BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

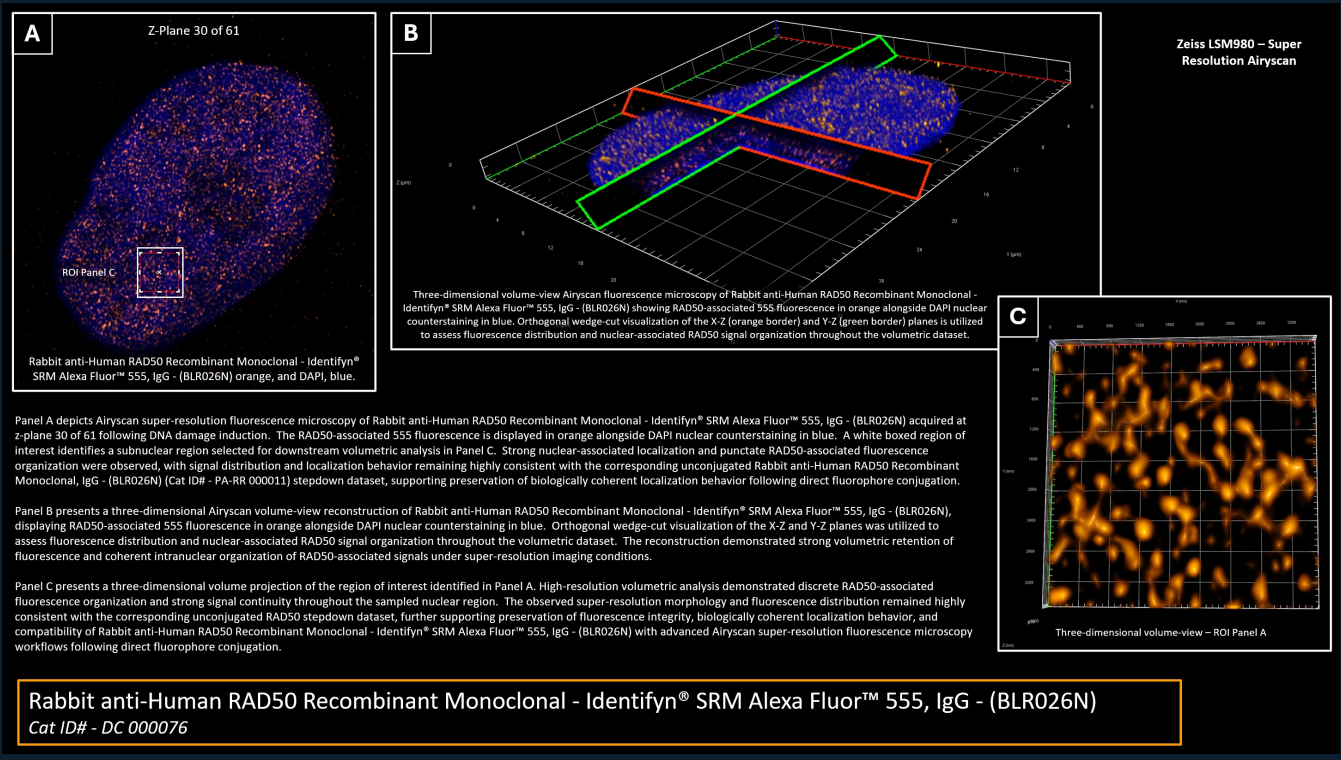
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / AIRYSCAN

DC-000076 | 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
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

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

EVIDENCE AVAILABLE

Western blot -> Widefield -> Confocal -> Airyscan

BENNETT ASSESSMENT

The preserved DC-000076 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000076 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 4 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 553
Confocal	405/DAPI; 488; 555	2; None; 561 nm @ 2.60%; 405 nm @ 0.3%; 553; 353; 568; 465
Airyscan	405/DAPI; 488; 555	2; None; 561 nm @ 2.00%; 405 nm @ 0.3%; 553; 353; 568; 465

MODALITY ASSESSMENT / PART 1 OF 4

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 555.

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 4

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: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 598 X 540; Image Size (Pixels): 598 X 540; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 30 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 30.00%; X-Cite Xylis Lamp Intensity @ 15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

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; 555.

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 4

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: 61 Slices (1.80 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1121 X 1121; Image Size (Pixels): 1121 X 1121; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.30 µs; Frame Time: 3.69 s. Illumination: Laser Wavelength: 561 nm @ 2.60%; 405 nm @ 0.3%. Optical/scan: Pinhole: 1.00 AU / 55 µm; 1.00 AU / 44µm; Scan Zoom: 2.0; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.0 (3D, Auto); Filter: 6.0 (3D, Auto). Structured source metadata values: 47.

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; 555.

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 4 OF 4

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: 61 Slices (1.80 µm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 965 X 1004; 100 X 100; Image Size (Pixels): 965 X 1004; 100 X 100; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.10 µs; Frame Time: 18.77 s. Illumination: Laser Wavelength: 561 nm @ 2.00%; 405 nm @ 0.3%. Optical/scan: Pinhole: 5.00 AU / 309 µm; 5.00 AU / 215 µm; Scan Zoom: 2.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; 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); View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution: 9.4 (3D, Auto); SuperResolution: 9.8 (3D, Auto). Structured source metadata values: 61.

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; 555.

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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000076 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=965 X 1004; Image Size (Scaled)=34.06 µm X 35.44 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

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

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

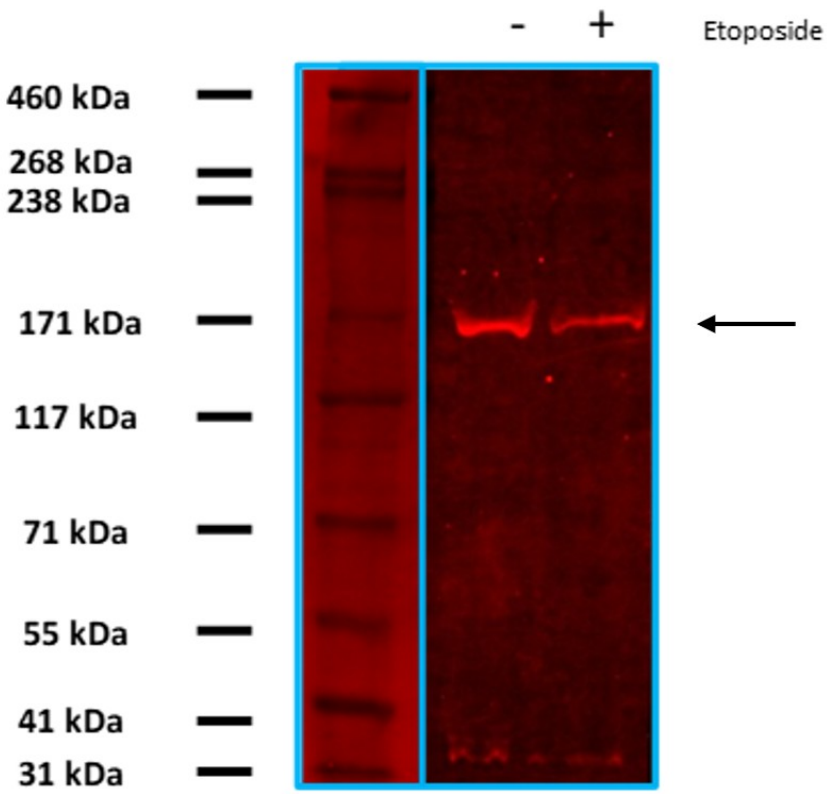
This dossier preserves the complete available evidence progression for DC-000076. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	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	555
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	532nm
Emission Filter	572±28nm
Detector	PMT
Intensity	2
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 Recombinant Monoclonal- Identifyn® SRM Alexa Fluor™ 555, IgG -(BLR026N)
Cat ID# - DC 000076

Expected MW: 154 kDa

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. 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 Recombinant Monoclonal- Identifyn™ SRM Alexa Fluor™ 555, IgG - (BLR026N) for 20 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence
Laser = 532nm
Emission Filter = 572±28nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

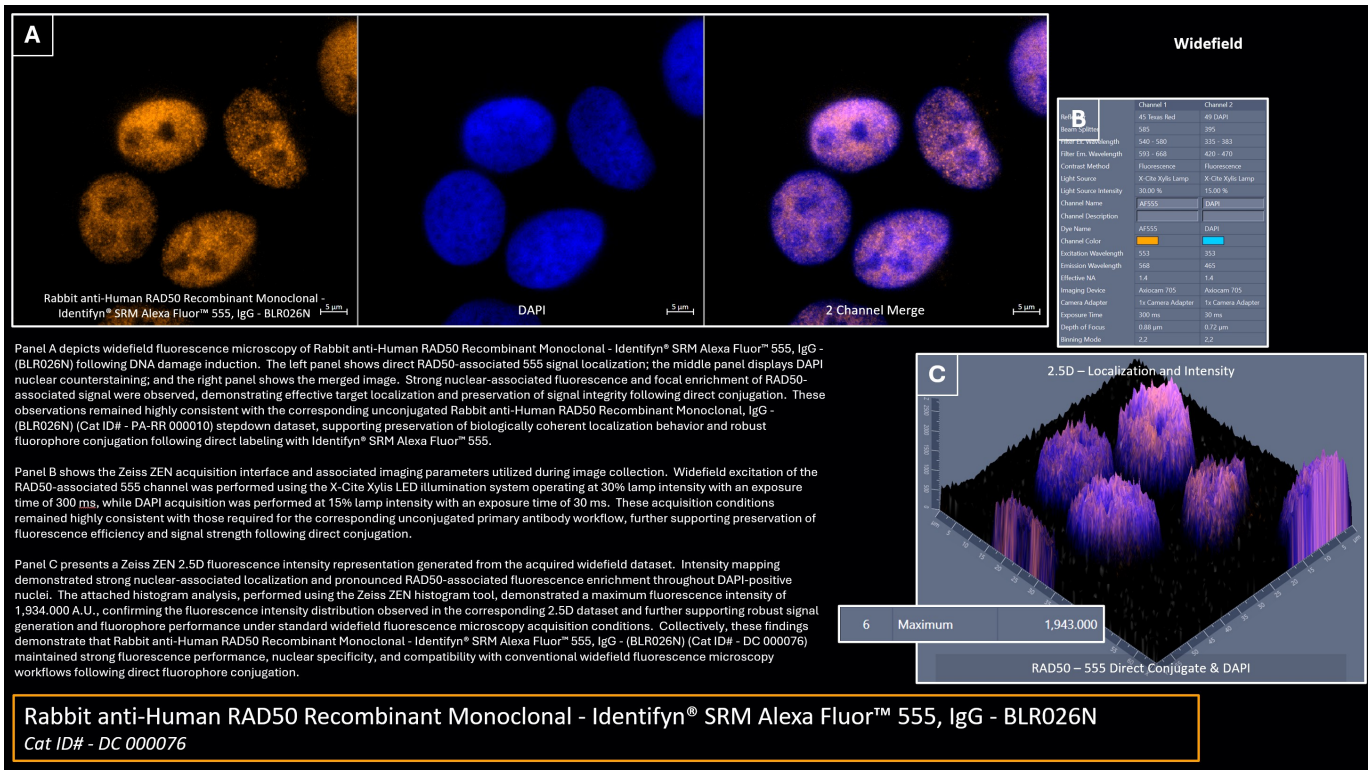
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000076
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	B2E485B18433F03689D4860348B240ED172F071E872D2C054055A2BC3B820A13
Method PDF SHA-256	F7C7E22DFE57013ACF22E477D11EC69545483720008ED5899CAC2B3D31223925

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) following DNA damage induction. The left panel shows direct RAD50-associated 555 signal localization; the middle panel displays DAPI nuclear counterstaining; and the right panel shows the merged image. Strong nuclear-associated fluorescence and focal enrichment of RAD50-associated signal were observed, demonstrating effective target localization and preservation of signal integrity following direct conjugation. These observations remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000010) stepdown dataset, supporting preservation of biologically coherent localization behavior and robust fluorophore conjugation following direct labeling with Identifyn® SRM Alexa Fluor™ 555.

Panel B shows the Zeiss ZEN acquisition interface and associated imaging parameters utilized during image collection. Widefield excitation of the RAD50-associated 555 channel was performed using the X-Cite Xylis LED illumination system operating at 30% lamp intensity with an exposure time of 300 ms, while DAPI acquisition was performed at 15% lamp intensity with an exposure time of 30 ms. These acquisition conditions remained highly consistent with those required for the corresponding unconjugated primary antibody workflow, further supporting preservation of fluorescence efficiency and signal strength following direct conjugation.

Panel C presents a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and pronounced RAD50-associated fluorescence enrichment throughout DAPI-positive nuclei. The attached histogram analysis, performed using the Zeiss ZEN histogram tool, demonstrated a maximum fluorescence intensity of 1,934.000 A.U., confirming the fluorescence intensity distribution observed in the corresponding 2.5D dataset and further supporting robust signal generation and fluorophore performance under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) (Cat ID# - DC 000076) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - BLR026N
Cat ID# - DC 000076

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image Size (Pixels)	598 X 540
Image Size (Scaled)	65.50 µm X 59.14 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	45 Texas Red | 49 DAPI
Beam Splitter	585 | 395
Filter Excitation Wavelength	540 - 580 | 335 - 383
Filter Emission Wavelength	593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 30.00% | X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time	300 ms | 30 ms
Excitation Wavelength	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X | 1x Camera Adapter
Depth of Focus	0.88 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	45
Angle Y	-45
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG -(BLR026N)
Cat ID# - DC 000076

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

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 598 X 540
Image Size (Scaled) = 65.50 µm X 59.14 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

555 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 30.00%
Exposure Time = 300 ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.88 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 @ 15.00%
Exposure Time = 30 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x Camera Adapter
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The left panel shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear localization and focal RAD50-associated fluorescence enrichment following DNA damage induction, supporting robust conjugation performance and preservation of target-specific fluorescence signal under widefield fluorescence microscopy conditions.

ZEN Acquisition Info Tab - Panel B

Shown is the acquisition interface for the Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) widefield dataset collected using the X-Cite Xylis Lamp and Zeiss Axiocam 705 camera. Imaging of the RAD50-associated 555 channel was performed using 30% X-Cite Xylis Lamp intensity with a 300 ms exposure time on the Axiocam 705, while DAPI acquisition was performed using 15% lamp intensity with a 30 ms exposure time. These acquisition conditions demonstrated robust fluorescence signal generation and strong nuclear-associated RAD50 localization under standard widefield excitation and collection settings. The ability to generate strong focal RAD50-associated fluorescence enrichment and target-specific signal under these moderate acquisition parameters further supports the robustness of direct conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 555.

2.5D Image Processing - Panel C

2.5D Display

Render Mode = Surface
Grid distance = 5
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 45
Angle Y = -45
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

ZEN Acquisition Histogram Data - Panel C

Shown is the maximum fluorescence intensity measurement obtained from the Zeiss ZEN Histogram tool for the Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) direct conjugate, demonstrating a peak fluorescence intensity of 1,934.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield fluorescence microscopy acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) following DNA damage induction. The left panel shows direct RAD50-associated 555 signal localization; the middle panel displays DAPI nuclear counterstaining; and the right panel shows the merged image. Strong nuclear-associated fluorescence and focal enrichment of RAD50-associated signal were observed, demonstrating effective target localization and preservation of signal integrity following direct conjugation. These observations remained highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of biologically coherent localization behavior and robust fluorophore conjugation following direct labeling with Identifyn® SRM Alexa Fluor™ 555.

Panel B shows the Zeiss ZEN acquisition interface and associated imaging parameters utilized during image collection. Widefield excitation of the RAD50-associated 555 channel was performed using the X-Cite Xylis LED illumination system operating at 30% lamp intensity with an exposure time of 300 ms, while DAPI acquisition was performed at 15% lamp intensity with an exposure time of 30 ms. These acquisition conditions remained highly

consistent with those required for the corresponding unconjugated primary antibody workflow, further supporting preservation of fluorescence efficiency and signal strength following direct conjugation.

Panel C presents a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and pronounced RAD50-associated fluorescence enrichment throughout DAPI-positive nuclei. The attached histogram analysis, performed using the Zeiss ZEN histogram tool, demonstrated a maximum fluorescence intensity of 1,934.000 A.U., confirming the fluorescence intensity distribution observed in the corresponding 2.5D dataset and further supporting robust signal generation and fluorophore performance under standard widefield fluorescence microscopy acquisition conditions. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) (Cat ID# - DC 000076) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

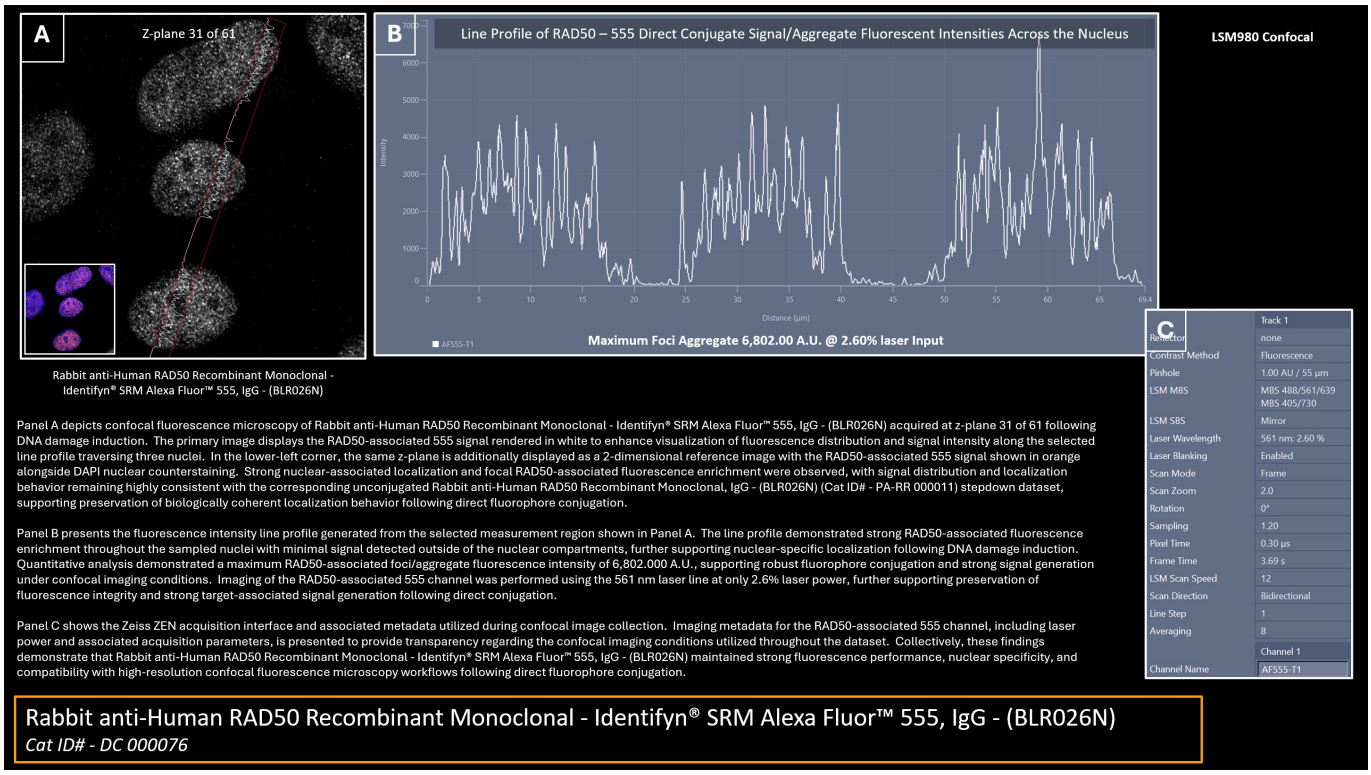
NONE

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

SOURCE PROVENANCE

Catalog	DC-000076
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B2AD776518A71C916055568F854099A0030816A57D225DE227DB1DEC8CDCE159
Method PDF SHA-256	24C51687AB17576A11A46B678107D185A5B3F063E22FB7828B67F0BDE861A9FC

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N)

Panel A depicts confocal fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) acquired at z-plane 31 of 61 following DNA damage induction. The primary image displays the RAD50-associated 555 signal rendered in white to enhance visualization of fluorescence distribution and signal intensity along the selected line profile traversing three nuclei. In the lower-left corner, the same z-plane is additionally displayed as a 2-dimensional reference image with the RAD50-associated 555 signal shown in orange alongside DAPI nuclear counterstaining. Strong nuclear-associated localization and focal RAD50-associated fluorescence enrichment were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents the fluorescence intensity line profile generated from the selected measurement region shown in Panel A. The line profile demonstrated strong RAD50-associated fluorescence enrichment throughout the sampled nuclei with minimal signal detected outside of the nuclear compartments, further supporting nuclear-specific localization following DNA damage induction. Quantitative analysis demonstrated a maximum RAD50-associated foci/aggregate fluorescence intensity of 6,802.000 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal imaging conditions. Imaging of the RAD50-associated 555 channel was performed using the 561 nm laser line at only 2.6% laser power, further supporting preservation of fluorescence integrity and strong target-associated signal generation following direct conjugation.

Panel C shows the Zeiss ZEN acquisition interface and associated metadata utilized during confocal image collection. Imaging metadata for the RAD50-associated 555 channel, including laser power and associated acquisition parameters, is presented to provide transparency regarding the confocal imaging conditions utilized throughout the dataset. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) maintained strong fluorescence performance, nuclear specificity, and compatibility with high-resolution confocal fluorescence microscopy workflows following direct fluorophore conjugation.

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N)
Cat ID# - DC 000076

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	47

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	61 Slices (1.80 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1121 X 1121
Image Size (Scaled)	65.93 µm X 65.93 µm
Bit Depth	16 Bit
Image Center Position	X: 91.25 mm, Y: 51.03 mm
Stage Position	X: 91.25 mm, Y: 51.03 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 55 µm | 1.00 AU / 44µm
LSM MBS	MBS 488/561/639 & 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @ 2.60% | 405 nm @ 0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	1.20
Pixel Time	0.30 µs
Frame Time	3.69 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Detection Wavelength	550 - 600 | 430 - 490
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.0 (3D, Auto) | Filter: 6.0 (3D, Auto)
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	Mirror
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 68.9), Y (Min 0.0 and Max 11036)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG -(BLR026N)
Cat ID# - DC 000076

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

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 31 of 61, Line Profile Panel B

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1121 X 1121
Image Size (Scaled) = 65.93 µm X 65.93 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.25 mm, Y: 51.03 mm
Stage Position = X: 91.25 mm, Y: 51.03 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 55 µm
LSM MBS = MBS 488/561/639 & 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @ 2.60%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30 µs
Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 550 - 600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.30 µs
Frame Time = 3.69 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.0 (3D, Auto)

2-Dimensional View - Panel A

Panel A, 2-Dimensional view at Z-plane 31 of 61, shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N). A line profile traverses three nuclei to define the region selected for quantitative fluorescence analysis, with the corresponding intensity profile further examined in Panels B and C.

Profile View Display - Panels B & C

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

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 68.9), Y (Min 0.0 and Max 11036)

Measured at Z-plane 31 of 61

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 555 channel (RAD50 555 direct conjugate) using the 561 nm laser at 2.60%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A depicts confocal fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) acquired at z-plane 31 of 61 following DNA damage induction. The primary image displays the RAD50-associated 555 signal, rendered in white to enhance visualization of fluorescence distribution and signal intensity along the selected line profile that traverses three nuclei. In the lower-left corner, the same z-plane is additionally displayed as a 2-dimensional reference image with the RAD50-associated 555 signal shown in orange alongside DAPI nuclear counterstaining. Strong nuclear-associated localization and focal RAD50-associated fluorescence enrichment were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents the fluorescence intensity line profile generated from the selected measurement region shown in Panel A. The line profile demonstrated strong RAD50-associated fluorescence enrichment throughout the sampled nuclei with minimal signal detected outside of the nuclear compartments, further supporting nuclear-specific localization following DNA damage induction. Quantitative analysis demonstrated a maximum RAD50-associated foci/aggregate fluorescence intensity of 6,802.000 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal imaging conditions. Imaging of the RAD50-associated 555 channel was performed using the 561 nm laser line at only 2.6% laser power, further supporting the preservation of fluorescence integrity and the generation of a strong target-associated signal following direct conjugation.

Panel C shows the Zeiss ZEN acquisition interface and the associated metadata used during confocal image acquisition. Imaging metadata for the RAD50-associated 555 channel, including laser power and acquisition parameters, is provided to ensure transparency regarding the confocal imaging conditions used throughout the dataset. Collectively, these findings demonstrate that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) maintained strong fluorescence performance, nuclear specificity, and compatibility with high-resolution confocal fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

Identifyn® Rabbit anti-Human RAD50 Recombinant Monoclonal- Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) was pseudocolored in Zen Software from orange, the default color, to white, to provide visual contrast in Panel A.

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

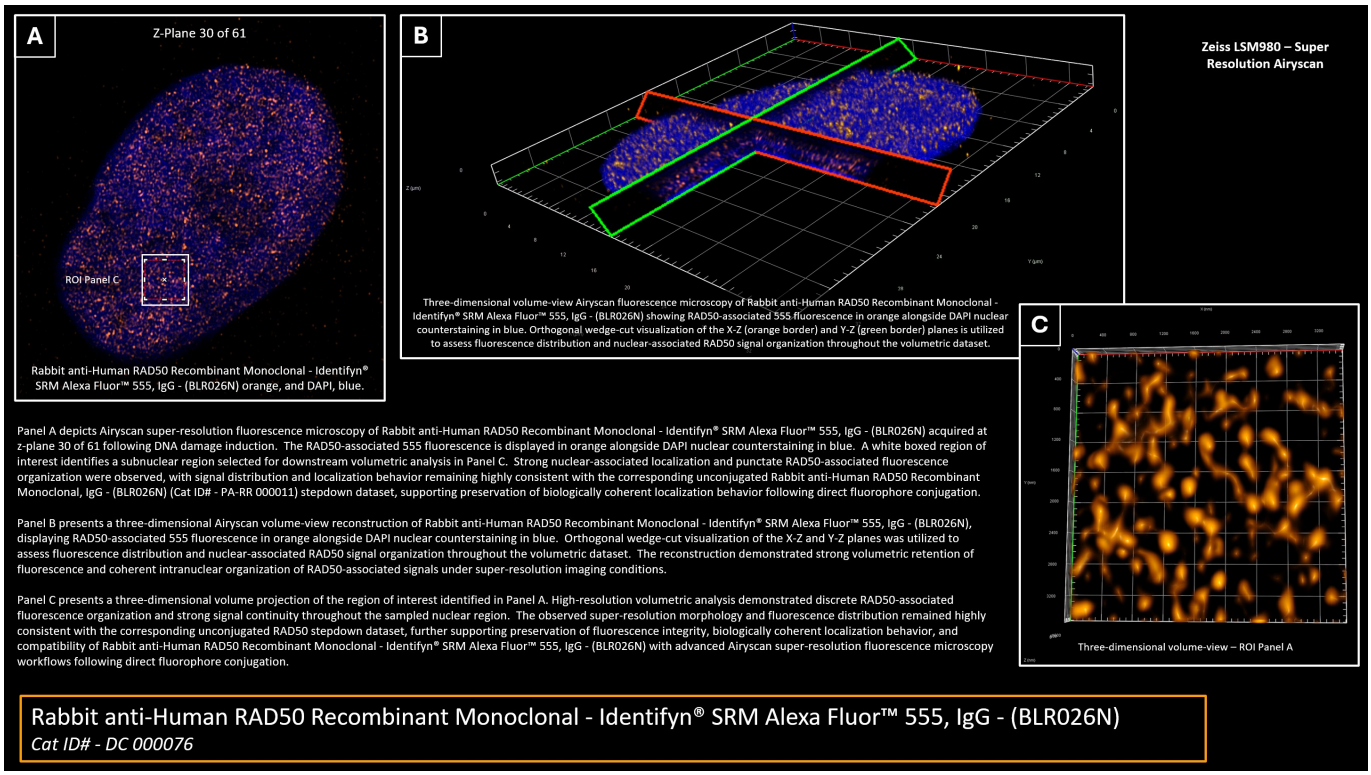
SOURCE PROVENANCE

Catalog	DC-000076
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	47

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F79CF74870703EA0987FE2CDBA202297B6DD3E820B52F5C743D39AAED37A84A8
Method PDF SHA-256	1DCC9D44168E2C43783D684BFCAC13327E57E74582302A225B993A61B343D110

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



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

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	61 Slices (1.80 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	965 X 1004 | 100 X 100
Image Size (Scaled)	34.06 µm X 35.44 µm | 3.53 µm X 3.53 µm
Bit Depth	16 Bit
Image Center Position	X: 91.66 mm, Y: 50.97 mm
Stage Position	X: 91.66 mm, Y: 50.97 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 309 µm | 5.00 AU / 215 µm
LSM MBS	MBS 488/561 & MBS 405/730
LSM SBS	SBS LP 525 | SBS SP 505
Laser Wavelength	561 nm @ 2.00% | 405 nm @ 0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	2.00
Pixel Time	1.10 µs
Frame Time	18.77 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	553 | 353
Emission Wavelength	568 | 465
Effective NA	1.4
Detection Wavelength	527-735 | 350-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution: 9.4 (3D, Auto) | SuperResolution: 9.8 (3D, Auto)
LMS MBS	MBS 488/561 & MBS 405/730
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels | Threshold 2%, Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Z	Activate was selected, textured opaque was chosen, and an orange outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	14% | -9%
Clip Y	-45° | 45°
Clip Z	0°
Y-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.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N)
Cat ID# - DC 000076

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

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A, 2-Dimensional view at Z-plane 30 of 61, and Panel B, 3-Dimensional View

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 965 X 1004
Image Size (Scaled) = 34.06 µm X 35.44 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.66 mm, Y: 50.97 mm
Stage Position = X: 91.66 mm, Y: 50.97 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C 3-Dimensional Volume View

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 100 X 100
Image Size (Scaled) = 3.53 µm X 3.53 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.66 mm, Y: 50.97 mm
Stage Position = X: 91.66 mm, Y: 50.97 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 309 µm
LSM MBS = MBS 488/561 & MBS 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @ 2.00%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 527-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution: 9.4 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 215 µm
LMS MBS = MBS 488/561 & MBS 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution: 9.8 (3D, Auto)

2-Dimensional View - Panel A

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) acquired at z-plane 30 of 61 following DNA damage induction. The RAD50-associated 555 fluorescence is displayed in orange alongside DAPI nuclear counterstaining displayed in blue, demonstrating strong nuclear localization, punctate RAD50-associated fluorescence organization, and robust signal integrity, consistent with the corresponding unconjugated, widefield, and confocal datasets within this direct conjugate workflow. A white boxed region of interest identifies a subnuclear region selected for downstream volumetric analysis in Panel C.

3D Image Processing - Panel B

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

Clipping Image Processing - Panel B

Clipping planes are introduced in Panel B to highlight Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) within the nuclear compartment through orthogonal wedge-cut visualization. Portions of the volumetric dataset were selectively removed across the X-Z and Y-Z planes to facilitate assessment of intranuclear RAD50-associated fluorescence distribution and volumetric signal organization.

X-Z = Activate was selected, textured opaque was chosen, and an orange 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°

Y-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 = -9%
Clip Y = 45°
Clip Z = 0°

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 55%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR026N) acquired at z-plane 30 of 61 following DNA damage induction. The RAD50-associated 555 fluorescence is displayed in orange, while DAPI nuclear counterstaining is displayed in blue. A white boxed region of interest identifies a subnuclear area selected for downstream volumetric analysis in Panel C. Strong nuclear-associated localization and punctate RAD50-associated fluorescence organization were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (BLR026N) (Cat ID# - PA-RR 000011) stepdown dataset, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents a three-dimensional wedge-cut Airyscan volume reconstruction demonstrating the organization of intranuclear RAD50-associated fluorescence following DNA damage induction. Orthogonal wedge-cut visualization is displayed with the X-Z plane shown in orange and the Y-Z plane shown in green to facilitate assessment of volumetric fluorescence distribution and intranuclear signal intensity throughout the sampled nuclear volume. The reconstruction further demonstrates preservation of volumetric RAD50-associated fluorescence organization under super-resolution imaging conditions following direct conjugation.

Panel C presents a three-dimensional Airyscan volume projection of the region of interest identified in Panel A, displaying both the RAD50-associated 555 fluorescence and DAPI nuclear counterstaining throughout the sampled volumetric dataset. The super-resolution morphology, fluorescence intensity, and intranuclear RAD50-associated signal organization remained highly consistent with the corresponding unconjugated RAD50 dataset, further

supporting preservation of fluorescence integrity, biologically coherent localization behavior, and robust super-resolution imaging performance following direct labeling with Identifyn® SRM Alexa Fluor™ 555.

Image Correction

NONE

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

SOURCE PROVENANCE

Catalog	DC-000076
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	61
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BC54E9E2FAF6718429211DC92EF3F35CF906F099004CAAB3FEC1AD7EE8B47846
Method PDF SHA-256	2AD2BF567C5D5E3A4D8E235C863F7E0A3E8206DF4B32FD004B42744EBEB8FB10