BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

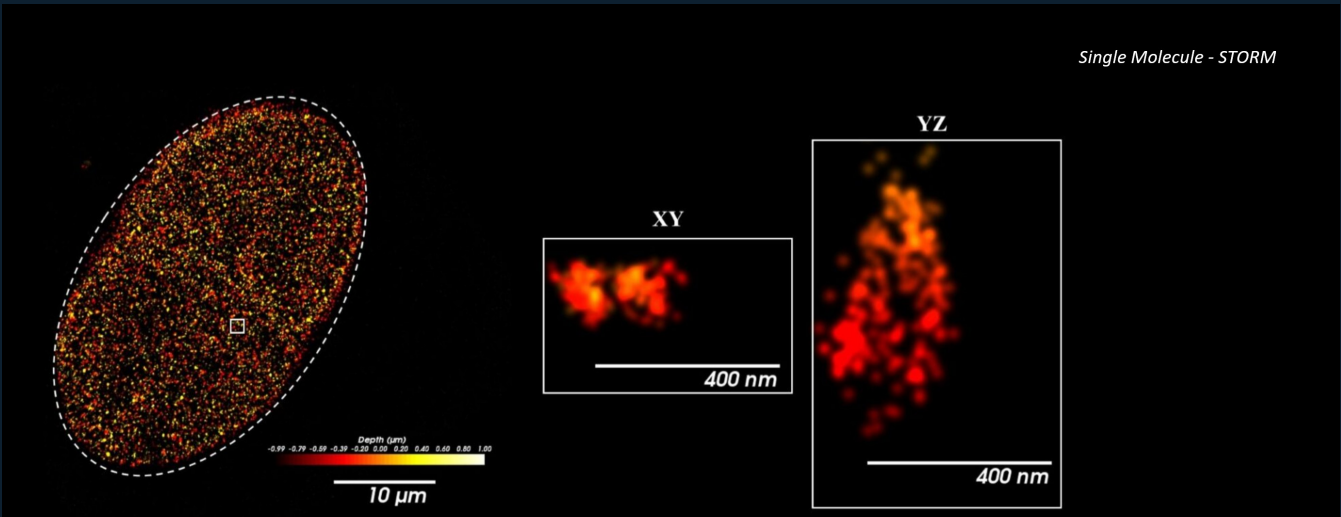
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647

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

8	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Single-molecule localization microscopy (STORM) of RAD50 following DNA damage.

Rabbit anti-Human RAD50 Polyclonal detected with Identifyn® SRM Alexa Fluor™ 647 (Cat. ID# DC-000061) imaged using the Vutara VXL STORM platform reveals that RAD50 foci unresolved by diffraction-limited and structured illumination modalities resolve into discrete, quantifiable subunits following etoposide-induced DNA damage. At single-molecule resolution, RAD50 assemblies are spatially separable, enabling direct measurement of sub-focal organization, density, and distribution within the nuclear compartment. In the CCR step-down CDx framework, STORM serves as the structural adjudication reference, confirming that aggregate signals observed at lower resolution represent true biological assemblies rather than resolution-limited composites. These data provide a quantitative foundation for pharmacodynamic and pharmacokinetic assessment of DDR engagement, supporting CDx-level evaluation of RAD50 recruitment, accumulation, and temporal response under genotoxic or therapeutic conditions.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000061 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

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

EVIDENCE AVAILABLE

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

BENNETT ASSESSMENT

The preserved DC-000061 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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 / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000061 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	3; None; 639nm @2.0%; 488nm @0.2%; 405nm @0.3%; 653; 493; 353
Airyscan	405/DAPI; 488; 647	3; None; 639nm @0.9%; 488nm @0.3%; 405nm @0.2%; 653; 493; 353
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s). Structured source metadata values: 11.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 904 X 557; Image Size (Pixels): 904 X 557; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @25.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 42 Slices (4.1 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 780 X 786; Image Size (Pixels): 780 X 786; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 200 ms; 25 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25; 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 161 Slices (4.80 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1302 X 1123; Image Size (Pixels): 1302 X 1123; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsp-Pmt3; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 0.26µs; Frame Time: 5.63s. Illumination: Laser Wavelength: 639nm @2.0%; 488nm @0.2%. Optical/scan: Pinhole: 1.00AU / 65µm; 1.00AU / 51µm; Scan Zoom: 1.5; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 8.0 (3D, Auto). Structured source metadata values: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 121 Slices (3.60 µm); Channels: 3; Scaling (Per Pixel): 35.00 nm X 35.00 nm X 30.00 nm; Image size (Pixels): 1711 X 1711; 303 X 249; Image Size (Pixels): 1711 X 1711; 303 X 249; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT; GaAsp-PMT. Timing: Pixel Time: 1.21µs; Frame Time: 17.06s. Illumination: Laser Wavelength: 639nm @0.9%; 488nm @0.3%. Optical/scan: Pinhole: 5.00AU / 328µm; 5.00AU / 250µm; Scan Zoom: 2.2; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 47 Slices (1.38 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1931 X 2035; 345 X 336; Image Size (Pixels): 1931 X 2035; 345 X 336; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @1.0%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 35%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 40 Slices (1.17 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1816 X 2010; 201 X 186; Image Size (Pixels): 1816 X 2010; 201 X 186; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120 ms; 100 ms; Frame Time: 1.59 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @1.0%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 35%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 8

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 80.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000061 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.00 nm X 35.00 nm X 30.00 nm -> 0.03500 µm X 0.03500 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1711 X 1711; Image Size (Scaled)=60.37 µm X 60.37 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.80%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1931 X 2035; Image Size (Scaled)=40.77 µm X 42.96 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1816 X 2010; Image Size (Scaled)=38.34 µm X 42.44 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Single-molecule STORM": ["12 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

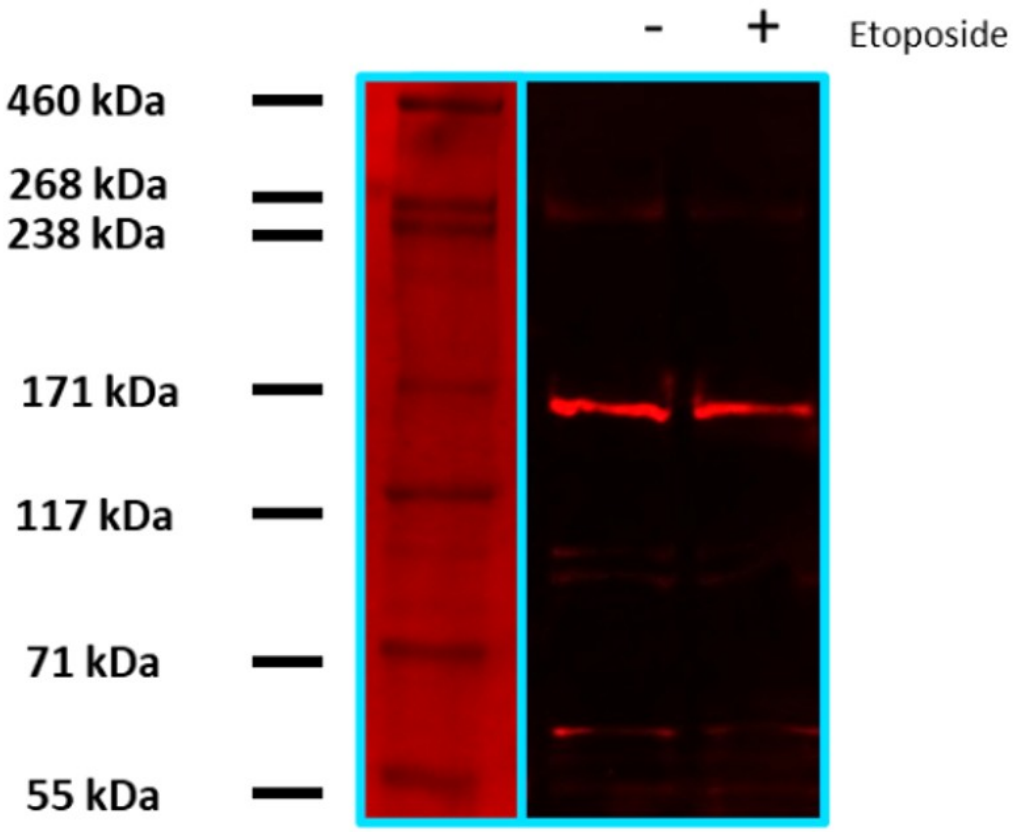
This dossier preserves the complete available evidence progression for DC-000061. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
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(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	632nm
Emission Filter	676±37nm
Detector	PMT
Intensity	3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Expected MW: 153 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. The Thermo Fisher's HiMark™ Pre-stained Protein Standard was used as a molecular weight marker.

Post-electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG for 20 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence
Laser = 632nm
Emission Filter = 676±37nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

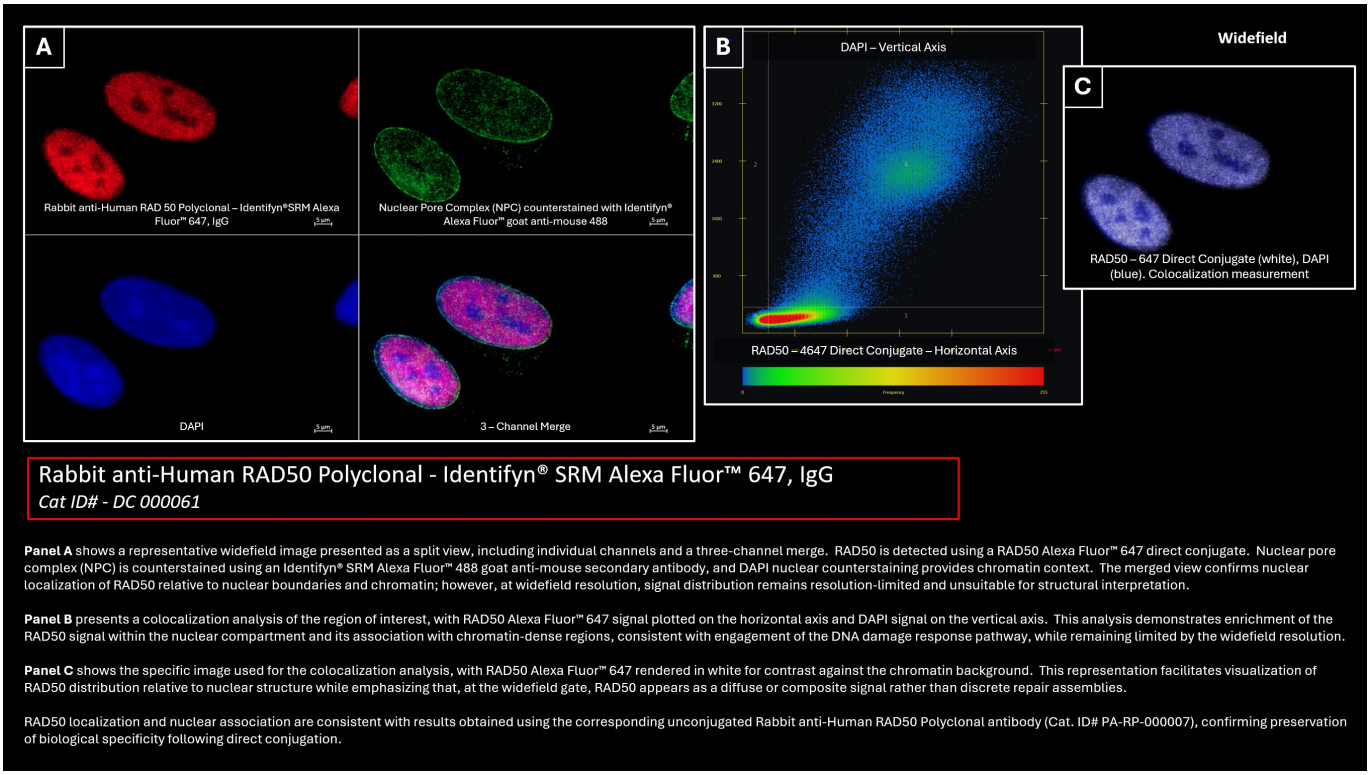
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000061
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0CDE7B77AC682E9C55DE41D18F774B5A4F4A9410A5F6A6303E3D536C77854BCF
Method PDF SHA-256	153BDEFC513F7DB5FDC849B1365DD42C278D372D7B028EA35DB9FC1E19CCF11C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	904 X 557
Image Size (Scaled)	99.01 µm X 61.00 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 @15.00% | X-Cite Xylis Lamp Intensity @25.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	150 ms | 250 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

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 904 X 557
Image Size (Scaled) = 99.01 µm X 61.00 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 @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647 IgG. The top-right panel shows NPC monoclonal antibody, visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L) (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

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

Horizontal Axis - AF647, Threshold 100, Range Auto
Vertical Axis - DAPI, Threshold 366, Range Auto

Summary

Panel A shows a representative widefield image presented as a split view, including individual channels and a three-channel merge. RAD50 is detected using a RAD50 Alexa Fluor™ 647 direct conjugate. Nuclear pore complex (NPC) is counterstained using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody, and DAPI nuclear counterstaining provides chromatin context. The merged view confirms nuclear localization of RAD50 relative to nuclear boundaries and chromatin; however, at widefield resolution, signal distribution remains resolution-limited and unsuitable for structural interpretation.

Panel B presents a colocalization analysis of the region of interest, with RAD50 Alexa Fluor™ 647 signal plotted on the horizontal axis and DAPI signal on the vertical axis. This analysis demonstrates enrichment of the RAD50 signal within the nuclear compartment and its association with chromatin-dense regions, consistent with engagement of the DNA damage response pathway, while remaining limited by the widefield resolution.

Panel C shows the specific image used for the colocalization analysis, with RAD50 Alexa Fluor™ 647 rendered in white for contrast against the chromatin background. This representation facilitates visualization of RAD50 distribution relative to nuclear structure while emphasizing that, at the widefield gate, RAD50 appears as a diffuse or composite signal rather than discrete repair assemblies.

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

Image Correction

None

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

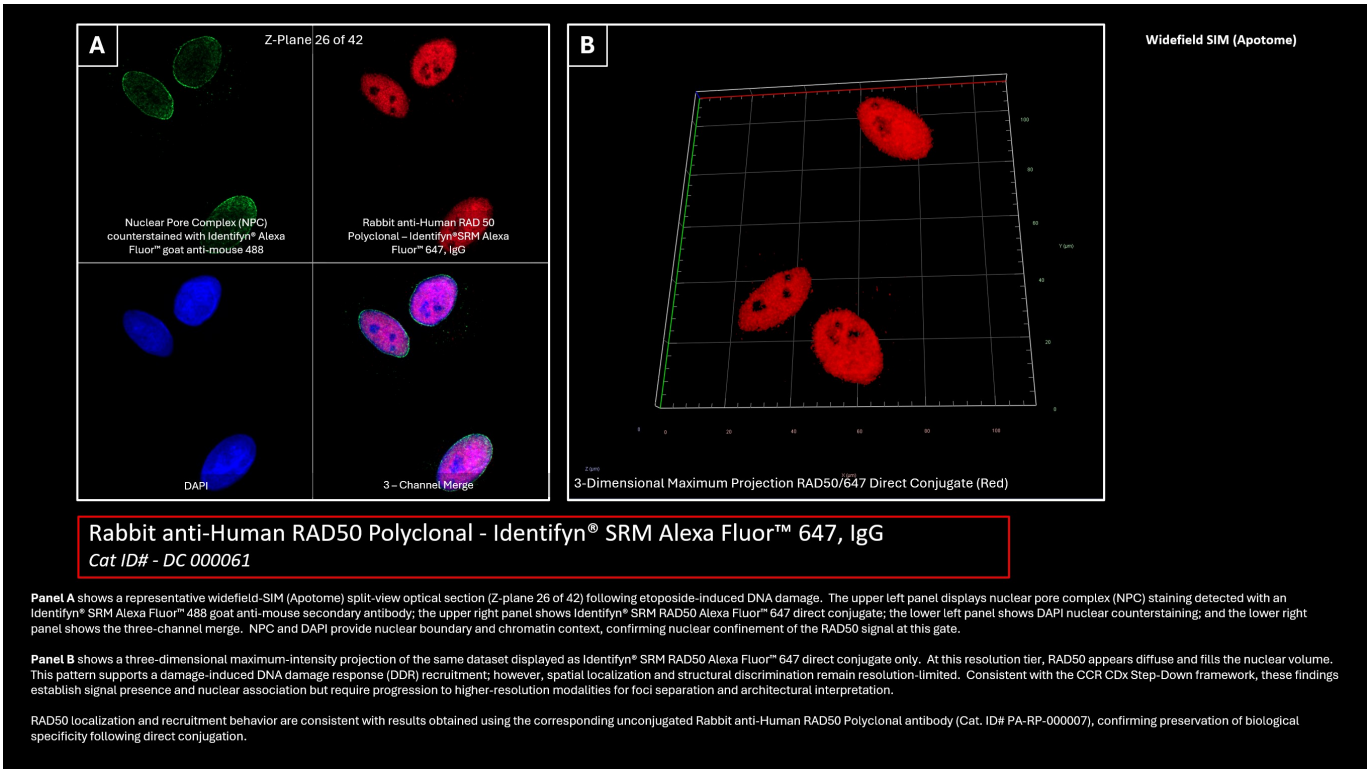
SOURCE PROVENANCE

Catalog	DC-000061
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9365B567AA2FC889A2D654F3087916705F07B2DF5B4530429827D0685398EE20
Method PDF SHA-256	A02B68223354559985483A3D7C3C57506D5B8DA50A7CC05427B35AE8ED9D3139

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	46

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	42 Slices (4.1 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	780 X 786
Image Size (Scaled)	113.43 µm X 112.29 µm
Bit Depth	14 Bit
Image Center Position	X: 64.48 µm, Y: 50.21 µm
Stage Position	X: 64.48 µm, Y: 50.21 µm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	200 ms | 25 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.25 | 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panels A and B

Z-Stack = 42 Slices (4.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 780 X 786
Image Size (Scaled) = 113.43 µm X 112.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 64.48 µm, Y: 50.21 µm
Stage Position = X: 64.48 µm, Y: 50.21 µm
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 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

Split View - Panel A

The top-left panel shows NPC monoclonal antibody, visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L) (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The top-right panel shows Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647 IgG. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

3D Image Processing - Panel B

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

Summary

Panel A shows a representative widefield-SIM (Apotome) split-view optical section (Z-plane 26 of 42) following etoposide-induced DNA damage. The upper left panel displays nuclear pore complex (NPC) staining detected with an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody; the upper right panel shows Identifyn® SRM RAD50 Alexa Fluor™ 647 direct conjugate; the lower left panel shows DAPI nuclear counterstaining; and the lower right panel shows the three-channel merge. NPC and DAPI provide nuclear boundary and chromatin context, confirming nuclear confinement of the RAD50 signal at this gate.

Panel B shows a three-dimensional maximum-intensity projection of the same dataset displayed as Identifyn® SRM RAD50 Alexa Fluor™ 647 direct conjugate only. At this resolution tier, RAD50 appears diffuse and fills the nuclear volume. This pattern supports a damage-induced DNA damage response (DDR) recruitment; however, spatial localization and structural discrimination remain resolution-limited. Consistent with the CCR CDx Step-Down framework, these findings establish signal presence and nuclear association but require progression to higher-resolution modalities for foci separation and architectural interpretation.

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

Image Correction

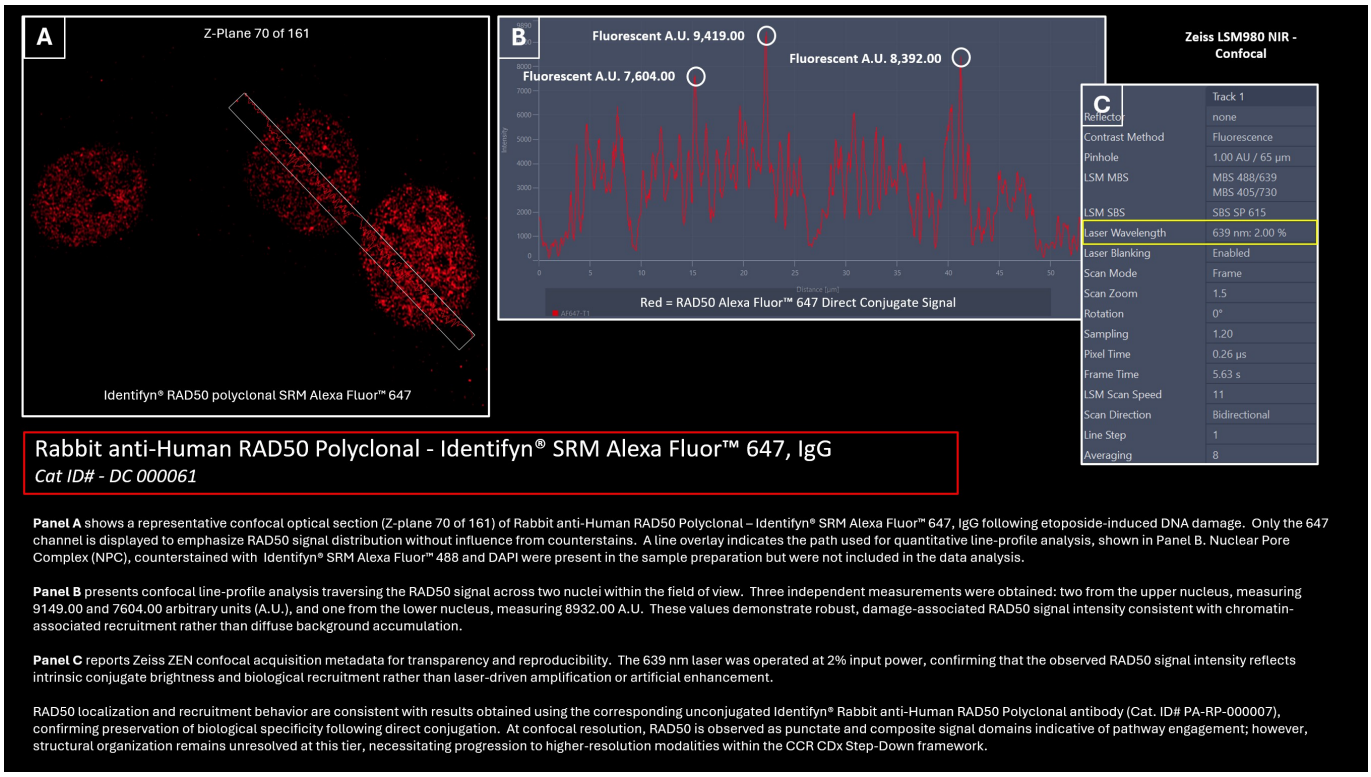
NONE

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

SOURCE PROVENANCE

Catalog	DC-000061
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D8CE6100F91C21B6ABC2285AA02540404EDD57C1444363BC32665CC4DFEE5621
Method PDF SHA-256	589316D9B7843C3E7F0E2BC1AF2546B193DC08403B369A7A3AE8509C3AB607A9

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A shows a representative confocal optical section (Z-plane 70 of 161) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. Only the 647 channel is displayed to emphasize RAD50 signal distribution without influence from counterstains. A line overlay indicates the path used for quantitative line-profile analysis, shown in Panel B. Nuclear Pore Complex (NPC), counterstained with Identifyn® SRM Alexa Fluor™ 488 and DAPI were present in the sample preparation but were not included in the data analysis.

Panel B presents confocal line-profile analysis traversing the RAD50 signal across two nuclei within the field of view. Three independent measurements were obtained: two from the upper nucleus, measuring 9149.00 and 7604.00 arbitrary units (A.U.), and one from the lower nucleus, measuring 8932.00 A.U. These values demonstrate robust, damage-associated RAD50 signal intensity consistent with chromatin-associated recruitment rather than diffuse background accumulation.

Panel C reports Zeiss ZEN confocal acquisition metadata for transparency and reproducibility. The 639 nm laser was operated at 2% input power, confirming that the observed RAD50 signal intensity reflects intrinsic conjugate brightness and biological recruitment rather than laser-driven amplification or artificial enhancement.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation. At confocal resolution, RAD50 is observed as punctate and composite signal domains indicative of pathway engagement; however, structural organization remains unresolved at this tier, necessitating progression to higher-resolution modalities within the CCR CDx Step-Down framework.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	161 Slices (4.80 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	1302 X 1123
Image Size (Scaled)	76.56 µm X 66.04 µm
Bit Depth	16 Bit
Image Center Position	X: 87.92 µm, Y: 47.93 µm
Stage Position	X: 87.92 µm, Y: 47.93 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 65µm | 1.00AU / 51µm | 1.00AU / 44µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615 | SBS SP 505 | Mirror
Laser Wavelength	639nm @2.0% | 488nm @0.2% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.5
Rotation	0°
Sampling	1.20
Pixel Time	0.26µs
Frame Time	5.63s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	640 - 732 | 511 - 550 | 430 - 485
Imaging Device	GaAsP-Pmt3 | GaAsp-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	500 V | 550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.5 (3D, Auto) | Filter: 8.0 (3D, Auto) | Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 54.5), Y (Min 0 and Max 9890)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complex (NPC) and DAPI were not shown in the data analysis, but were present in the sample.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 70 of 161, Line Profile Panel B

Z-Stack = 161 Slices (4.80 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 1302 X 1123
Image Size (Scaled) = 76.56 µm X 66.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 87.92 µm, Y: 47.93 µm
Stage Position = X: 87.92 µm, Y: 47.93 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 65µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @2.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26µs
Frame Time = 5.63s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

488 - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26µs
Frame Time = 5.63s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.0 (3D, Auto)

DAPI - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 44µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.26µs
Frame Time = 5.63s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 485
Imaging Device = GaAsp-Pmt3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

2- Dimensional View - Panel A

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, shown in default Zeiss Zen red.

Image from Z-plane 70 of 161 and the Line Profile graphic showing the location where the measurement was taken.

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 54.5), Y (Min 0 and Max 9890)

Zen Acquisition Info Tab -

Shown is the acquisition of the 647 channel (RAD50 647 direct conjugate) using the 639 nm laser at 2.0%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A shows a representative confocal optical section (Z-plane 70 of 161) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. Only the 647 channel is displayed to emphasize RAD50 signal distribution without influence from counterstains. A line overlay indicates the path used for quantitative line-profile analysis, shown in Panel B. Nuclear Pore Complex (NPC), counterstained with Identifyn® SRM Alexa Fluor™ 488 and DAPI, were present in the sample preparation but were not included in the data analysis.

Panel B presents confocal line-profile analysis traversing the RAD50 signal across two nuclei within the field of view. Three independent measurements were obtained: two from the upper nucleus, measuring 9149.00 and 7604.00 arbitrary units (A.U.), and one from the lower nucleus, measuring 8932.00 A.U. These values demonstrate robust, damage-associated RAD50 signal intensity consistent with chromatin-associated recruitment rather than diffuse background accumulation.

Panel C reports Zeiss ZEN confocal acquisition metadata for transparency and reproducibility. The 639 nm laser was operated at 2% input power, confirming that the observed RAD50 signal intensity reflects intrinsic conjugate brightness and biological recruitment rather than laser-driven amplification or artificial enhancement.

RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation. At confocal resolution, RAD50 is observed as punctate and composite signal domains indicative of pathway engagement; however, structural organization remains unresolved at this tier, necessitating progression to higher-resolution modalities within the CCR CDx Step-Down framework.

Image Correction

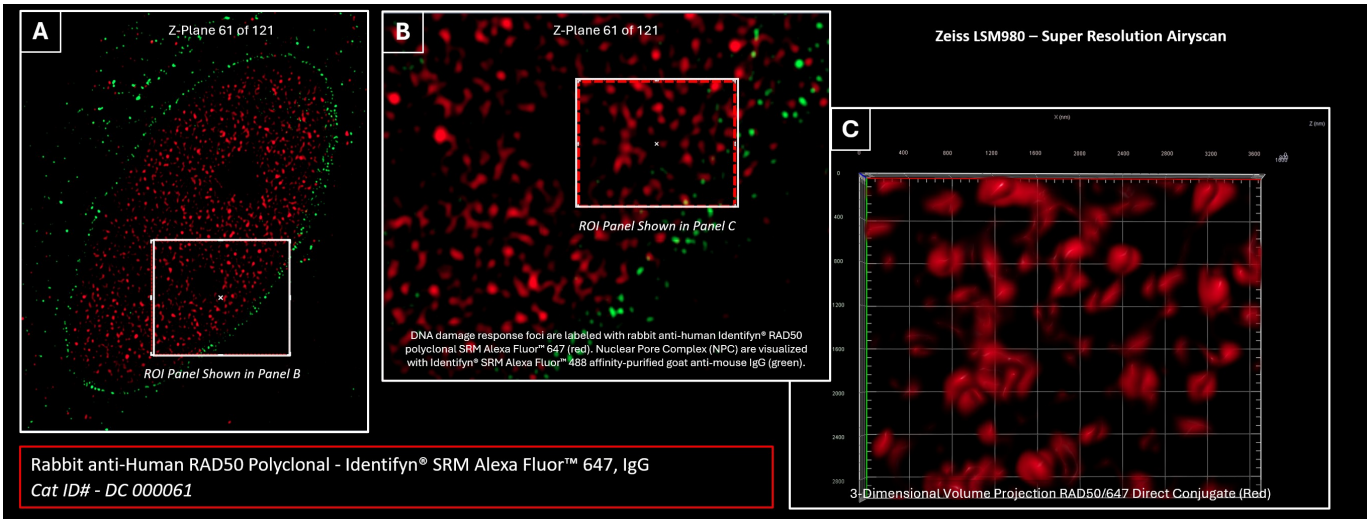
NONE

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

SOURCE PROVENANCE

Catalog	DC-000061
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2FB6513B28FB83354289A8553CFF3FE6DDF05F21A040B519D1CEB2948EF2DEC4
Method PDF SHA-256	04C0E5458163E5F1AF07FB29D45E34DAC1FE07440FE6EC022EE4804BEA8D3035

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A presents super-resolution Airyscan imaging of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 647, IgG, together with nuclear pore complex (NPC) staining detected using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody. A white boxed region denotes the region of interest (ROI) advanced for further interrogation in Panel B. NPC staining provides nuclear boundary context and confirms confinement of RAD50 signal to the nuclear compartment.

Panel B shows the same Z-plane and protein channels as Panel A, focused on the ROI. At the Airyscan resolution gate, RAD50 signal domains that appear as singular, circular foci at the confocal tier are revealed to possess internal structure and spatial complexity. A secondary white boxed region identifies a representative RAD50 assembly advanced for volumetric assessment in Panel C.

Panel C displays a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 direct conjugate alone. In three dimensions, RAD50 assemblies no longer appear as discrete puncta but instead resolve into composite, unresolved structures within the nuclear volume. This transition demonstrates that the confocal-to-Airyscan threshold defines a definitive resolution boundary, in which apparent single foci are revealed to be structurally complex assemblies rather than isolated repair units.

Together, these panels establish that Airyscan imaging provides a clear and reproducible gain over confocal resolution for RAD50, enabling discrimination between true singular foci and composite repair structures. However, despite this gain, sub-structural organization remains unresolved at the Airyscan gate. In accordance with the CCR CDx Step-Down framework, these findings warrant progression to higher-resolution modalities, including SIM, SIM^2, and STORM, for definitive spatial and structural interpretation of RAD50 organization. RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	62

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	121 Slices (3.60 µm)
Channels	3
Scaling (Per Pixel)	0.03500 µm X 0.03500 µm X 0.03000 µm
Image size (Pixels)	1711 X 1711 | 303 X 249 | 105 X 85
Image Size (Scaled)	60.37 µm X 60.37 µm | 10.69 µm X 8.79 µm | 3.71 µm X 3.0 µm
Bit Depth	16 Bit
Image Center Position	X: 88.12 µm, Y: 52.52 µm
Stage Position	X: 88.12 µm, Y: 52.52 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 328µm | 5.00AU / 250µm | 5.00AU / 214µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 550 | SBS SP 505
Laser Wavelength	639nm @0.9% | 488nm @0.3% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	2.00
Pixel Time	1.21µs
Frame Time	17.06s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 499 - 548 | 422 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT | GaAsp-PMT
Detector Gain	800 V | 550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	9.5 (3D, Auto) | 9.8 (3D, Auto) | 8.0 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complex (NPC) and DAPI were not shown in the data analysis, but were present in the sample.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 61 of 121

Z-Stack = 121 Slices (3.60 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 1711 X 1711
Image Size (Scaled) = 60.37 µm X 60.37 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.12 µm, Y: 52.52 µm
Stage Position = X: 88.12 µm, Y: 52.52 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, 2-Dimensional view at Z-plane 61 of 121

Z-Stack = 121 Slices (3.60 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 303 X 249
Image Size (Scaled) = 10.69 µm X 8.79 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.12 µm, Y: 52.52 µm
Stage Position = X: 88.12 µm, Y: 52.52 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 121 Slices (3.60 µm)

Channels = 3
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 30.00 nm
Image size (Pixels) = 105 X 85
Image Size (Scaled) = 3.71 µm X 3.0 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.12 µm, Y: 52.52 µm
Stage Position = X: 88.12 µm, Y: 52.52 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @0.9%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21µs
Frame Time = 17.06s
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 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.5 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21µs
Frame Time = 17.06s
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 = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.8 (3D, Auto)

DAPI - Not Shown in Data Analysis

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 214µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.21µs
Frame Time = 17.06s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 8.0 (3D, Auto)

2- Dimensional View - Panel A

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

Image from Z-plane 61 of 121, Panels A and B.

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 25%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A presents super-resolution Airyscan imaging of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, together with nuclear pore complex (NPC) staining detected using an Identifyn® SRM Alexa Fluor™ 488 goat anti-mouse secondary antibody. A white boxed region denotes the region of interest (ROI) advanced for further interrogation in Panel B. NPC staining provides nuclear boundary context and confirms confinement of RAD50 signal to the nuclear compartment.

Panel B shows the same Z-plane and protein channels as Panel A, focused on the ROI. At the Airyscan resolution gate, RAD50 signal domains that appear as singular, circular foci at the confocal tier are revealed to possess internal structure and spatial complexity. A secondary white boxed region identifies a representative RAD50 assembly advanced for volumetric assessment in Panel C.

Panel C displays a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 direct conjugate alone. In three dimensions, RAD50 assemblies no longer appear as discrete puncta but instead resolve into composite, unresolved structures within the nuclear volume. This transition demonstrates that the confocal-to-Airyscan threshold defines a definitive resolution boundary, in which apparent single foci are revealed to be structurally complex assemblies rather than isolated repair units.

Together, these panels establish that Airyscan imaging provides a clear and reproducible gain over confocal resolution for RAD50, enabling discrimination between true singular foci and composite repair structures. However, despite this gain, sub-structural organization remains unresolved at the Airyscan gate. In accordance with the CCR CDx Step-Down framework, these findings warrant progression to higher-resolution modalities, including SIM, SIM², and STORM, for definitive spatial and structural interpretation of RAD50 organization.

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

Image Correction

NONE

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

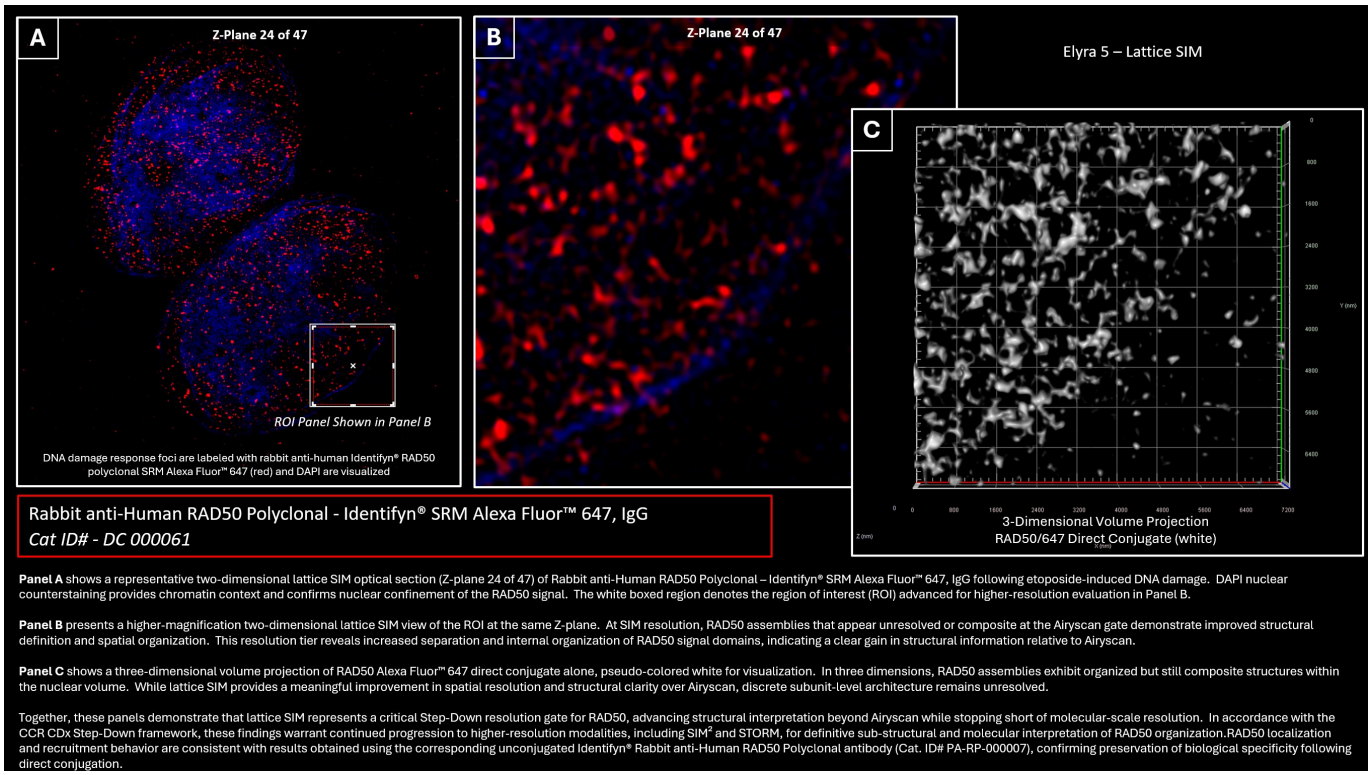
SOURCE PROVENANCE

Catalog	DC-000061
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	4237C9295B86C60125B7037CBAF80DEBD845808BA39EF32CC228FF278754A6B7
Method PDF SHA-256	855B2667A3AAA7EF596FDF7CFF01A8D6484AB50EEB98611F51AE1FD539BE2B49

ORIGINAL IMAGE EVIDENCE / SIM



Panel A shows a representative two-dimensional lattice SIM optical section (Z-plane 24 of 47) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. DAPI nuclear counterstaining provides chromatin context and confirms nuclear confinement of the RAD50 signal. The white boxed region denotes the region of interest (ROI) advanced for higher-resolution evaluation in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM view of the ROI at the same Z-plane. At SIM resolution, RAD50 assemblies that appear unresolved or composite at the Airyscan gate demonstrate improved structural definition and spatial organization. This resolution tier reveals increased separation and internal organization of RAD50 signal domains, indicating a clear gain in structural information relative to Airyscan.

Panel C shows a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 direct conjugate alone, pseudo-colored white for visualization. In three dimensions, RAD50 assemblies exhibit organized but still composite structures within the nuclear volume. While lattice SIM provides a meaningful improvement in spatial resolution and structural clarity over Airyscan, discrete subunit-level architecture remains unresolved.

Together, these panels demonstrate that lattice SIM represents a critical Step-Down resolution gate for RAD50, advancing structural interpretation beyond Airyscan while stopping short of molecular-scale resolution. In accordance with the CCR CDx Step-Down framework, these findings warrant continued progression to higher-resolution modalities, including SIM^2 and STORM, for definitive sub-structural and molecular interpretation of RAD50 organization.RAD50 localization and recruitment behavior are consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	62

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	47 Slices (1.38 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1931 X 2035 | 345 X 336
Image Size (Scaled)	40.77 µm X 42.96 µm | 7.28 µm X 7.09 µm
Bit Depth	16 Bit
Image Center Position	X: 41.09 mm, Y: 34.55 mm
Stage Position	X: 41.09 mm, Y: 34.55 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.0% | 488 nm @1.50% | 405 nm @1.0%
Frame Time	1.59 s | 1.33 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.2500 (647), 11.2120 (488), 10.2093 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 35%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complex (NPC) and DAPI were not shown in the data analysis, but were present in the sample.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, Z-plane 24 of 47

Z-Stack = 47 Slices (1.38 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1931 X 2035
Image Size (Scaled) = 40.77 µm X 42.96 µm
Bit Depth = 16 Bit
Image Center Position = X: 41.09 mm, Y: 34.55 mm
Stage Position = X: 41.09 mm, Y: 34.55 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI, Z-plane 24 of 47, and Panel C, 3-Dimensional Volume Projection

Z-Stack = 47 Slices (1.38 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 345 X 336
Image Size (Scaled) = 7.28 µm X 7.09 µm
Bit Depth = 16 Bit
Image Center Position = X: 41.09 mm, Y: 34.55 mm
Stage Position = X: 41.09 mm, Y: 34.55 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 @1.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 - Not Shown in Data Analysis

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

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @1.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.2500 (647), 11.2120 (488), 10.2093 (DAPI)
Fitting = Fast Fit
Normalization = Auto

2- Dimensional View - Panels A and B

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG is shown in red, DAPI is shown in blue.

Image from Z-plane 24 of 47, and the white box denotes the region of interest in Panel A

3D Image Processing - Panel C

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

Summary

Panel A shows a representative two-dimensional lattice SIM optical section (Z-plane 24 of 47) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. DAPI nuclear counterstaining provides chromatin context and confirms nuclear confinement of the RAD50 signal. The white boxed region denotes the region of interest (ROI) advanced for higher-resolution evaluation in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM view of the ROI at the same Z-plane. At SIM resolution, RAD50 assemblies that appear unresolved or composite at the Airyscan gate demonstrate improved structural definition and spatial organization. This resolution tier reveals increased separation and internal organization of RAD50 signal domains, indicating a clear gain in structural information relative to Airyscan.

Panel C shows a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 direct conjugate alone, pseudo-colored white for visualization. In three dimensions, RAD50 assemblies exhibit organized but still composite structures within the nuclear volume. While lattice SIM provides a meaningful improvement in spatial resolution and structural clarity over Airyscan, discrete subunit-level architecture remains unresolved.

Together, these panels demonstrate that lattice SIM represents a critical Step-Down resolution gate for RAD50, advancing structural interpretation beyond Airyscan while stopping short of molecular-scale resolution. In accordance with the CCR CDx Step-Down framework, these findings warrant continued progression to higher-resolution modalities, including SIM² and STORM, for definitive sub-structural and molecular interpretation of RAD50 organization.

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

Image Correction

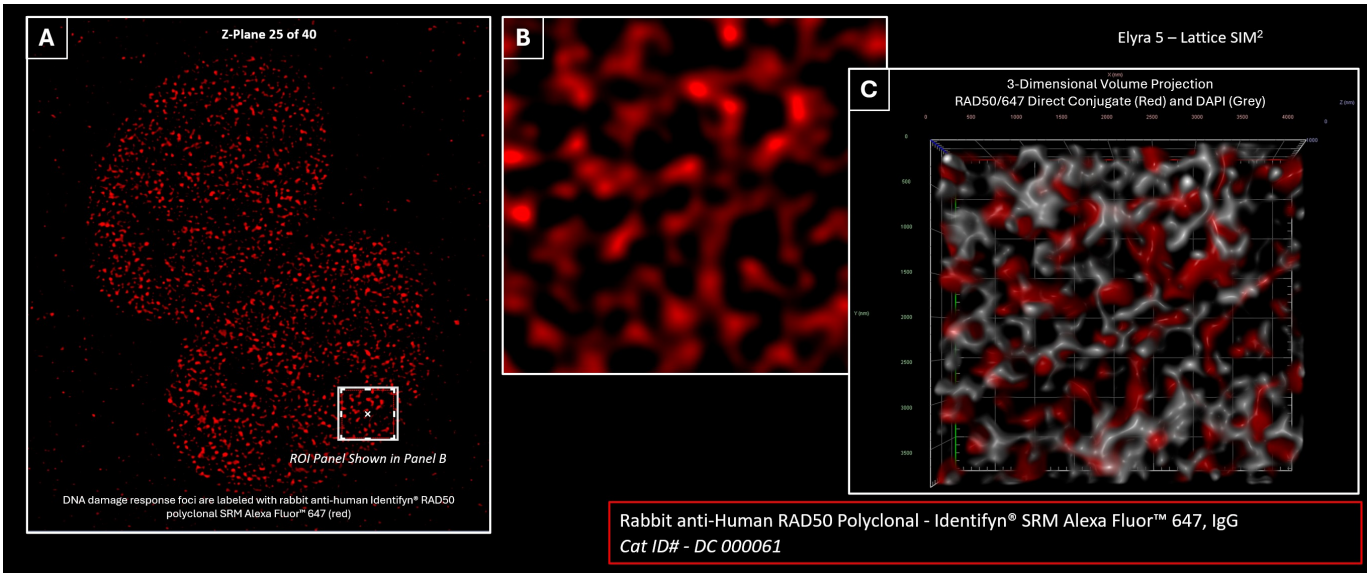
NONE

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

SOURCE PROVENANCE

Catalog	DC-000061
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	55FD3F4831ACAB38D61AD82EDD4400594E40673ECA1DC6704C74F67ADA5FB4EF
Method PDF SHA-256	27AA219981C46C7D487DCE7E03989B8B9D27D64B6975658837F051D4C8D7307F

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A shows a representative two-dimensional lattice SIM² optical section (Z-plane 25 of 40) of Rabbit anti-Human RAD50 Polyclonal – Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. Only the RAD50 647 channel is displayed to emphasize signal organization at this resolution tier. A white boxed region denotes the region of interest (ROI) advanced for further analysis in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM² view of the same ROI at the identical Z-plane. Relative to lattice SIM, SIM² reconstruction reveals enhanced structural definition and spatial refinement of RAD50 assemblies. Signal domains that appeared broader and less defined at the SIM gate now demonstrate increased organization and narrowing, indicating a measurable gain in spatial resolution.

Panel C displays a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 together with DAPI, with DAPI pseudo-colored light grey to provide chromatin context. In three dimensions, RAD50 assemblies demonstrate clear inter-localization with chromatin architecture, revealing organized recruitment patterns within and around chromatin domains. This represents the highest level of structural detail attainable prior to single-molecule localization.

Together, these panels demonstrate that lattice SIM² provides a meaningful improvement in resolution and spatial context over SIM, refining RAD50 organization and chromatin association. However, despite this gain, discrete subunit-level architecture and molecular-scale organization remain unresolved. In accordance with the CCR CDx Step-Down framework, definitive resolution of the structural features observed here requires progression to single-molecule localization microscopy (STORM) for biological ground-truth determination.RAD50 localization and recruitment behavior remain consistent with results obtained using the corresponding unconjugated Identifyn® Rabbit anti-Human RAD50 Polyclonal antibody (Cat. ID# PA-RP-000007), confirming preservation of biological specificity following direct conjugation.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	62

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	40 Slices (1.17 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1816 X 2010 | 201 X 186
Image Size (Scaled)	38.34 µm X 42.44 µm | 4.24 µm X 3.93 µm
Bit Depth	16 Bit
Image Center Position	X: 41.09 mm, Y: 34.55 mm
Stage Position	X: 41.09 mm, Y: 34.55 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms | 100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.0% | 488 nm @1.50% | 405 nm @1.0%
Frame Time	1.59 s | 1.33 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% 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 35%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Identifyn® Secondary Antibodies

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3 times in PBS.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Nuclear Pore Complex (NPC) and DAPI were not shown in the data analysis, but were present in the sample.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, Z-plane 25 of 40

Z-Stack = 40 Slices (1.17 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1816 X 2010
Image Size (Scaled) = 38.34 µm X 42.44 µm
Bit Depth = 16 Bit
Image Center Position = X: 41.09 mm, Y: 34.55 mm
Stage Position = X: 41.09 mm, Y: 34.55 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI, Z-plane 25 of 40, and Panel C, 3-Dimensional Volume Projection

Z-Stack = 40 Slices (1.17 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 201 X 186
Image Size (Scaled) = 4.24 µm X 3.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 41.09 mm, Y: 34.55 mm
Stage Position = X: 41.09 mm, Y: 34.55 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 @1.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 - Not Shown in Data Analysis

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

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @1.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

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

2- Dimensional View - Panels A and B

Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG is shown in red.

Image from Z-plane 25 of 40, and the white box denotes the region of interest in Panel A.

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% 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 35%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A shows a representative two-dimensional lattice SIM² optical section (Z-plane 25 of 40) of Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG following etoposide-induced DNA damage. Only the RAD50 647 channel is displayed to emphasize signal organization at this resolution tier. A white boxed region denotes the region of interest (ROI) advanced for further analysis in Panel B.

Panel B presents a higher-magnification two-dimensional lattice SIM² view of the same ROI at the identical Z-plane. Relative to lattice SIM, SIM² reconstruction reveals enhanced structural definition and spatial refinement of RAD50 assemblies. Signal domains that appeared broader and less defined at the SIM gate now demonstrate increased organization and narrowing, indicating a measurable gain in spatial resolution.

Panel C displays a three-dimensional volume projection of RAD50 Alexa Fluor™ 647 together with DAPI, with DAPI pseudo-colored light grey to provide chromatin context. In three dimensions, RAD50 assemblies demonstrate clear inter-localization with chromatin architecture, revealing organized recruitment patterns within and around chromatin domains. This represents the highest level of structural detail attainable prior to single-molecule localization.

Together, these panels demonstrate that lattice SIM² provides a meaningful improvement in resolution and spatial context over SIM, refining RAD50 organization and chromatin association. However, despite this gain, discrete subunit-level architecture and molecular-scale organization remain unresolved. In accordance with the CCR CDx Step-Down framework, definitive resolution of the structural features observed here requires progression to single-molecule localization microscopy (STORM) for biological ground-truth determination.

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

Image Correction

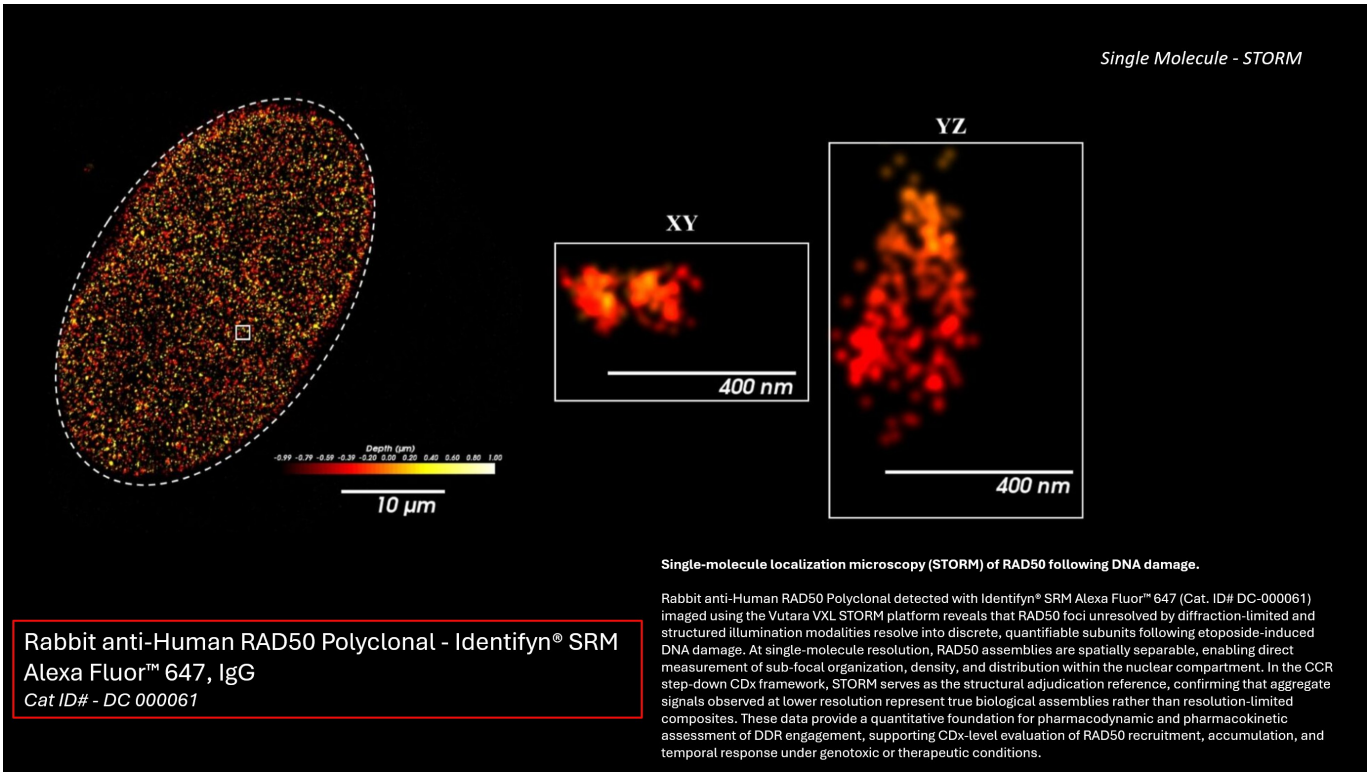
NONE

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

SOURCE PROVENANCE

Catalog	DC-000061
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	055641190A3476F911F3BC4E69BF11DB10C23FA8C0450219DA2A79DE16EC55CF
Method PDF SHA-256	175D9BE4750C2932842EDA5180F8EB558ED90A9711EF37F0ED415D54DCEE5EC3

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

Single-molecule localization microscopy (STORM) of RAD50 following DNA damage.

Rabbit anti-Human RAD50 Polyclonal detected with Identifyn® SRM Alexa Fluor™ 647 (Cat. ID# DC-000061) imaged using the Vutara VXL STORM platform reveals that RAD50 foci unresolved by diffraction-limited and structured illumination modalities resolve into discrete, quantifiable subunits following etoposide-induced DNA damage. At single-molecule resolution, RAD50 assemblies are spatially separable, enabling direct measurement of sub-focal organization, density, and distribution within the nuclear compartment. In the CCR step-down CDx framework, STORM serves as the structural adjudication reference, confirming that aggregate signals observed at lower resolution represent true biological assemblies rather than resolution-limited composites. These data provide a quantitative foundation for pharmacodynamic and pharmacokinetic assessment of DDR engagement, supporting CDx-level evaluation of RAD50 recruitment, accumulation, and temporal response under genotoxic or therapeutic conditions.

EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	80

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	635nm 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	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640nm (635nm Dichroic); First reference probe: 640nm (635nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	179.5µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" 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	50µm is selected
Piezo Record	Begin: 179.5; End: 179.5
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	30000
Z Positions	1
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	40%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
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
Probe1	BG threshold: 15
Sizing Mode	Radial Precision
Particle size	5nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.20
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	10 Intervals
Pixel Size	30nm
Smoothing	8.00
Radial precision	35
Axial precision	70
Clustering Algorithm	Not Selected
Maximum Particle Distance	Not Selected
Maximum Particle Count to Form Cluster	Not Selected
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	Not Selected
Compute	Not 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 Polyclonal - Identifyn® SRM Alexa Fluor™ 647, IgG
Cat ID# - DC 000061

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 200µM Etoposide-spiked complete growth media for 12 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD50 Polyclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:100. 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: 635nm 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: 100ms
Super Resolution Camera Exposure Time: 20ms
Widefield Camera Binning: 1
Per-Probe Exposure Time: Selected

Probe Configuration

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

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200µm
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 a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 179.5µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100ms
Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640nm" 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: 50µm 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 optimally focused.

Piezo Record: Begin: 179.5; End: 179.5

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: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: 30000
Probe 1
Z Positions: 1
Cycles: 1
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: 40%

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1px
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:

Probe1: BG threshold: 15

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: 5nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.20
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: 30nm
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: Not Selected

Parameters

Maximum Particle Distance: Not Selected
Maximum Particle Count to Form Cluster: Not Selected
Computer Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Hull Alpha shape Radius: Not Selected
Compute: Not Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Summary

Single-molecule localization microscopy (STORM) of RAD50 following DNA damage.
Rabbit anti-Human RAD50 Polyclonal detected with Identifyn® SRM Alexa Fluor™ 647 (Cat. ID# DC-000061) imaged using the Vutara VXL STORM platform reveals that RAD50 foci unresolved by diffraction-limited and structured illumination modalities resolve into discrete, quantifiable subunits following etoposide-induced DNA damage. At single-molecule resolution, RAD50 assemblies are spatially separable, enabling direct measurement of sub-focal organization, density, and distribution within the nuclear compartment. In the CCR step-down CDx framework, STORM serves as the structural adjudication reference, confirming that aggregate signals observed at lower resolution represent true biological assemblies rather than resolution-limited composites. These data provide a quantitative foundation for pharmacodynamic and pharmacokinetic assessment of DDR engagement, supporting CDx-level evaluation of RAD50 recruitment, accumulation, and temporal response under genotoxic or therapeutic conditions.

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	DC-000061
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	80
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AA02C77545842F63FEF741306C8F875192385F382DED5FB07B2A0A6887D87B9A
Method PDF SHA-256	6F12C95F21009B54F1A47D5A0C8DF5EDD60B65CBF882DCC4E322B0AAA4B501A3