

IDENTIFYN RESOLUTION STEPDOWN

RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Polyclonal

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

8

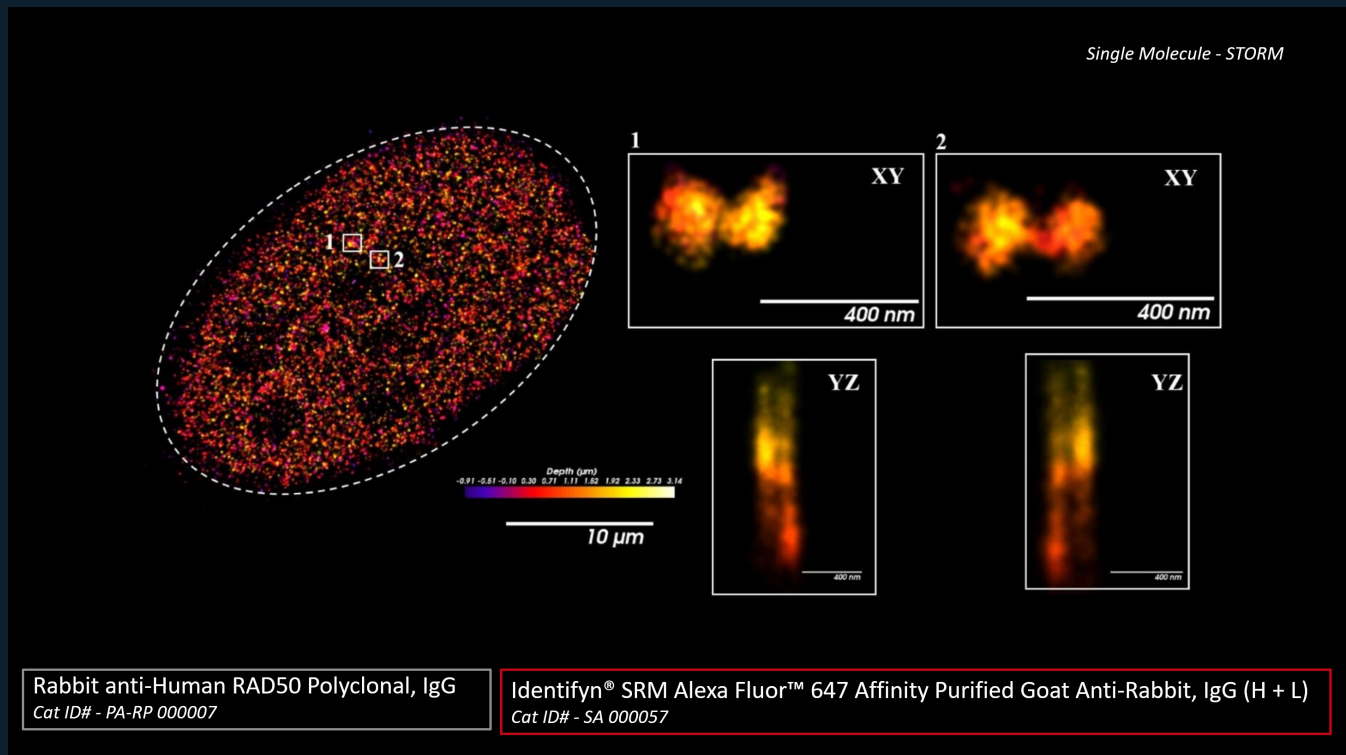
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RP-000007 | 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
- Preserved control experiment
- 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
-> Control

BENNETT ASSESSMENT

The preserved PA-RP-000007 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 PA-RP-000007 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

A true preserved control record is present and appears last.

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 @0.10%; 488nm @0.10%; 405nm @0.2%; 653; 493; 353
Airyscan	405/DAPI; 488; 647; 750	3; None; 639nm @0.10%; 488nm @0.08%; 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
SIM ²	405/DAPI; 488; 647	3 - (488 and DAPI not shown); 2; 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
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	3; 1 (647); None; 639nm @0.10%; 653; 668

MODALITY ASSESSMENT / PART 1 OF 9

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 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 μm X 0.110 μm ; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 100 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

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: 89 Slices (8.8 μm); Channels: 3; Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm ; Image size (Pixels): 1002 X 887; 365 X 375; Image Size (Pixels): 1002 X 887; 365 X 375; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 Mono. Timing: Exposure Time: 100 ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @20.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

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 9

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): 1017 X 1017; Image Size (Pixels): 1017 X 1017; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; GaAsP-Pmt3; Detector Type: GaAsP-PMT; GaAsP-PMT. Timing: Pixel Time: 0.33 μs ; Frame Time: 3.36s. Illumination: Laser Wavelength: 639nm @0.10%; 488nm @0.10%. Optical/scan: Pinhole: 1.00AU / 65 μm ; 1.00AU / 50 μm ; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 6.7 (3D, Auto). Structured source metadata values: 59.

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 9

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: 77 Slices (2.28 μm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; 35.90 nm X 35.90 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 796 X 783; Image Size (Pixels): 1566 X 1566; 796 X 783; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66 μs ; Frame Time: 7.82s. Illumination: Laser Wavelength: 639nm @0.10%; 488nm @0.08%. Optical/scan: Pinhole: 5.00AU / 328 μm ; 5.00AU / 250 μm ; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Filter: 9.6 (3D, Auto); Filter: 8.5 (3D, Auto). Structured source metadata values: 71.

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; 750.

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 9

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: 165 Slices (4.89 μ m); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 3126 X x 2929; 1268 X x 1258; Image Size (Pixels): 3126 X x 2929; 1268 X x 1258; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820; AxioCam 820 #2. Timing: Exposure Time: 100 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 15°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 70.

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 9

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: Slices 63 (1.86 μ m); Channels: 3 - (488 and DAPI not shown); 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1220 X x 1049; 318 X x 286; Image Size (Pixels): 1220 X x 1049; 318 X x 286; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.00%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 50°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 68.

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 9

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: 79.

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.

MODALITY ASSESSMENT / PART 9 OF 9

Control / 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: Channels: 3; 1 (647); Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; 0.059 μm X 0.059 μm ; Image size (Pixels): 1017 X 1017; 939 X 758; Image Size (Pixels): 1017 X 1017; 939 X 758; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.33 μs ; 0.30 μs ; Frame Time: 3.36s; 3.69s. Illumination: Laser Wavelength: 639nm @0.10%. Optical/scan: Pinhole: 1.00AU / 65 μm ; 1.00AU / 66 μm ; Scan Zoom: 2.2; 2.0; LSM Scan Speed: 12; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 6.6 (3D, Auto). Structured source metadata values: 54.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Control 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 PA-RP-000007 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): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 μ m X 0.03530 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1566 X 1566; Image Size (Scaled)=55.27 μ m X 55.27 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.90 nm X 35.90 nm X 30.00 nm -> 0.03590 μ m X 0.03590 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1566 X 1566; Image Size (Scaled)=55.27 μ m X 55.27 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 1.72%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3126 X x 2929; Image Size (Scaled)=66.00 μ m X 61.84 μ m; 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 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1220 X x 1049; Image Size (Scaled)=25.76 μ m X 22.15 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 μ M; 200 μ M -> Etoposide = 2 μ M. The governing scientist identified every 200 μ M occurrence as a technician transcription error. The canonical historical concentration is 2 μ M. 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 EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

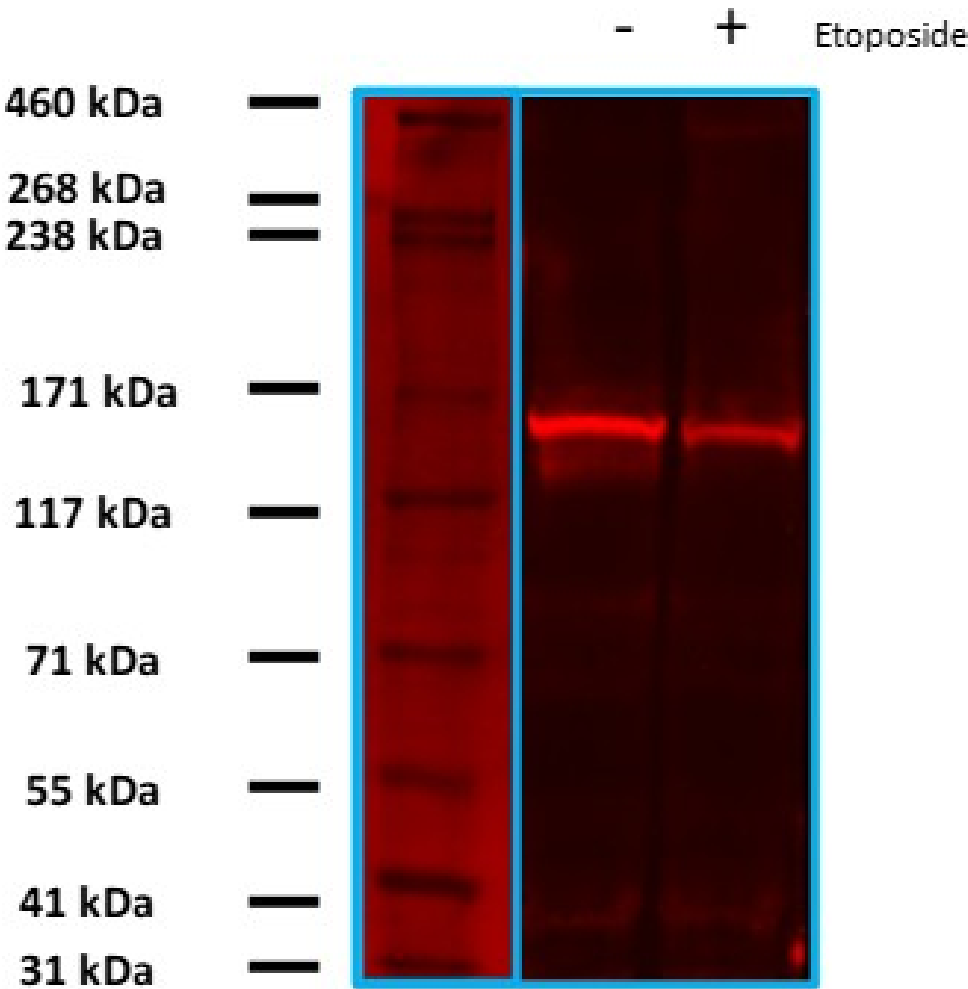
This dossier preserves the complete available evidence progression for PA-RP-000007. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(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, IgG

Cat ID# - PA-RP 000007

Expected MW: 153 kDa

HeLa cells were treated with 200 μ M etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and RIPA lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 μ g) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis 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 for 20 hours, followed by 5 washes in 5X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with 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 $\pm$ 37nm

Detector = PMT

Intensity = 3

Pixel size = 100 μ m

Scan Speed = High

Quality = High

Focus = Membrane (0 μ m in Z-axis)

Post Processing

None

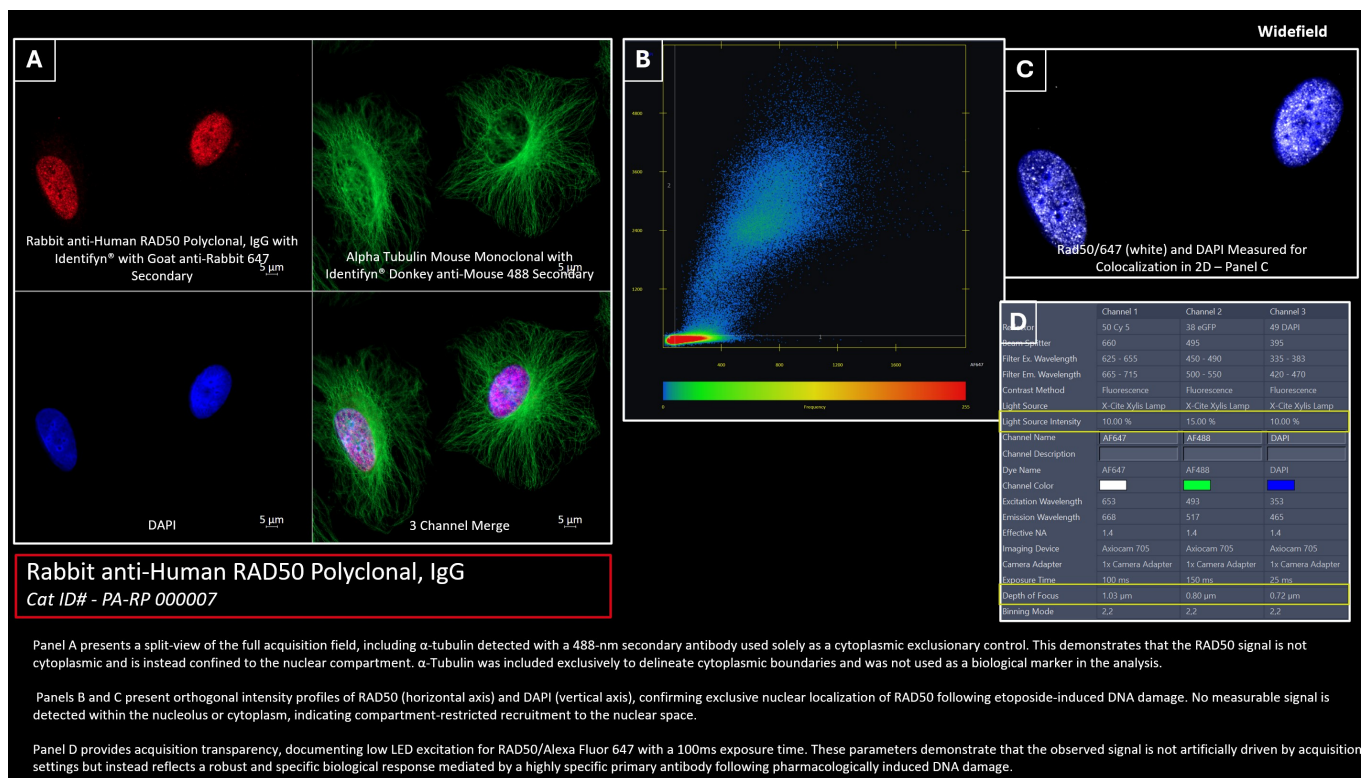
Image: K. Small

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SOURCE PROVENANCE

Catalog	PA-RP-000007
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	9CA29D551D54C8501AA8EEE578F4B790C6EB7A942BE51EFEB4975B9F326A3A3C
Method PDF SHA-256	DC1C8872227952EA597CC463308C56FB85523D0653D0446A74B0EC1D823145A6

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)	1216 X 1028
Image Size (Scaled)	133.18 μm X 112.59 μm
Bit Depth	14 Bit
Image Center Position	X: 0.00 μm , Y: 0.00 μm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	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 @10.00% X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	100 ms 150 ms 25 ms
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 μm 0.80 μm 0.72 μm
Binning Mode	2,2
Conclusion	RAD50 exhibits exclusive nuclear recruitment following etoposide-induced DNA damage. Whole-cell context and orthogonal intensity profiling confirm the absence of RAD50 signal in the cytoplasm and nucleolus, demonstrating compartment-restricted localization. α -Tubulin is used solely as a cytoplasmic exclusionary control to define cellular boundaries and is not interpreted as a biological marker. Acquisition parameters employ low excitation and short exposure times, confirming that the observed RAD50 signal reflects a robust and specific biological response rather than acquisition-driven amplification.

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, IgG
Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
 Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
 Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

Microscope

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

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110 µm X 0.110 µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18 µm X 112.59 µm

Bit Depth = 14 Bit

Image Center Position = X: 0.00 µm, Y: 0.00 µm

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

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 @10.00%
 Exposure Time = 100 ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 1.03 μm
 Binning Mode = 2,2

488 -

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

DAPI -

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

Split View - Panel A

The top-left panel features Rabbit anti-Human RAD50 Polyclonal, IgG, visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the top-right panel shows Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L), the bottom-left panel features DAPI nuclear staining, and the bottom-right panel features the merged image of all channels.

Colocalization View - Panel B, and Image Panel C, Measurement was of the entire field

Horizontal Axis - AF647, Threshold 82, Range Auto

Vertical Axis - DAPI, Threshold 254, Range Auto

Zeiss Zen Acquisition Settings - Panel D

Shown are the acquisition settings across all three channels, including LED intensity and exposure times, along with all other relevant parameters, provided for methodological transparency.

Summary

Panel A presents a split-view of the full acquisition field, including α -tubulin detected with a 488-nm secondary antibody used solely as a cytoplasmic exclusionary control. This demonstrates that the RAD50 signal is not cytoplasmic and is instead confined to the nuclear compartment. α -Tubulin was included exclusively to delineate cytoplasmic boundaries and was not used as a biological marker in the analysis.

Panels B and C present orthogonal intensity profiles of RAD50 (horizontal axis) and DAPI (vertical axis), confirming exclusive nuclear localization of RAD50 following etoposide-induced DNA damage. No measurable signal is detected within the nucleolus or cytoplasm, indicating compartment-restricted recruitment to the nuclear space.

Panel D provides acquisition transparency, documenting low LED excitation for RAD50/Alexa Fluor 647 with a 100 ms exposure time. These parameters demonstrate that the observed signal is not artificially driven by acquisition settings but instead reflects a robust, specific biological response mediated by a highly specific primary antibody following pharmacologically induced DNA damage.

Conclusion: RAD50 exhibits exclusive nuclear recruitment following etoposide-induced DNA damage. Whole-cell context and orthogonal intensity profiling confirm the absence of RAD50 signal in the cytoplasm and nucleolus, demonstrating compartment-restricted localization. α -Tubulin is used solely as a cytoplasmic exclusionary control to define cellular boundaries and is not interpreted as a biological marker. Acquisition parameters employ low excitation and short exposure times, confirming that the observed RAD50 signal reflects a robust and specific biological response rather than acquisition-driven amplification.

Image Correction

Panel C - Rabbit anti-Human Rad50 Polyclonal counterstained with Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

Image by E.F.Simon

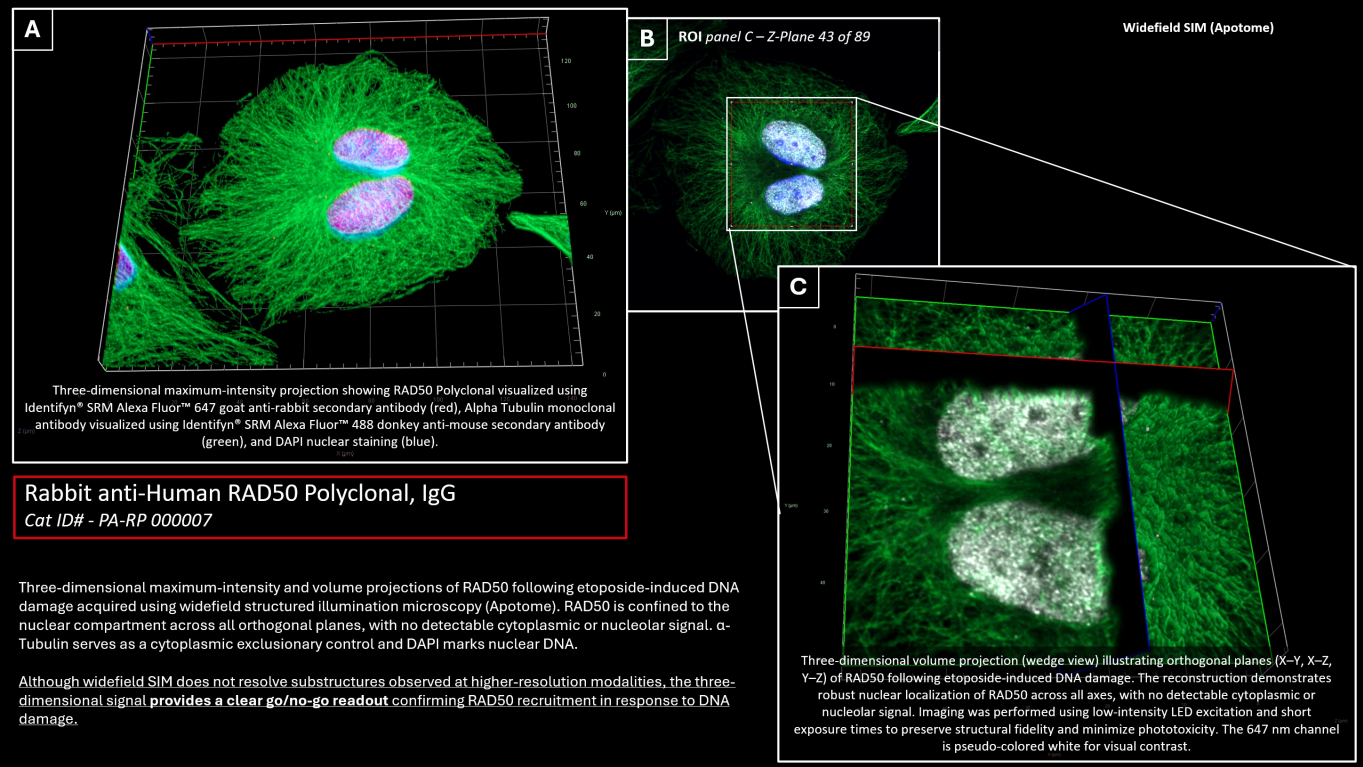
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	PA-RP-000007
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 Xylys Lamp, was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	12DB92062C5AA3F9DCD09579D60B910BB7ACEDCCF542A885B06D9AE496465A8A
Method PDF SHA-256	3E7D2F6DFE6DE13541238760FFDFF4F4A893EFE73F81E6B1AD0780624CB0CB47

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	62

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	89 Slices (8.8 μm)
Channels	3
Scaling (Per Pixel)	0.143 μm X 0.143 μm X 0.100 μm
Image size (Pixels)	1002 X 887 365 X 375
Image Size (Scaled)	143.14 μm X 126.71 μm 52.14 μm X 53.57 μm
Bit Depth	14 Bit
Image Center Position	X: 87.68 μm , Y: 53.20 μm
Stage Position	X: 87.68 μm , Y: 53.20 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 @15.00% X-Cite Xylis Lamp Intensity @20.00% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	100 ms 200 ms 25 ms
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.25
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 13% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

FIELD	SOURCE-DOCUMENTED VALUE
3D Volume Projection	Precise
X-Y	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-3% -33% -14%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	45° -45°
Y-Z	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

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, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 89 Slices (8.8 µm)

Channels = 3

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

Image size (Pixels) = 1002 X 887

Image Size (Scaled) = 143.14 µm X 126.71 µm

Bit Depth = 14 Bit

Image Center Position = X: 87.68 µm, Y: 53.20 µm

Stage Position = X: 87.68 µm, Y: 53.20 µm

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

Single Plane (2D) - Panel B, Z-Plane 43 of 89, (Dimension Shown in Panel A)

Z Stack - (3D) - Panel C

Z-Stack = 89 Slices (8.8 µm)

Channels = 3

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

Image size (Pixels) = 365 X 375

Image Size (Scaled) = 52.14 µm X 53.57 µm

Bit Depth = 14 Bit

Image Center Position = X: 87.68 µm, Y: 53.20 µm

Stage Position = X: 87.68 µm, Y: 53.20 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 @15.00%

Exposure Time = 100 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 = 1.25
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 0.90 μm
 Binning Mode = 2,2

Post Processing - SIM Processing

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

3D Image Processing - Panel A

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

3D Image Processing - Panel C

3D Volume Projection = Precise

Appearance = Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

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

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

Position of Clip = -3%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = -33%

Clip Y = 45°

Clip Z = 0°

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

Position of Clip = -14%

Clip Y = -45°

Clip Z = 0°

Summary - RAD50 Recruitment Assessed by Widefield SIM (Apotome)

Three-dimensional maximum-intensity projection showing RAD50 detected with an Identifyn® polyclonal primary antibody and visualized using Identifyn® SRM Alexa Fluor™ 647 goat anti-rabbit secondary antibody (pseudo-colored white). α -Tubulin is visualized with an Identifyn® SRM Alexa Fluor™ 488 donkey anti-mouse secondary antibody (green) and serves as a cytoplasmic exclusionary control, while DAPI marks nuclear DNA (blue).

ROI Panel C corresponds to Z-plane 43 of 89. A three-dimensional volume reconstruction (wedge view) illustrates orthogonal planes (X-Y, X-Z, Y-Z) of RAD50 following etoposide-induced DNA damage. Across all axes, the RAD50 signal is confined to the nuclear compartment, with no detectable cytoplasmic or nucleolar localization. Imaging was performed using low-intensity LED excitation and short exposure times to preserve structural fidelity and avoid acquisition-driven signal amplification.

Acquired using widefield structured illumination microscopy (Apotome), this dataset demonstrates that three-dimensional imaging at SIM resolution enables visualization of RAD50 aggregates that are not discernible in conventional widefield imaging. While these aggregates do not resolve the substructures observed at higher-resolution modalities downstream in the step-down workflow (SIM² and STORM), the signal provides a clear go / no-go readout confirming that etoposide induces a measurable RAD50 DNA damage response.

Image Correction

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white, to provide visual contrast in Panels B and C.

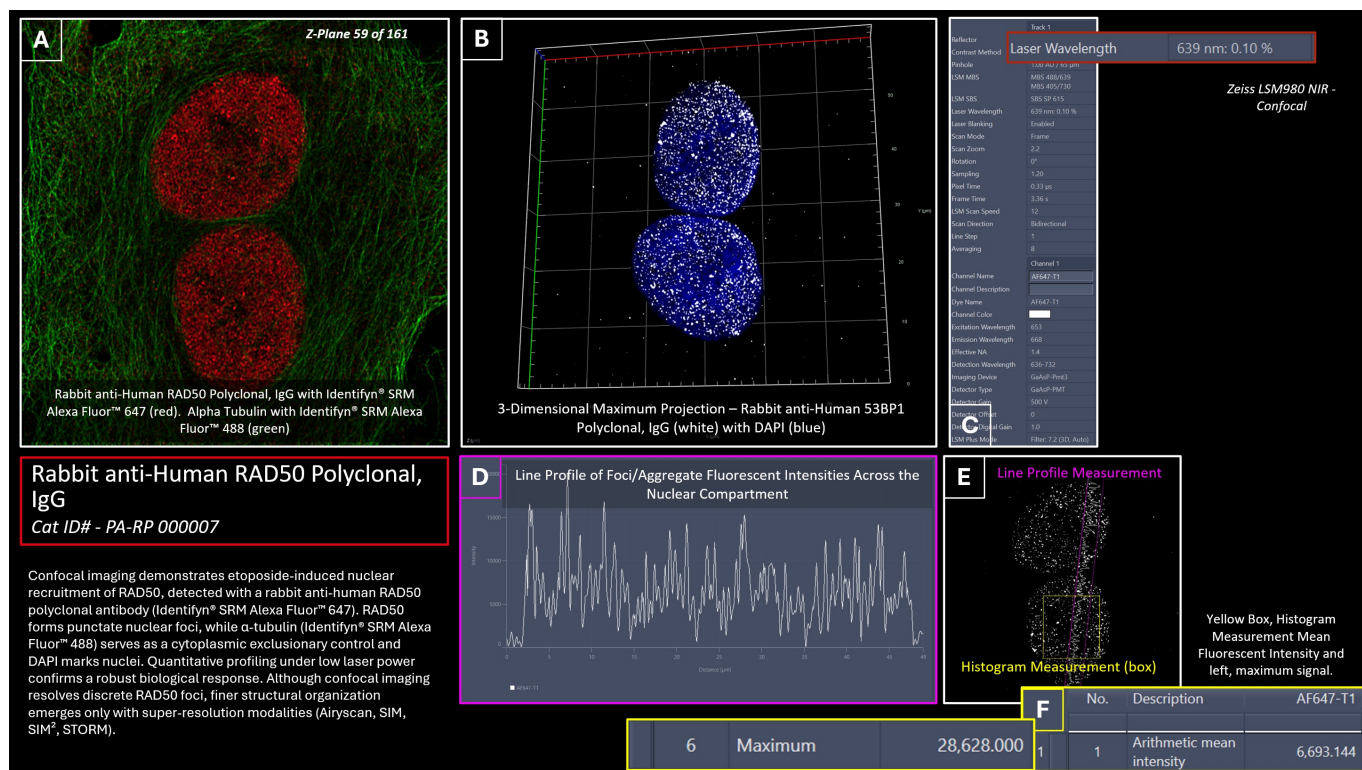
Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	PA-RP-000007
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	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2AAE10B7F5ADE0B7F46B0E31940C30135762B779702BFFD45731B27F7A79E1E0
Method PDF SHA-256	0E9AA9200A8EFD9F5E5BD09EACBCC10565FB5B6D64F7A5000B603B93B14642E7

ORIGINAL IMAGE EVIDENCE / CONFOCAL



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

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)	1017 X 1017
Image Size (Scaled)	59.81 μm X 59.81 μm
Bit Depth	16 Bit
Image Center Position	X: 92.75 mm, Y: 50.66 mm
Stage Position	X: 92.75 mm, Y: 50.66 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 65 μm 1.00AU / 50 μm 1.00AU / 43 μm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615 Mirror
Laser Wavelength	639nm @0.10% 488nm @0.10% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.20
Pixel Time	0.33 μs
Frame Time	3.36s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.4
Detection Wavelength	636 - 732 489 - 550 430 - 470
Imaging Device	GaAsP-Pmt3 GaAsP-Pmt3
Detector Type	GaAsP-PMT GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) Filter: 6.7 (3D, Auto) Filter: 6.1 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% 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)
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 49.0), Y (Min 0 and Max 21092)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000)

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, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

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:

Single Plane (2D) - Z-Plane 59 of 161- Panels A & E

Z Stack - (3D) - 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) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm

Bit Depth = 16 Bit

Image Center Position = X: 92.75 mm, Y: 50.66 mm

Stage Position = X: 92.75 mm, Y: 50.66 mm

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 @0.10%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.2

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.33µs

Frame Time = 3.36s

LSM Scan Speed = 12

Scan Direction = Bidirectional

Line Step = 1

Averaging - 8

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Detection Wavelength = 636 - 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: 7.2 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 50µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 488nm @0.10%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.33µs
 Frame Time = 3.36s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 489 - 550
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.7 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 43µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.33µs
 Frame Time = 3.36s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 430 - 470
 Imaging Device = GaAsp-Pmt3
 Detector Type = GaAsp-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.1 (3D, Auto)

2-Dimensional View - Panel A

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L).

The image was taken from Z-stack Acquisition at plane 59 of 161.

3D Image Processing - Panel B

3D Maximum Projection = Precise
 Appearance = Threshold 2% 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)

Note that the 53BP1, counterstained with 647, was pseudo-colored white from red in Zeiss Zen, to enhance visual contrast.

LSM 980 Zen Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel (RAD50 Rabbit Polyclonal with Identifyn 647 secondary) using the 639nm laser at 0.10%.

Not shown, the 488 channel (Alpha Tubulin) using the 488nm laser at 0.10%, and DAPI using the 405nm laser at 0.2%.

Profile View Display - Panels D & E

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 49.0), Y (Min 0 and Max 21092)

This panel measures RAD50 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 2'K A.U. at its maximum.

Measured at Z-plane 59 of 161.

Histogram Display - Panels E & F

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000)

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

Summary -

Panel A - Confocal Imaging of RAD50

Confocal imaging of RAD50 following etoposide-induced DNA damage. RAD50 is detected using a rabbit anti-human RAD50 polyclonal antibody conjugated to Identifyn® SRM Alexa Fluor™ 647 (red). α -Tubulin is visualized with Identifyn® SRM Alexa Fluor™ 488 (green) and serves as a cytoplasmic exclusionary control.

Panel B - Nuclear Damage Context

Three-dimensional maximum-intensity projection of rabbit anti-human 53BP1 polyclonal antibody (white) with DAPI nuclear staining (blue), providing confirmation of DNA damage-associated nuclear responses under the same treatment conditions.

Panel C - Acquisition Parameters

Laser power settings for the 639 nm channel (0.10) were used during confocal acquisition, demonstrating low excitation conditions.

Panels D and E - Quantitative Signal Analysis

Line profile and histogram measurements of RAD50 signal intensity under low laser power conditions, yielding an arithmetic mean intensity of 6,693.144 and a maximum signal of 28,623.8, confirming robust signal induction without acquisition-driven amplification.

Summary -

Panel A - Confocal Imaging of RAD50

Confocal imaging of RAD50 following etoposide-induced DNA damage. RAD50 is detected using a rabbit anti-human RAD50 polyclonal antibody conjugated to Identifyn® SRM Alexa Fluor™ 647 (red). α -Tubulin is visualized with Identifyn® SRM Alexa Fluor™ 488 (green) and serves as a cytoplasmic exclusionary control.

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Three-dimensional maximum-intensity projection of rabbit anti-human 53BP1 polyclonal antibody (white) with DAPI nuclear staining (blue), providing confirmation of DNA damage-associated nuclear responses under the same treatment conditions.

Panel C - Acquisition Parameters

Laser power settings for the 639 nm channel (0.10) were used during confocal acquisition, demonstrating low excitation conditions.

Panels D and E - Quantitative Signal Analysis

Line profile and histogram measurements of RAD50 signal intensity under low laser power conditions, yielding an arithmetic mean intensity of 6,693.144 and a maximum signal of 28,623.8, confirming robust signal induction without acquisition-driven amplification.

Confocal imaging demonstrates etoposide-induced recruitment of RAD50 to the nuclear compartment, where it appears as discrete punctate foci consistent with activation of the DNA damage response. Quantitative intensity measurements obtained under low laser power confirm a genuine biological signal rather than forced amplification. At confocal resolution, the assay provides a clear go/no-go readout for RAD50 recruitment; however, finer structural organization of RAD50 foci is not resolved until crossing the super-resolution threshold using Airyscan, SIM, SIM², and STORM, where sub-focal architecture becomes progressively apparent.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and E.

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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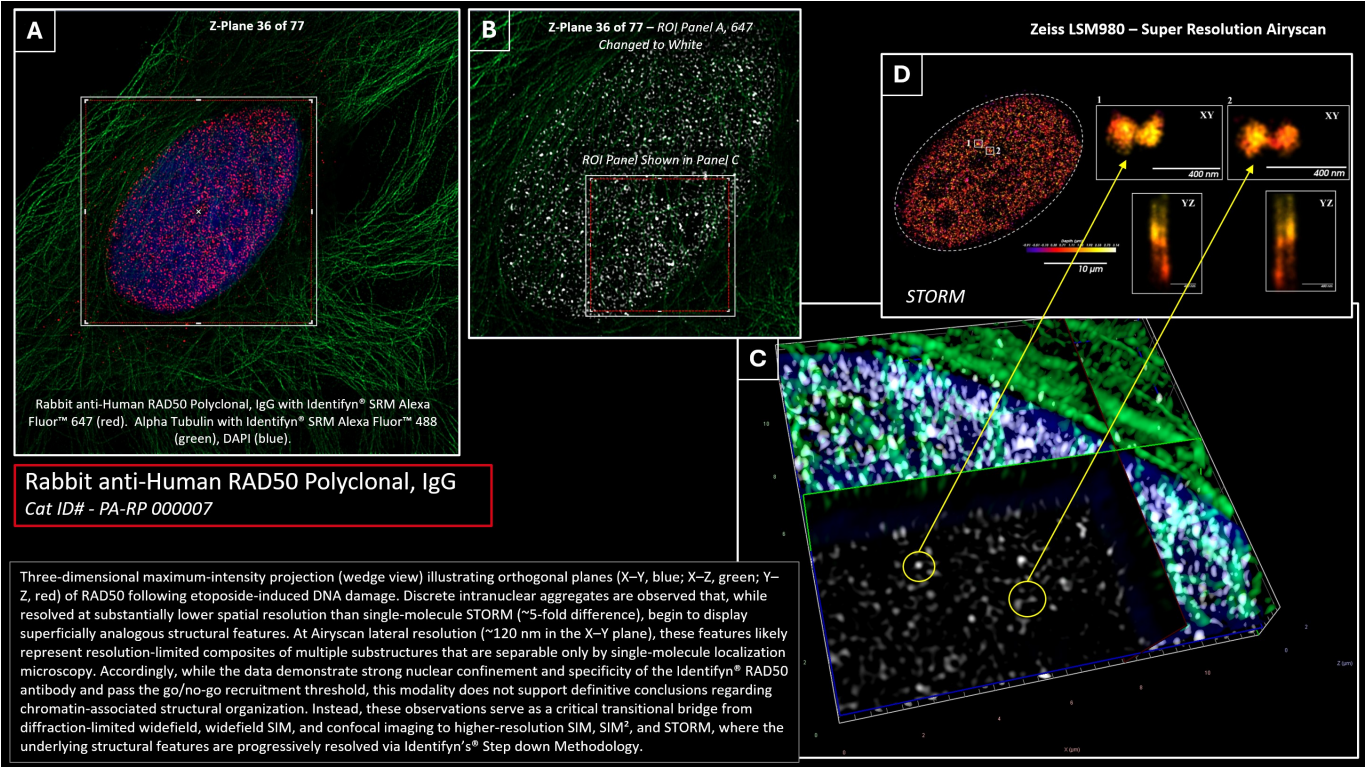
SOURCE PROVENANCE

Catalog	PA-RP-000007
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	59

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C0DEFB1FE97C8C4599A8874FE1095720BF40A28D9324D95EAE1DA53DFC699A79
Method PDF SHA-256	23219A92B8C6C39CAE0F9944E05839DAD907D1B79EF5131A1A5DDC8325913DDE

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 750
METADATA VALUES	71

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
Channels	3
Scaling (Per Pixel)	0.03530 μm X 0.03530 μm X 0.03000 μm 0.03590 μm X 0.03590 μm X 0.03000 μm
Image Size (Pixels)	1566 X 1566 796 X 783 332 X 317
Image Size (Scaled)	55.27 μm X 55.27 μm 28.10 μm X 27.64 μm 11.72 μm X 11.19 μm
Bit Depth	16 Bit
Image Center Position	X: 89.34 mm, Y: 54.32 mm X: 85.47 mm, Y: 48.09 mm
Stage Position	X: 89.34 mm, Y: 54.32 mm X: 85.47 mm, Y: 48.09 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Z-Stack	77 Slices (2.28 μm)
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 328 μm 5.00AU / 250 μm 5.00AU / 214 μm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 640 SBS SP 550 SBS SP 505
Laser Wavelength	639nm @0.10% 488nm @0.08% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66 μs
Frame Time	7.82s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
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
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V 750 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Filter: 9.6 (3D, Auto) Filter: 8.5 (3D, Auto) Filter: 7.9 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 3% 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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-50% -8% 38%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	30°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human RAD50 Polyclonal, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

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:

Single Plane (2D) - Z-Plane 36 of 77- Panel A

Channels = 3

Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm

Image Size (Pixels) = 1566 X 1566

Image Size (Scaled) = 55.27 μ m X 55.27 μ m

Bit Depth = 16 Bit

Image Center Position = X: 89.34 mm, Y: 54.32 mm

Stage Position = X: 89.34 mm, Y: 54.32 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Single Plane (2D) - Z-Plane 36 of 77- Panel B, ROI Panel A

Channels = 3

Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm

Image Size (Pixels) = 796 X 783

Image Size (Scaled) = 28.10 μ m X 27.64 μ m

Bit Depth = 16 Bit

Image Center Position = X: 89.34 mm, Y: 54.32 mm

Stage Position = X: 89.34 mm, Y: 54.32 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

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

Z-Stack = 77 Slices (2.28 μ m)

Channels = 3

Scaling (Per Pixel) = 35.90 nm X 35.90 nm X 30.00 nm

Image Size (Pixels) = 332 X 317

Image Size (Scaled) = 11.72 μ m X 11.19 μ m

Bit Depth = 16 Bit

Image Center Position = X: 85.47 mm, Y: 48.09 mm

Stage Position = X: 85.47 mm, Y: 48.09 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 328µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = SBS SP 640
 Laser Wavelength = 639nm @0.10%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66µs
 Frame Time = 7.82s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 643 - 735
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Filter: 9.6 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 250µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = SBS SP 550
 Laser Wavelength = 488nm @0.08%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66µs
 Frame Time = 7.82s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 499 - 548
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Filter: 8.5 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 214µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = SBS SP 505
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66µs
 Frame Time = 7.82s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 422 - 477
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 750 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Filter: 7.9 (3D, Auto)

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI.

Panel B, the 647 channel (53BP1) is changed from the default red to white to enhance visual contrast.

Image was taken from Z-stack Acquisition at plane 36 of 77.

3D Image Processing

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

Clipping Image Process - Panel C

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

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

Position of Clip = -50%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = -8%

Clip Y = 30°

Clip Z = 0°

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

Position of Clip = 38%

Clip Y = 30°

Clip Z = 0°

Panel D - STORM Image from the same data set.

Summary -

Gate - Recruitment / Specificity (Go-No-Go)

Using the Identifyn® Standards for Optical Microscopy Methods™, a step-down, multi-modal imaging workflow was applied to evaluate RAD50 recruitment following etoposide-induced DNA damage. HeLa cells were labeled with a rabbit anti-human RAD50 polyclonal IgG, detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, and α -tubulin and DAPI served as cytoplasmic and nuclear references, respectively. Across all imaging views, RAD50 signal was robustly confined to the nuclear compartment, with no detectable cytoplasmic or extranuclear labeling, establishing antibody specificity and passing the go/no-go recruitment gate.

Enrichment - Spatial Context and Signal Validation

Two-dimensional optical sections (Panels A and B) acquired from a 77-slice Z-stack demonstrate a discrete intranuclear RAD50 signal relative to α -tubulin. For visualization, the 647-nm channel was pseudo-colored white instead of its default red, without altering the underlying intensity values. Three-dimensional volume reconstructions (Panel C) reveal punctate and aggregated RAD50 distributions throughout the nuclear volume. Orthogonal clipping and wedge-based projections across X-Y, X-Z, and Y-Z planes confirm spatial continuity and nuclear confinement, enriching the recruitment signal with three-dimensional context.

All data were acquired on a Zeiss LSM 980 with Airyscan detection under low-excitation conditions (639 nm at 0.10%, 488 nm at 0.08%, 405 nm at 0.2%). Quantitative line-profile and histogram analyses show strong signal-to-background ratios and reproducible nuclear intensity distributions, validating signal robustness under conservative acquisition parameters.

Structural Resolution - Deferred Interpretation (Step-Down Progression)

While three-dimensional Airyscan imaging begins to reveal features superficially analogous to those observed by single-molecule localization microscopy, the lateral resolution limit of Airyscan (~120 nm in X-Y) constrains structural interpretation. Apparent RAD50 aggregates at this stage likely represent resolution-limited composites of multiple substructures that are separable only at higher resolution, as demonstrated by STORM imaging of the same dataset (Panel D). Accordingly, although the data confirm nuclear recruitment and antibody specificity, definitive chromatin-associated structural organization is intentionally deferred to subsequent super-resolution steps.

Aligned Interpretation

Within a CDx framework, confocal and Airyscan imaging establish a binary recruitment gate (presence/absence), provide enrichment through validated three-dimensional localization, and define the transition point at which structural claims require higher-resolution modalities. The Airyscan dataset thus serves as a rigorously controlled intermediate step that preserves assay integrity while enabling progression to SIM, SIM², and STORM for structural stratification of DNA damage-associated RAD50 architecture.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and C.

Image by J.K. Bennett

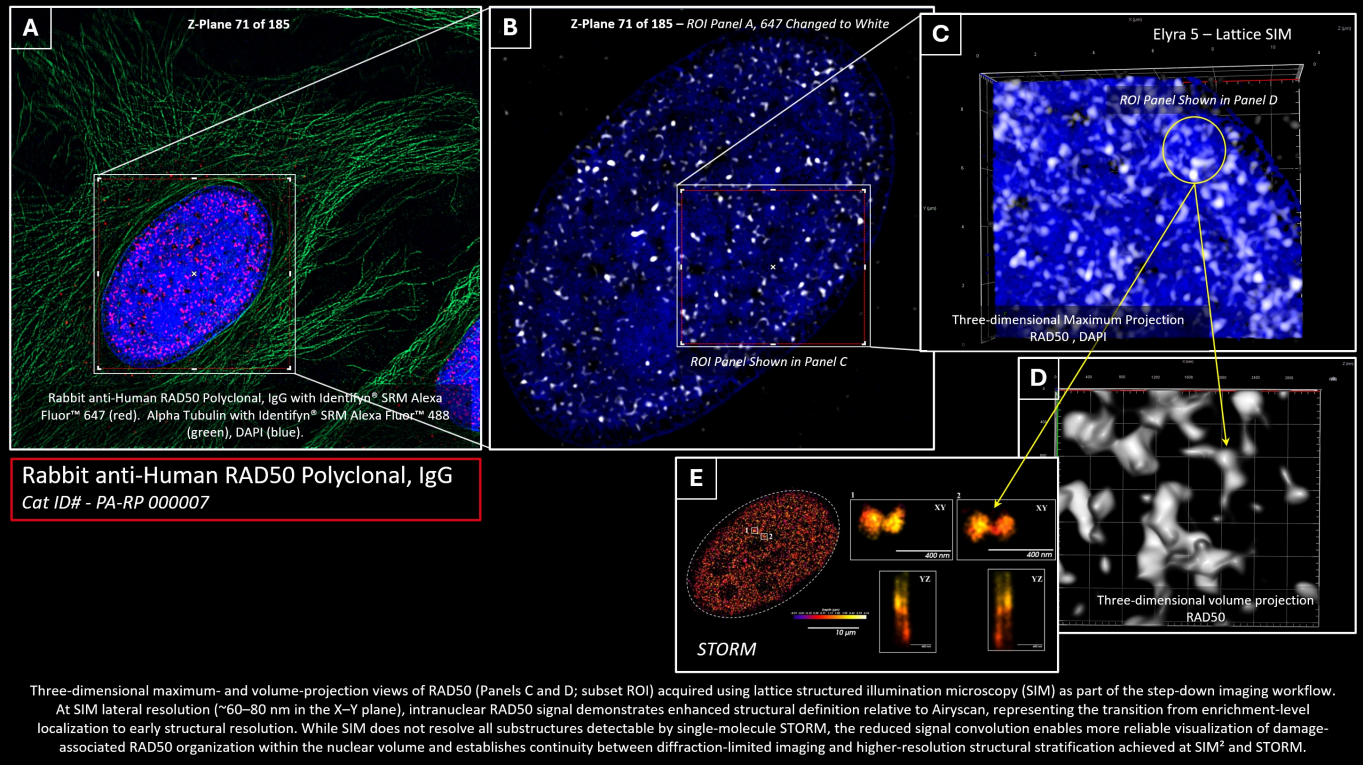
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	PA-RP-000007
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	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	07C0073E16075EB869371973616D51E55A4ECE965B450547AD261D46B7342EDA
Method PDF SHA-256	304E6745DCE1BE63C81FEEB9A1E65D996512E2DAA1586FD94A9B5CB54A15E19F

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	70

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
Channels	3
Scaling (Per Pixel)	0.02111 μm X 0.02111 μm X 0.03000 μm
Image Size (Pixels)	3126 X x 2929 1268 X x 1258 522 X x 438 151 X x 135
Image Size (Scaled)	66.00 μm X 61.84 μm 26.77 μm X 25.56 μm 11.02 μm X 9.25 μm 3.19 μm X 2.85 μm
Bit Depth	16 Bit
Image Center Position	X: 60.64 mm, Y: 39.26 mm
Stage Position	X: 60.64 mm, Y: 39.26 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Z-Stack	165 Slices (4.89 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	100 ms 50 ms
Depth of Focus	1.13 μm 0.81 μm 0.69 μm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% 488 nm @1.00% 405 nm @2.00%
Frame Time	1.33 s 676.00 ms
Scan Direction	Unidirectional
Grating	27.5 μm 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	9.6423 (647), 11.5105 (488), 10.1126 (DAPI)
Fitting	Fast Fit
Normalization	Auto
Panel B	the 647 channel (RAD50) is changed from the default red to white to enhance visual contrast, and tubulin (488) is not shown.
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 15°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

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, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

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:

Single Plane (2D) - Z-plane 71 of 185 total - Panel A

Channels = 3

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 3126 X x 2929

Image Size (Scaled) = 66.00 µm X 61.84 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.64 mm, Y: 39.26 mm

Stage Position = X: 60.64 mm, Y: 39.26 mm

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

Single Plane (2D) - Z-plane 71 of 185 total - Panel B

Channels = 3

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 1268 X x 1258

Image Size (Scaled) = 26.77 µm X 25.56 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.64 mm, Y: 39.26 mm

Stage Position = X: 60.64 mm, Y: 39.26 mm

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

Z Stack - (3D) - Panel C

Z-Stack =165 Slices (4.89 µm)

Channels = 3

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 522 X x 438

Image Size (Scaled) = 11.02 µm X 9.25 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.64 mm, Y: 39.26 mm

Stage Position = X: 60.64 mm, Y: 39.26 mm

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

Z Stack - (3D) - Panel D

Z-Stack =165 Slices (4.89 µm)

Channels = 3

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 151 X x 135

Image Size (Scaled) = 3.19 µm X 2.85 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.64 mm, Y: 39.26 mm

Stage Position = X: 60.64 mm, Y: 39.26 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 = 100 ms
 Depth of Focus = 1.13 μm
 Binning Mode = 2,2
 Laser Wavelength = 640 nm @1.00%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 27.5 μm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 100 ms
 Depth of Focus = 0.81 μm
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.00%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 27.5 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 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @2.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m 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 = 9.6423 (647), 11.5105 (488), 10.1126 (DAPI)
 Fitting = Fast Fit
 Normalization = Auto

2-Dimensional View - Panels A and B

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) combined with a Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI,

Panel B: the 647 channel (RAD50) is changed from the default red to white to enhance visual contrast, and tubulin (488) is not shown.

Image was taken from Z-stack Acquisition at Z-plane 71 of 185.

3D Image Processing - Panel C

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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Volume Projection = Precise

Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 15°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - STORM

Summary -

Super-resolution lattice structured illumination microscopy (SIM) was used to further characterize the intranuclear distribution of RAD50 following etoposide-induced DNA damage within the Identifyn® Standards for Optical Microscopy Methods™ step-down framework. HeLa cells were labeled with a rabbit anti-human RAD50 polyclonal IgG, detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, and α -tubulin and DAPI served as cytoskeletal and nuclear references, respectively. Across all lattice SIM views, RAD50 signal was strictly confined to the nuclear compartment, consistent with its established role as a core component of the MRN complex involved in DNA double-strand break (DSB) detection and signaling.

Two-dimensional lattice SIM optical sections (Panels A and B) acquired from a 185-slice Z-stack reveal punctate and aggregated intranuclear RAD50 distributions with substantially increased spatial definition relative to Airyscan imaging. Three-dimensional volume reconstructions (Panel C) and higher-magnification region-of-interest analyses (Panel D) further resolve damage-associated RAD50 assemblies throughout the nuclear volume. At lattice SIM lateral resolution (~60-80 nm in the X-Y plane), these signal patterns display reproducible organizational features consistent with RAD50 accumulation at DNA damage sites coordinated by the MRN complex.

Functionally, RAD50 plays a central role in DNA double-strand break sensing, tethering, and signaling through its interaction with MRE11 and NBS1, facilitating ATM activation and repair pathway engagement. While lattice SIM does not resolve all substructures detectable by single-molecule localization microscopy, the improved resolution reduces signal convolution and enables more reliable visualization of damage-associated RAD50 organization within the nuclear volume. Direct comparison with STORM imaging from the same dataset (Panel E) indicates that apparent aggregates at SIM resolution may represent resolution-limited composites of multiple RAD50 sub-assemblies that are separable only at the single-molecule level.

Collectively, these data position lattice SIM as a critical intermediate modality within the step-down workflow, bridging enrichment-level localization (confocal/Airyscan) and definitive structural resolution (SIM²/STORM). Although chromatin-level architectural conclusions require higher-resolution localization approaches, accurate mapping of RAD50 nuclear organization at this stage provides essential context for interpreting DNA damage sensing, MRN complex engagement, and early repair pathway activation following genotoxic stress.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B, C, and D.

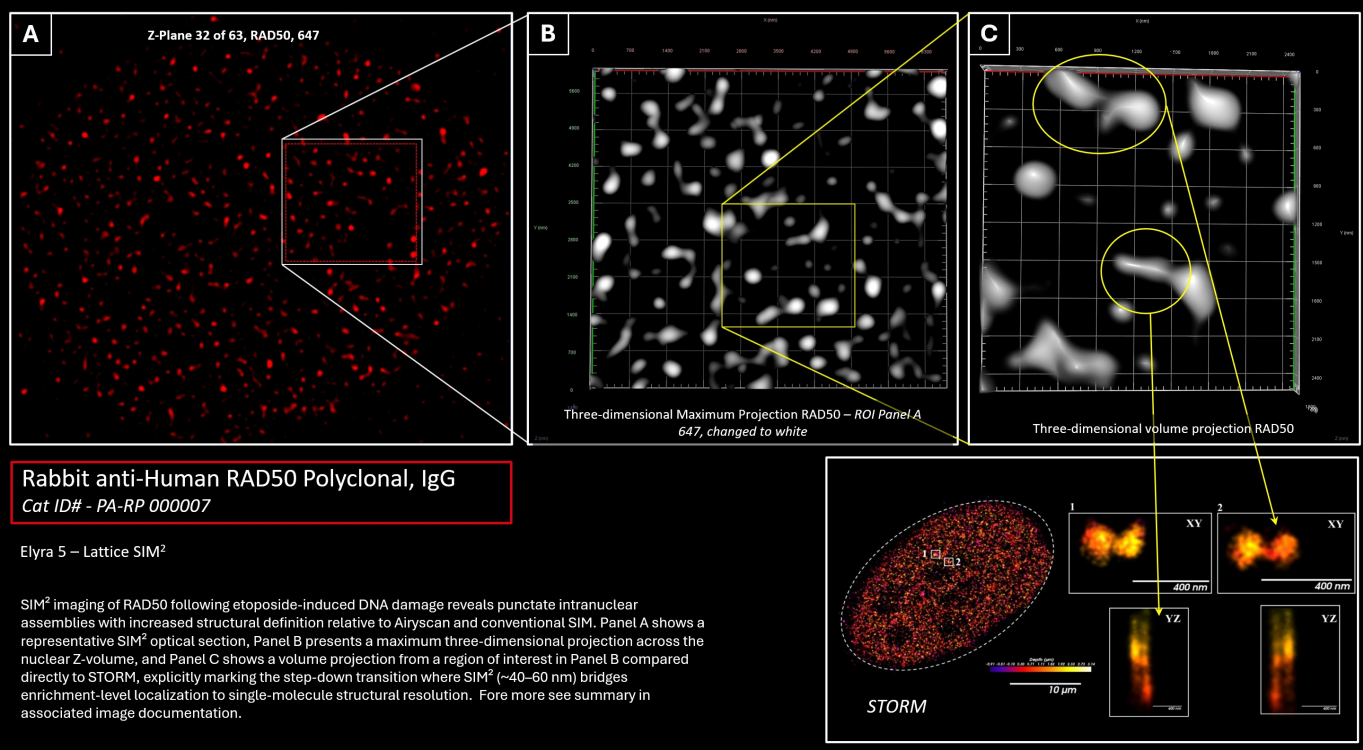
Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	PA-RP-000007
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	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1E65AC68DE7E605AD3770AA3DA33C17C83DD7C325044509F77665583D2E93BAE
Method PDF SHA-256	322F61CFCD8047588C15691B52EDDE370A632198E4E4D68223EBCB122E7A3F20

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	68

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
Channels	3 - (488 and DAPI not shown) 2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1220 X x 1049 318 X x 286 118 X x 120
Image Size (Scaled)	25.76 µm X 22.15 µm 6.71 µm X 6.04 µm 2.49 µm X 2.53 µm
Bit Depth	16 Bit
Image Center Position	X: 61.21 mm, Y: 36.03 mm
Stage Position	X: 61.21 mm, Y: 36.03 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	Slices 63 (1.86 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	100 ms 50 ms
Depth of Focus	1.13 µm 0.81 µm 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% 488 nm @1.00% 405 nm @2.00%
Frame Time	1.33 s 676.00 ms
Scan Direction	Unidirectional
Grating	27.5 µm 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 Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 50°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

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, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000015

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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

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

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:

Single Plane (2D) - Z-plane 32 of 63 total - Panel A

Channels = 3 - (488 and DAPI not shown)
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1220 X x 1049
Image Size (Scaled) = 25.76 µm X 22.15 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.21 mm, Y: 36.03 mm
Stage Position = X: 61.21 mm, Y: 36.03 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = Slices 63 (1.86 µm)

Channels = 3 - (488 and DAPI not shown)
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 318 X x 286
Image Size (Scaled) = 6.71 µm X 6.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.21 mm, Y: 36.03 mm
Stage Position = X: 61.21 mm, Y: 36.03 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = Slices 63 (1.86 µm)

Channels = 3 - (488 and DAPI not shown)
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 118 X x 120
Image Size (Scaled) = 2.49 µm X 2.53 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.21 mm, Y: 36.03 mm
Stage Position = X: 61.21 mm, Y: 36.03 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 = 100 ms
 Depth of Focus = 1.13 μm
 Binning Mode = 2,2
 Laser Wavelength = 640 nm @1.00%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 27.5 μm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 100 ms
 Depth of Focus = 0.81 μm
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.00%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 27.5 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 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @2.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM2 Processing

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

2-Dimensional View - Panel A

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L).

Not Shown but Aquired - Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI,

Image was taken from Z-stack Acquisition at Z-plane 32 of 63.

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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 50°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Panel E - STORM

Summary -

Super-resolution lattice structured illumination microscopy squared (SIM²) was used to characterize the intranuclear distribution and organization of RAD50 following etoposide-induced DNA damage within the Identifyn® Standards for Optical Microscopy Methods™ step-down framework. HeLa cells were labeled with a rabbit anti-human RAD50 polyclonal IgG, detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, along with α -tubulin and DAPI (not shown in the data set). Across all SIM² views, the RAD50 signal was strictly confined to the nuclear compartment, consistent with its established role as a core component of the MRE11-RAD50-NBS1 (MRN) complex, which is involved in DNA double-strand break (DSB) detection and signaling.

Two-dimensional SIM² optical sections (Panel A) acquired from a 63-slice Z-stack reveal punctate and aggregated intranuclear RAD50 distributions with markedly increased spatial definition relative to Airyscan and Lattice SIM imaging. Panel B presents a maximum three-dimensional projection, illustrating the full nuclear distribution and continuity of RAD50 signal across the entire Z-volume. Three-dimensional volume reconstructions and higher-magnification region-of-interest analyses (Panel C) further resolve damage-associated RAD50 assemblies throughout the nuclear space. At SIM² lateral resolution (~40-60 nm in the X-Y plane), these signal patterns exhibit reproducible organizational features that closely approximate those observed by single-molecule localization microscopy.

Biologically, RAD50 functions as a structural and mechanochemical component of the MRN complex, mediating DNA end tethering, stabilizing broken DNA ends, and initiating ATM-dependent damage signaling. While SIM² does not resolve all molecular substructures detectable by STORM, the substantially improved resolution reduces signal convolution and enables more precise separation of adjacent RAD50 assemblies within damage-associated foci. Direct comparison with STORM imaging from the same dataset indicates that some SIM²-resolved features remain composites of multiple RAD50 sub-assemblies, entirely separable only at the single-molecule level.

Collectively, these data position SIM² as a critical structural-resolution stage within the step-down workflow, bridging enrichment-level localization (confocal/Airyscan) and definitive molecular resolution (STORM). By resolving RAD50 organization beyond diffraction-limited and conventional SIM modalities, SIM² provides essential structural context for interpreting MRN complex engagement and early DNA damage sensing following genotoxic stress.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and C.

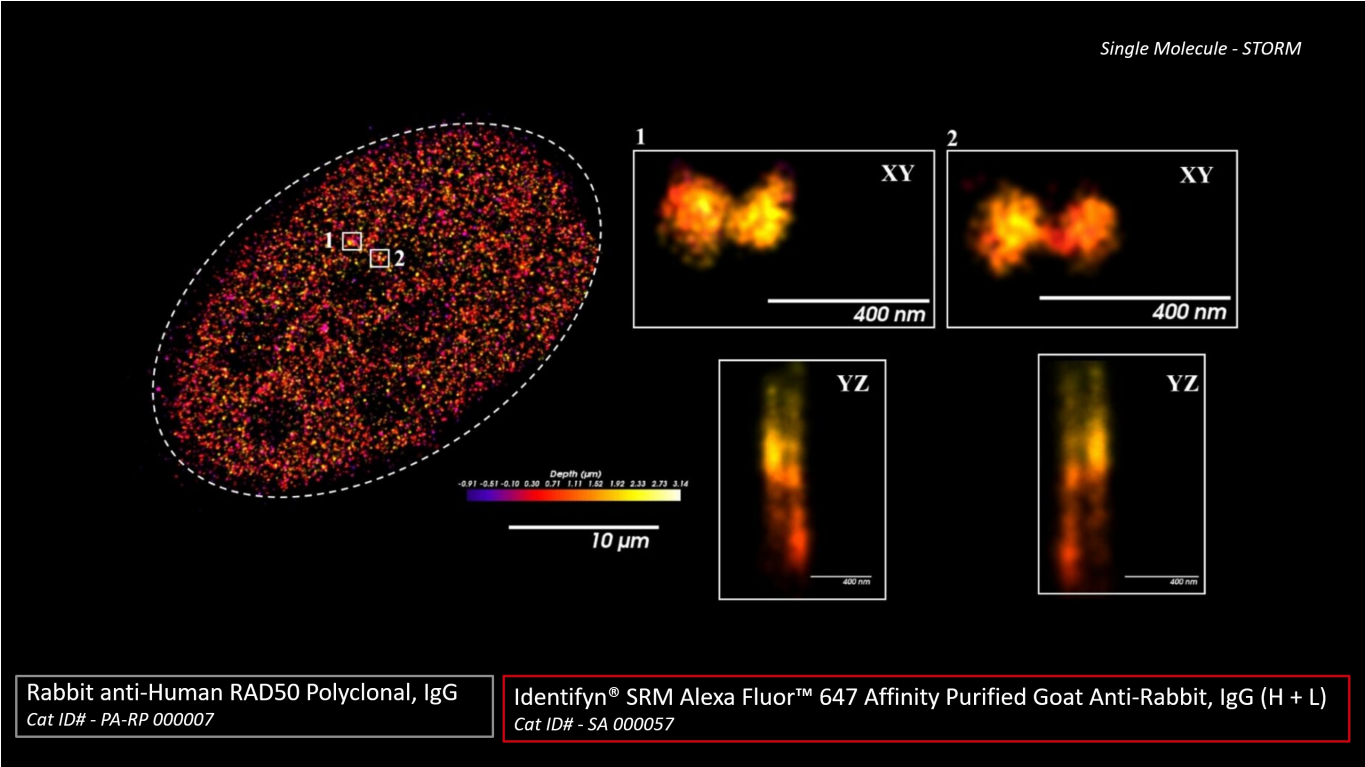
Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	PA-RP-000007
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	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4EE373C597A3D30C6AC0110F0444D47DD899FF9320F40366963BFC0D2C48CCB7
Method PDF SHA-256	A5D7B5FB8EBED8E3A3FAA204FD83905850560D3F35882F271B4F950AA44A2DA0

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	79

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: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	180.5 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	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; End: 181.2
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	200
Z Positions	12
Cycles	12
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	80
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: 10
Sizing Mode	Radial Precision
Particle size	5nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.12
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	10 Intervals
Pixel Size	50nm
Smoothing	8.00
Radial precision	30
Axial precision	65
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1um
Maximum Particle Count to Form Cluster	10
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Polyclonal, IgG

Cat ID# - PA-RP 000007

Identifyn® Secondary Antibody

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

Cat ID# - SA 000057

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

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 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 was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn®'s proprietary Wash Buffer, and Identifyn®'s 647 secondary antibody was incubated at room temperature for 1 hour 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: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm 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: 180.5 μ m

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 640 nm (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: 50 μ m is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 179; End: 181.2

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: 200

Z Positions: 12

Cycles: 12

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635nm Dichroic)

Laser Power: 80

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: 10

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: 0.12

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: 50nm

Smoothing: 8.00

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

Radial precision: 30

Axial precision: 65

The other parameters were not applied.

Statistical analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1um

Maximum Particle Count to Form Cluster: 10

Computer Hulls: Not Selected

Compute Density Isosurfaces: Not Selected

Edit Settings: All Probes

Compute: Selected

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

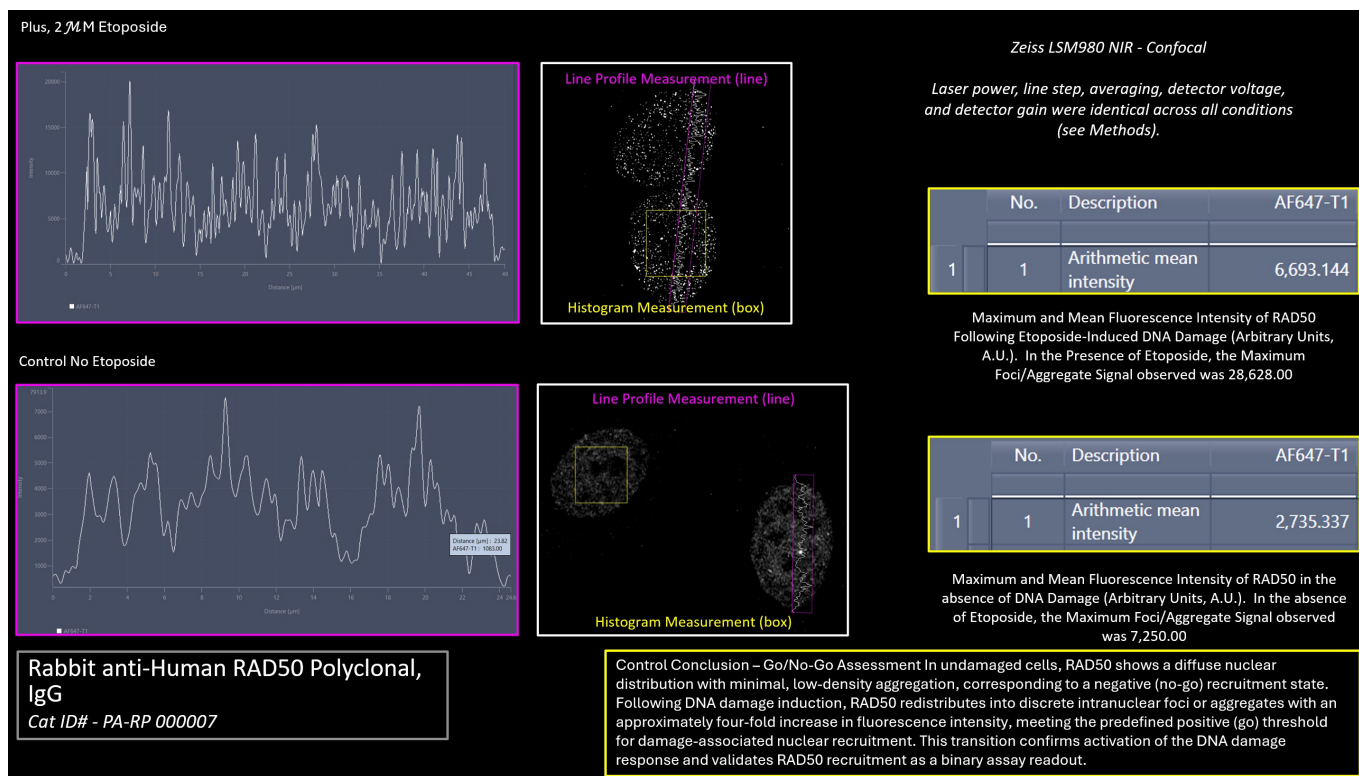
Image by P. Raut

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SOURCE PROVENANCE

Catalog	PA-RP-000007
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	79
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1088A73E67AC04BF7D657DF0F1A2B6058DA8C1C4D14148F704936BB329E27A1E
Method PDF SHA-256	7C3B62F18E21E6AEC7E7E47691E05A2DC1DEA6C09013817CF5B5DFC31A682D38

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	54

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	3 1 (647)
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm 0.059 μm X 0.059 μm
Image Size (Pixels)	1017 X 1017 939 X 758
Image Size (Scaled)	59.81 μm X 59.81 μm 55.23 μm X 44.58 μm
Bit Depth	16 Bit
Image Center Position	X: 92.75 mm, Y: 50.66 mm X: 88.95 mm, Y: 49.33 mm
Stage Position	X: 92.75 mm, Y: 50.66 mm X: 88.95 mm, Y: 49.33 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 65 μm 1.00AU / 66 μm
LMS MBS	MBS 488/639 & 405/730 MBS 488/561 & 405/730
LMS SBS	SBS SP 615 Mirror
Laser Wavelength	639nm @0.10%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2 2.0
Rotation	0°
Sampling	1.20
Pixel Time	0.33 μs 0.30 μs
Frame Time	3.36s 3.69s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	636 - 732 635 - 735
Imaging Device	GaAsP-Pmt3
Detector Type	GaAsP-PMT
Detector Gain	500 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) Filter: 6.6 (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 49.0), Y (Min 0 and Max 21092) X and Y axes were set to Auto - X (Min 0.0 and 24.6 Max), Y (Min 163 and Max 7914)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000) X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000)

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, IgG Cat ID# - PA-RP 000007

Identifyn® Secondary Antibodies

Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

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

Method

HeLa cells were grown on 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, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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. (Not Shown, but in sample)

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:

Single Plane (2D) - Z-Plane 59 of 161 - Plus DNA Damage, Top

Channels = 3

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

Image Size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm

Bit Depth = 16 Bit

Image Center Position = X: 92.75 mm, Y: 50.66 mm

Stage Position = X: 92.75 mm, Y: 50.66 mm

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

Single Plane (2D) - Single Plane- Minus DNA Damage, Bottom

Channels = 1 (647)

Scaling (Per Pixel) = 0.059 μm X 0.059 μm

Image Size (Pixels) = 939 X 758

Image Size (Scaled) = 55.23 μm X 44.58 μm

Bit Depth = 16 Bit

Image Center Position = X: 88.95 mm, Y: 49.33 mm

Stage Position = X: 88.95 mm, Y: 49.33 mm

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

647 - Etoposide-Induced DNA Damage, Top

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 65 μm

LMS MBS = MBS 488/639 & 405/730

LMS SBS = SBS SP 615

Laser Wavelength = 639nm @0.10%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.2

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.33 μs

Frame Time = 3.36s

LSM Scan Speed = 12

Scan Direction = Bidirectional

Line Step = 1

Averaging - 8

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Detection Wavelength = 636 - 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: 7.2 (3D, Auto)

647 - NO Induced DNA Damage, Bottom

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 66µm
 LMS MBS = MBS 488/561 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 639nm @0.10%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.30µs
 Frame Time = 3.69s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 635 - 735
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.6 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 49.0), Y (Min 0 and Max 21092)

This panel measures RAD50 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~28K A.U. at its maximum.

Measured at Z-plane 59 of 161.

Profile View Display - Minus DNA Damage, Bottom

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and 24.6 Max), Y (Min 163 and Max 7914)

This panel measures RAD50 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~7K A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 2000)

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

Histogram Display - Minus DNA Damage, Bottom

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

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000)

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

Control Conclusion -

In undamaged cells, RAD50 shows a diffuse nuclear distribution with minimal, low-density aggregation, corresponding to a negative (no-go) recruitment state. Following DNA damage induction, RAD50 redistributes into discrete intranuclear foci or aggregates with an approximately fourfold increase in fluorescence intensity, meeting the predefined positive (go) threshold for damage-associated nuclear recruitment. This transition confirms activation of the DNA damage response and validates RAD50 recruitment as a binary assay readout.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	PA-RP-000007
Stage	Control
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8F2C7C8295DAAB527D5D33C2D08B8892F0A0728A2458B8F468C6D96050E34AFD
Method PDF SHA-256	48E13160FC4D7CC71FC8E26B38C572E341D21B8B3EE8E62B3D506E20A3D2D3BD