Y: 35.66 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 488
Channel Name	T1-Axiocam 820 -SR T2-Axiocam 820-SR
Excitation Wavelength	640 488
Emission Wavelength	729 525
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	1.13 µm 0.81 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 2.0% 488 nm @ 1.50%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Transparency Projection	Precise
Appearance	Threshold 5% all channels, Ramp 20% all channels, Maximum 100% all channels
Background	Light Grey
Light	Brightness 100%, Disabled Tone Mapping
Projection	View Angle 35°, Scale Z 10%, 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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)

Cat ID# - PA-MM 000002

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000017

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 the identification and processing of 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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5 times in 1X PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3 times in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG, F(ab')₂ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

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) - 2D at Z-Plane 72 of 142, Panel A

Z-Stack = 142 Slices (4.23 µm)

Channels = 2

Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm

Image size (Pixels) = 1374 X 1593

Image Size (Scaled) = 29.01 µm X 33.63 µm

Bit Depth = 16 Bit

Image Center Position = X: 66.49 mm, Y: 35.66 mm

Stage Position = X: 66.49 mm, Y: 35.66 mm

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

Z Stack - (3D) - 3D Transparency Projection, Panel B

Z-Stack = 40 Slices (1.17 µm) - Gallery was reduced to the Z-Planes that have RAD50 Signal

Channels = 2

Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm

Image size (Pixels) = 232 X 227

Image Size (Scaled) = 4.90 µm X 4.75 µm

Bit Depth = 16 Bit

Image Center Position = X: 66.49 mm, Y: 35.66 mm

Stage Position = X: 66.49 mm, Y: 35.66 mm

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

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 = 120 ms

Depth of Focus = 1.13 µm

Binning Mode = 2,2

Laser Wavelength = 640 nm @ 2.0%

Frame Time = 1.59 s

Scan Direction = Unidirectional

Grating = 36.5 µm grating

488 - Acquired but not Shown in the SIM2 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 = 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 μ m
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @ 1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM2 Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 2
 Algorithm = SIM2
 Input SNR = Low
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Normalization = Auto

2-Dimensional View - Panel A

Shows Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Transparency Projection = Precise
 Appearance = Threshold 5% all channels, Ramp 20% all channels, Maximum 100% all channels
 Background = Light Grey
 Light = Brightness 100%, Disabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset, illustrating the resolution limitations of Lattice SIM² imaging. While SIM² provides improved localization precision and structural detail relative to conventional SIM, a more direct comparison with STORM demonstrates that the nanoscale organization of RAD50 assemblies remains only partially resolved under SIM² acquisition conditions.

Summary

Panel A shows a single optical section at the corresponding Z-plane used for SIM² acquisition, displaying RAD50 detected in the 647 channel (Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary). A white box denotes the region of interest (ROI) selected for subsequent analysis.

Panel B presents a three-dimensional transparency projection (Zeiss ZEN), which enables visualization of signal distribution throughout the z-volume by rendering voxel intensity without full surface occlusion. This approach is used to emphasize internal structure and spatial relationships within RAD50-associated assemblies that are not readily appreciated in 2D projections. Within this representation, multiple RAD50 foci exhibit apparent "doublet" or paired morphologies, which may suggest the presence of DNA break ends or tethered structures. However, these features must be interpreted with caution, as they may reflect resolution-limited separation or convolution of the signal rather than true discrete subunits.

Using SIM², structures that appear as single foci under confocal and Airyscan are partially resolved into more spatially distinct features, revealing increased heterogeneity in RAD50 organization. In several instances, patterns resembling paired or elongated structures are observed. While these may reflect underlying biological architecture-consistent with RAD50's role in bridging DNA ends within the MRN complex-they remain at the limit of SIM² resolution and are susceptible to overinterpretation.

Panel C shows single-molecule STORM imaging of RAD50, which provides substantially greater structural refinement. Although some qualitative similarities in spatial patterning are observed between SIM² and STORM, these correspondences are not definitive. Features appearing as "doublets" in SIM² may represent partially resolved structure, but they may also arise from point spread function effects and labeling geometry. Notably, STORM reveals nanoscale organization that SIM² does not fully capture, and even these structures may remain incompletely resolved.

Importantly, RAD50 (~153 kDa) forms extended coiled-coil dimers and higher-order assemblies spanning tens to hundreds of nanometers, thereby tethering and stabilizing the ends of DNA double-strand breaks. Despite this inherent structural scale, the apparent dimensions of SIM²-resolved features often exceed expected molecular architecture due to the combined effects of optical resolution limits and antibody-based labeling displacement. As such, while SIM² begins to reveal aspects of RAD50 organization beyond the diffraction limit of imaging, it does not provide definitive structural resolution.

Collectively, these data demonstrate that SIM² improves visualization of RAD50 spatial organization and suggest the presence of underlying structural features, including possible paired assemblies. However, accurate interpretation of these features requires higher-resolution approaches, such as STORM, because resolution limits and potential signal conflation constrain SIM².

Image Correction

NONE

Image by J. H. Cheong

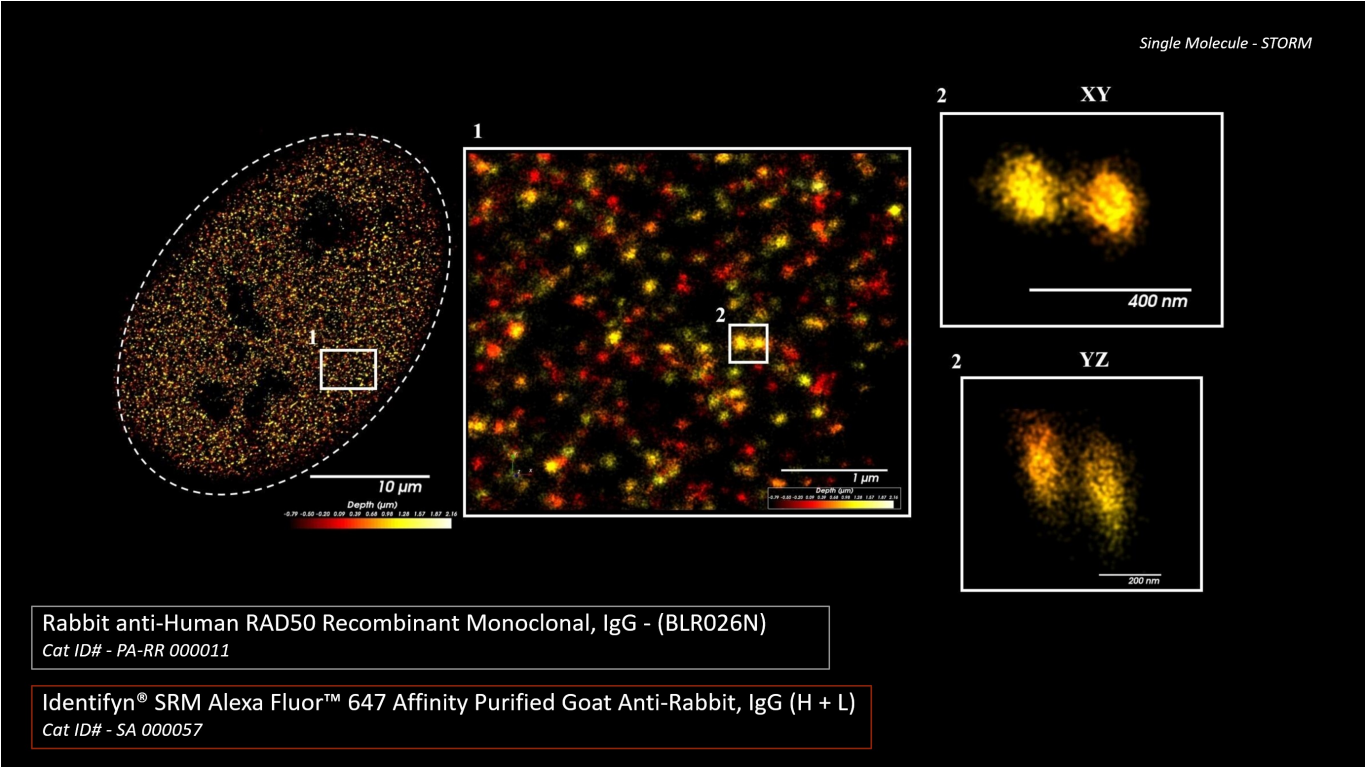
Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	PA-RR-000011
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 f
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C70C2F2477DE2ED55E4D78F2A95E2045B0D995C780B2122E2856C0B3D8CD679D
Method PDF SHA-256	3492BA9ACE0C8A77CC32A2F0AFFFA91EC7FF540660B15ED9EFC4ABA8D8785691

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	81

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Objective	Olympus 60x/1.30 Silicon Oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635 nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100 ms 20 ms
Super Resolution Camera Exposure Time	20 ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20 ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200 µm
Capture Mode	Live

FIELD	SOURCE-DOCUMENTED VALUE
Cell(s) or Cellular Structure Localization	The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused. Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Current	183.6 μ m
Widefield 640 nm	0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50 μ m is selected
Piezo Record	Begin: 182; End: 183.6
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	8
Cycles	19
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	50
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe 1	BG threshold: 20
Sizing Mode	Radial Precision
Particle size	4 nm
Color by	Depth
Color Table	Single

FIELD	SOURCE-DOCUMENTED VALUE
Opacity Mode	Constant; Opacity 1: 0.04
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	35
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 μm
Maximum Particle Count to Form Cluster	10
Compute Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000057

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 susceptibility to cancer, and other genetic disorders.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM Etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - (BLR026N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn®'s proprietary Wash Buffer, and Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:200. Cells were washed 5X in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn®'s Calibration Standard Operating Procedure, which can be found here.

<https://identifyn.com/storm-calibration>

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, 'Record' is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1
 Number of Simultaneous Probes: 1
 Number of Reference Probes: 1
 Camera: Both Cameras
 Color Channel: Both Channels
 Probe Capture Mode: Interleaved
 Z -Stack Capture Mode: Interleaved
 Multiple Location Capture: Not selected
 Post Record Reference: Not Selected
 Fluidics: Not Selected
 Autofocus Drift Correction: On

Microscope Configuration

Biplane Module: 635 nm Dichroic
 Objective: Silicon Selected
 Stage Insert: Rectangular Selected
 Course Focus Position: 8-Well Chamber Selected
 Dichroic: SuperRes
 PSF: (Red, Orange)
 Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%
 Widefield Camera Exposure Time: 100 ms
 Super Resolution Camera Exposure Time: 20 ms
 Widefield Camera Binning: 1
 Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
 Photoactivation/Imaging Laser: 0% Selected
 Exposure Time: 20 ms Selected

Experiment Configuration

Record Workflow: Selected Options
 View Mode: Widefield 200 µm
 Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization: The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 183.6 μ m

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640 nm: 0.1X Neutral Density Filter: Selected, @ 0.2% Laser Input

Reference Probe 1 Laser control 640 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100 ms

Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position: -0.979

Current Intensity: 4.5

Calibration Value: -5070 nm/unit

Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50 μ m is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization: Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.

Piezo Record: Begin: 182; End: 183.6

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input

Reference Probe 1 Laser control 640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20 ms

Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200

Z Positions: 8

Cycles: 19

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 50

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to default values.

Localization

Cutout Width: 16 px

Max Frame Accumulation: 1 frame

Max Accumulation Offset: 1 px

Fiducial at Max Accumulation: Not selected

Max Particles Per Frame: No Max

Background SLPE Removal: Not Selected

Cutout/Background Removal: Not Selected

Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe 1: BG threshold: 20

Region of Interest -

Not selected

Display -

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting

Sizing Mode: Radial Precision

Particle size: 4 nm

Color by: Depth

Color Table: Single

Opacity Mode: Constant; Opacity 1: 0.04

Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)

Time Intervals: 10 Intervals

Pixel Size: 50 nm

Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

Radial precision: 35

Axial precision: 70

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1 μm

Maximum Particle Count to Form Cluster: 10

Compute Hulls: Not Selected

Compute Density Isosurfaces: Not Selected

Edit Settings: All Probes

Compute: Selected

Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

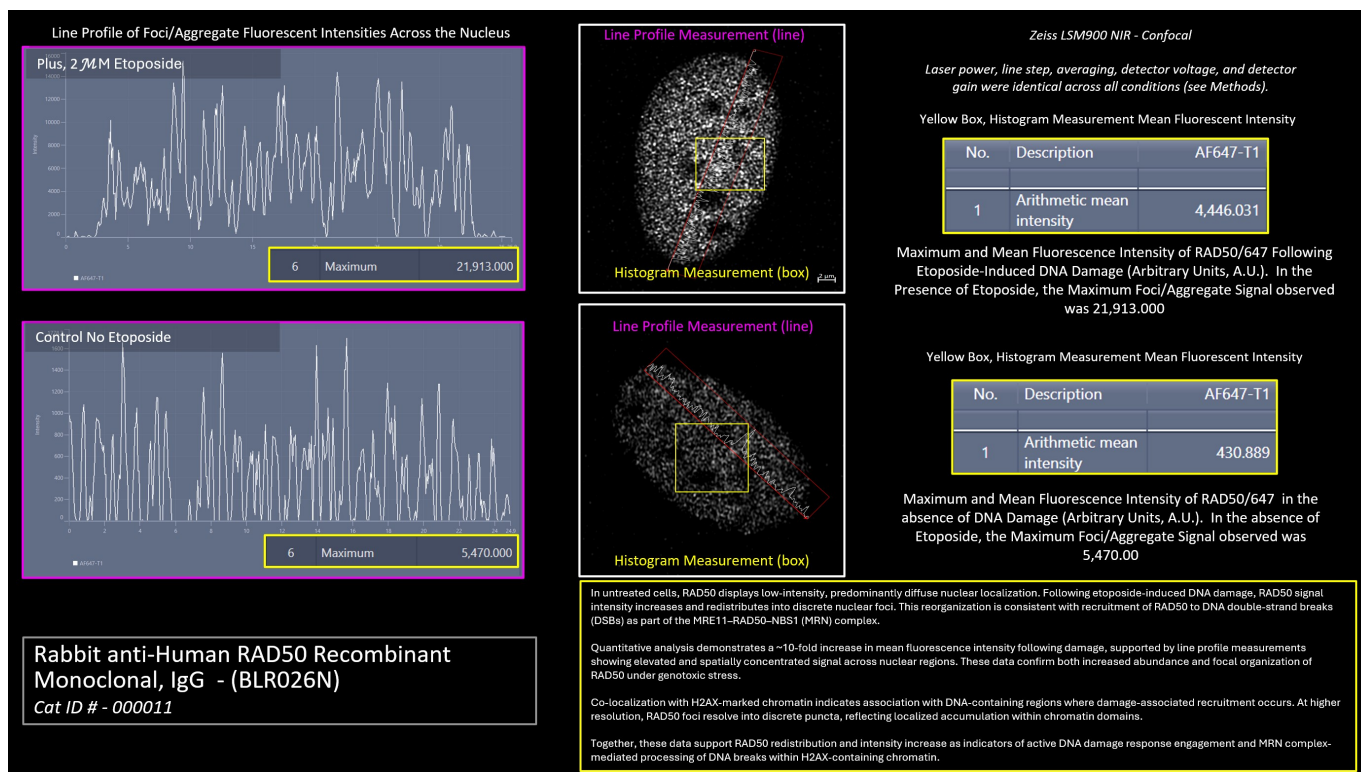
Image by P. Raut

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	PA-RR-000011
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil objective, was used with the following parameters:
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C052D5462857B1D7A32F5942FC031C29F5D983A5BECE6B0F1C986AE7326A50F5
Method PDF SHA-256	EA34F4226228FD1C0EDD69B6C03AEA3E0161291D129D6A6D889A1D233F05966C

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	52

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss 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 μ m) 151 Slices (4.5 μ m)
Channels	1
Scaling (Per Pixel)	0.059 μ m X 0.059 μ m X 0.030 μ m
Image size (Pixels)	528 X 673 515 X 560
Image Size (Scaled)	31.06 μ m X 39.59 μ m 30.30 μ m X 32.94 μ m
Bit Depth	16 Bit
Image Center Position	X: 72.53 mm, Y: 52.26 mm X: 79.20 mm, Y: 49.34 mm
Stage Position	X: 72.53 mm, Y: 52.26 mm X: 79.20 mm, Y: 49.34 mm
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 65 μ m 1.00 AU / 64 μ m
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS SP 615
Laser Wavelength	639 nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.20
Pixel Time	0.28 μ s
Frame Time	5.28 s
LSM Scan Speed	12 11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	645 - 700
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.0 (3D, Auto) Filter: 8.7 (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 34.70), Y (Min 0.0 and Max 15829.00) X and Y axes were set to Auto - X (Min 0.0 and Max 24.90), Y (Min 0.0 and Max 1779)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 100) X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 500000)
Arithmetic Mean intensity	4406.031 430.889

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 Recombinant Monoclonal, IgG - (BLR026N)

Cat ID# - PA-RR 000011

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000057

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 the identification and processing of 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 susceptibility to cancer, and other genetic disorders.

Method

Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 24 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - (BLR026N), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

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 Recombinant Monoclonal - (BLR026N), was incubated at room temperature for 1 hour at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 68 of 111, Plus Etoposide/DNA Damage.

Z-Stack = 111 Slices (3.30 µm)

Channels = 1

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image size (Pixels) = 528 X 673

Image Size (Scaled) = 31.06 µm X 39.59 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.53 mm, Y: 52.26 mm

Stage Position = X: 72.53 mm, Y: 52.26 mm

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

Z Stack - (3D) - 3D at Z-Plane 76 of 151, No Etoposide/Minus DNA Damage.

Z-Stack = 151 Slices (4.5 µm)

Channels = 1

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image size (Pixels) = 515 X 560

Image Size (Scaled) = 30.30 µm X 32.94 µm

Bit Depth = 16 Bit

Image Center Position = X: 79.20 mm, Y: 49.34 mm

Stage Position = X: 79.20 mm, Y: 49.34 mm

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

647 - Plus Damage, from Confocal Data Set

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 65 μ m
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS SP 615
 Laser Wavelength = 639 nm @ 0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 μ s
 Frame Time = 5.28 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 645 - 700
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 8.0 (3D, Auto)

647 - Minus Damage - noting the same laser input power and conditions

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 64 µm
 LMS MBS = MBS 488/561/639 & 405/730
 LMS SBS = SBS SP 615
 Laser Wavelength = 639 nm @ 0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28 µs
 Frame Time = 5.28 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 645 - 700
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 8.7 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

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 34.70), Y (Min 0.0 and Max 15829.00)

This panel measures RAD50 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~15K A.U. at its maximum.

Measured at the Z-plane 68 of 111, Plus Etoposide.

Profile View Display - Minus DNA Damage, Bottom

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 24.90), Y (Min 0.0 and Max 1779)

This panel measures RAD50 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~2K A.U. at its maximum.

3D at Z-Plane 76 of 151, No Etoposide.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 100)

Arithmetic Mean intensity = 4406.031

The histogram was generated from the yellow-boxed region to quantify the overall RAD50 protein response within a defined nuclear area following DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - Minus DNA Damage, Bottom

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 500000)

Arithmetic Mean intensity = 430.889

The histogram was generated from the yellow-boxed region to quantify the overall RAD50 protein within a defined nuclear area in the absence of DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In untreated cells, RAD50 displays low-intensity, predominantly diffuse nuclear localization. Following etoposide-induced DNA damage, RAD50 signal intensity increases and redistributes into discrete nuclear foci. This reorganization is consistent with recruitment of RAD50 to DNA double-strand breaks (DSBs) as part of the MRE11-RAD50-NBS1 (MRN) complex.

Quantitative analysis demonstrates a ~10-fold increase in mean fluorescence intensity following damage, supported by line-profile measurements showing an elevated, spatially concentrated signal across nuclear regions. These data confirm both increased abundance and focal organization of RAD50 under genotoxic stress.

Co-localization with H2AX-marked chromatin throughout the dataset indicates association with DNA-containing regions where damage-associated recruitment occurs. At higher resolution, RAD50 foci resolve into discrete puncta, reflecting localized accumulation within chromatin domains.

Together, these data support RAD50 redistribution and increased intensity as indicators of active DNA damage response engagement and MRN complex-mediated processing of DNA breaks within H2AX-containing chromatin.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	PA-RR-000011
Stage	Control
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
Structured source metadata values	
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	EEF591E429DCED7C108EEE6079E12CA56716471853FB5F097F852BED730BCC43
Method PDF SHA-256	CB222797B2EB988EDCC3D2B19141FD6E00C33BE8B2A2CDED36E4B2B1B29183F8