BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

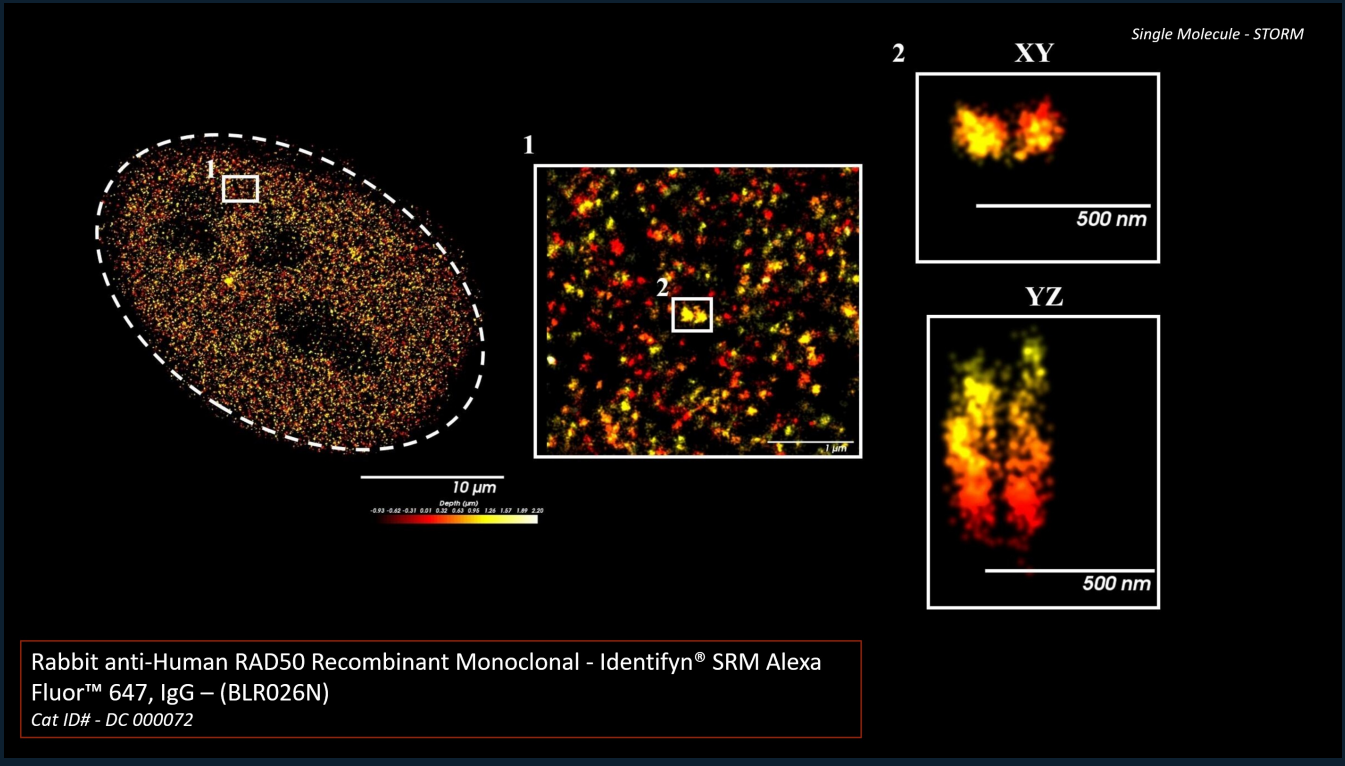
RAD50

MICROSCOPY EVIDENCE ASSESSMENT

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

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN -> SINGLE-MOLECULE STORM

5	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG – (BLR026N)
Cat ID# - DC 000072

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000072 | 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
- Single-molecule STORM presentation workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

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

EVIDENCE AVAILABLE

Western blot -> Widefield -> Confocal -> Airyscan -> Single-molecule STORM

BENNETT ASSESSMENT

The preserved DC-000072 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. 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-000072 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 5 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 647	50 Cy 5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653; 353
Confocal	405/DAPI; 488; 647	2; None; 639 nm @ 1.80%; 405 nm @ 0.3%; 653; 353; 668; 465
Airyscan	405/DAPI; 488; 647	2; None; 639 nm @ 1.80%; 405 nm @ 0.2%; 653; 353; 668; 465
Single-molecule STORM	647	Both Channels

MODALITY ASSESSMENT / PART 1 OF 5

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 Imaging.. 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 5

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:. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 25.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 24.

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; 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 5

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: 131 Slices (3.90 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 543 X 699; Image Size (Pixels): 543 X 699; 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: 639 nm @ 1.80%; 405 nm @ 0.3%. Optical/scan: Pinhole: 1.00 AU / 66 µm; 1.00 AU / 43µm; Scan Zoom: 2.0; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 6.5 (3D, Auto). Structured source metadata values: 44.

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 4 OF 5

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: 131 Slices (3.90 µm); Channels: 2; Scaling (Per Pixel): 0.035 nm X 0.035 nm X 0.030 nm; Image size (Pixels): 845 X 1016; Image Size (Pixels): 845 X 1016; 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: 639 nm @ 1.80%; 405 nm @ 0.2%. Optical/scan: Pinhole: 5.00 AU / 328 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 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution: 10.0 (3D, Auto); Super Resolution: 8.3 (3D, Auto). Structured source metadata values: 49.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 5

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: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 81.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000072 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. 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): 0.035 nm X 0.035 nm X 0.030 nm -> 0.03529 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)=845 X 1016; Image Size (Scaled)=29.82 µm X 35.86 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

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

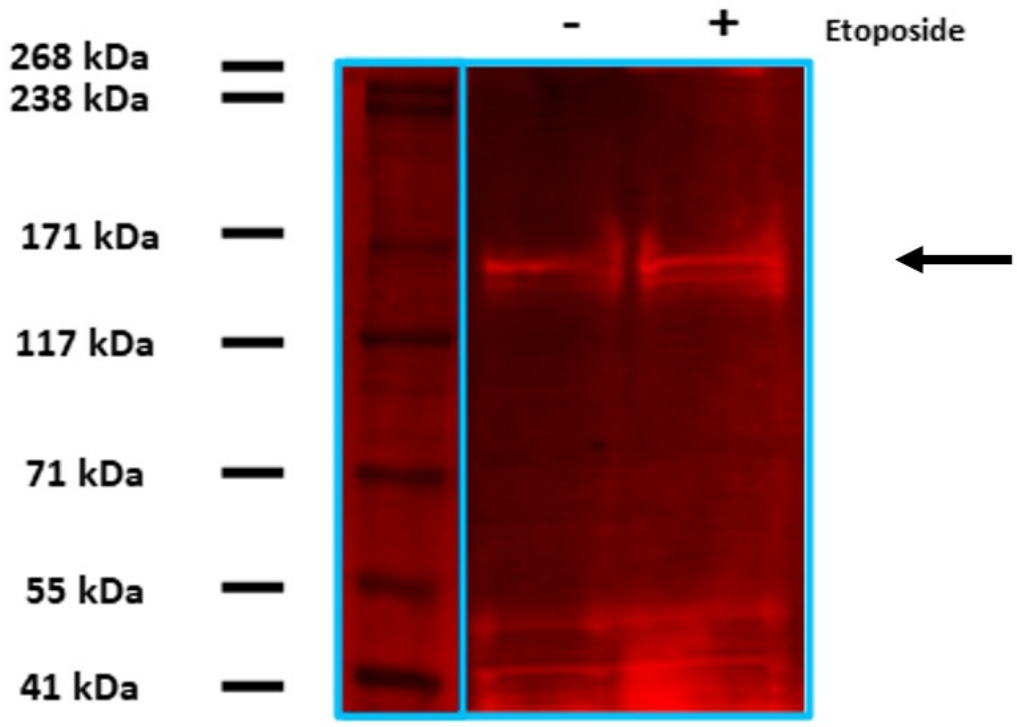
This dossier preserves the complete available evidence progression for DC-000072. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



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

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L2
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™ 647, IgG - (BLR026N)
Cat ID# - DC 000072

Expected MW: 154 kDa

HeLa cells were treated with 2 µM etoposide for 18 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a lysis buffer, then centrifuged to pellet cellular 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 on NuPAGE™ Tris-Acetate Mini Protein Gels (3-8%, 1.0 mm) in a Mini Gel Tank. Thermo Fisher™ HiMark™ Pre-stained Protein Standard was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed five times with 1X PBS. The blocked membrane was incubated with Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, 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 Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

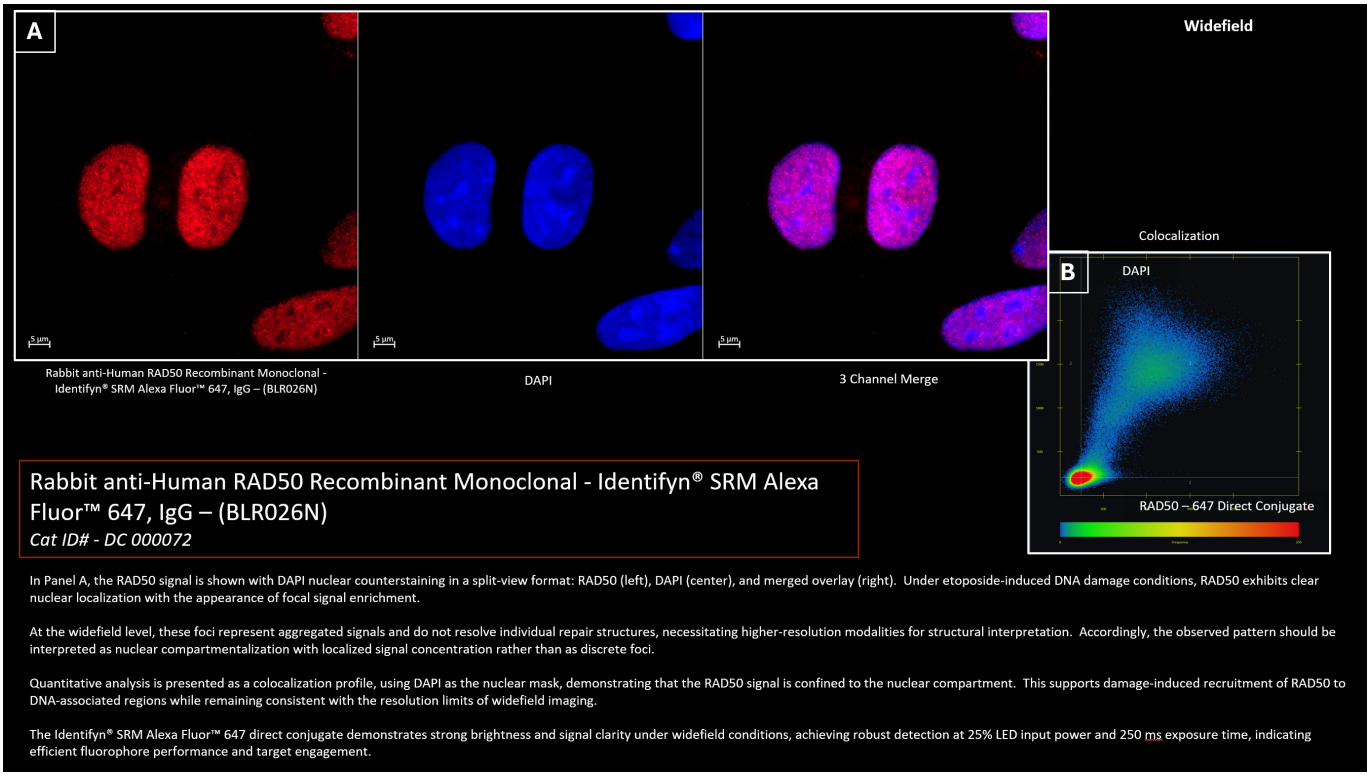
NONE

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000072
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1F9244947112CC1DFC9A326190491510CD8347060E4FDBEDB8E32FAFEB1473BD
Method PDF SHA-256	B6EF93EE995464A029451512005F8A2232F977AB5885B7E8C193D485EC0D198E

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	24

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
Reflector	50 Cy 5 | 49 DAPI
Beam Splitter	660 | 395
Filter Excitation Wavelength	625 - 655 | 335 - 383
Filter Emission Wavelength	665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 25.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	250 ms | 20 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.72 µm
Binning Mode	2,2
Image Measurement	Entire Field

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™ 647, IgG - (BLR026N)
Cat ID# - DC 000072

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. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth medium for 18 hours, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. 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:

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

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 = 20 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 left panel shows Rabbit anti-Human RAD50 Recombinant Monoclonal antibody (IgG), directly conjugated to Identifyn® SRM Alexa Fluor™ 647. The center panel shows DAPI nuclear staining. The right panel shows the merged image of RAD50 and DAPI, demonstrating nuclear localization of the RAD50 signal in response to etoposide-induced DNA damage, with focal signal enrichment within the nucleus.

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 146, Range Auto
Vertical Axis - DAPI, Threshold 206, Range Auto
Image Measurement = Entire Field

Colocalization analysis of the Identifyn® SRM Alexa Fluor™ 647-conjugated RAD50 signal (X-axis) and DAPI (Y-axis) was performed across the full field of view shown in Panel A. RAD50 localization remained confined to DAPI-positive nuclear regions with DAPI, confirming confinement of signal to the nuclear compartment and supporting damage-associated nuclear localization.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) following etoposide-induced DNA damage. The left panel shows direct visualization of the RAD50-associated 647 signal, the middle panel shows DAPI nuclear counterstaining, and the right panel shows the merged image. Consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (PA-RR 000026) stepdown dataset, the direct conjugate demonstrated strong nuclear-associated localization with discrete RAD50-associated focal signal enrichment and minimal nonspecific background fluorescence, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents colocalization analysis of the RAD50-associated 647 signal relative to DAPI nuclear staining across the full imaging field. RAD50 fluorescence remained strongly confined to DAPI-positive nuclear regions, supporting preservation of DNA damage-associated nuclear localization behavior and target-specific fluorescence enrichment following direct conjugation. The observed signal distribution and nuclear-associated localization patterns remained highly consistent with the corresponding unconjugated RAD50 workflow, further supporting preservation of antibody performance characteristics and fluorescence integrity under standard widefield fluorescence microscopy conditions.

Collectively, the dataset demonstrates that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) maintained strong fluorescence signal generation, low background-associated interference, and biologically coherent nuclear localization behavior following direct fluorophore conjugation. These findings further support the compatibility of the Identifyn® direct conjugation platform with conventional widefield fluorescence microscopy workflows while preserving localization fidelity and target-associated fluorescence organization consistent with the unconjugated antibody workflow.

Image Correction

NONE

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

SOURCE PROVENANCE

Catalog	DC-000072
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	24
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7E7D809B01504B3FE3E1B63A3F21241E16377989B2A774F5D373587D377982CC
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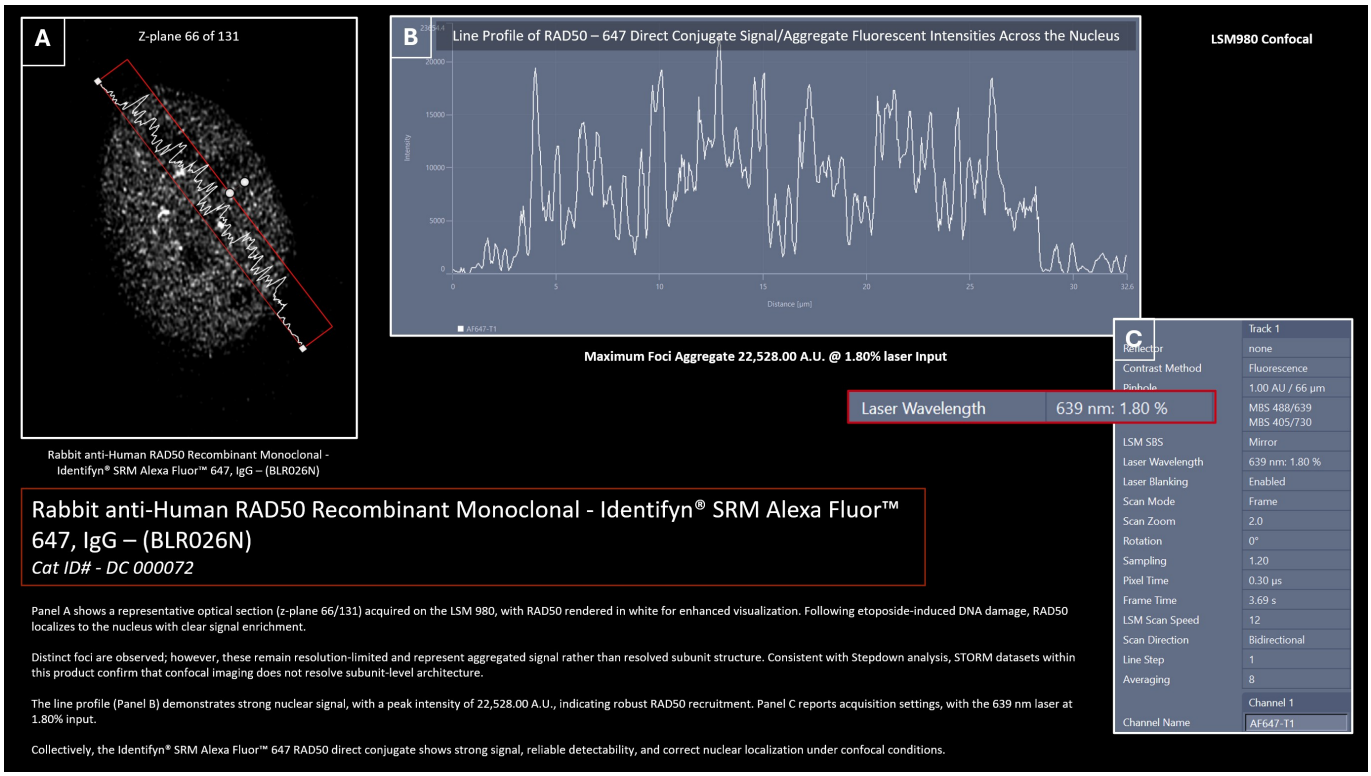
ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	44

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	131 Slices (3.90 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	543 X 699
Image Size (Scaled)	31.94 µm X 41.11 µm
Bit Depth	16 Bit
Image Center Position	X: 89.18 mm, Y: 52.00 mm
Stage Position	X: 89.18 mm, Y: 52.00 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 66 µm | 1.00 AU / 43µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	Mirror
Laser Wavelength	639 nm @ 1.80% | 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	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Detection Wavelength	635 - 668 | 430 - 480
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.5 (3D, Auto) | Filter: 6.5 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 32.6), Y (Min 0 and Max 23654)

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™ 647, IgG - (BLR026N)
Cat ID# - DC 000072

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. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth medium for 18 hours, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal- Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. 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 66 of 131, Line Profile Panel B

Z-Stack = 131 Slices (3.90 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 543 X 699
Image Size (Scaled) = 31.94 µm X 41.11 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.18 mm, Y: 52.00 mm
Stage Position = X: 89.18 mm, Y: 52.00 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 66 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 639 nm @ 1.80%
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 = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 635 - 668
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.5 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43µm
LMS MBS = MBS 488/639 & 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 - 480
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.5 (3D, Auto)

2-Dimensional View - Panel A

Panel A, 2-Dimensional view at Z-plane 66 of 131, shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N). A line profile is drawn across the image 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 32.6), Y (Min 0 and Max 23654)

Measured at Z-plane 66 of 131

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel (RAD50 647 direct conjugate) using the 639 nm laser at 1.80%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A shows a representative optical section (z-plane 66 of 131) acquired on the LSM 980 confocal system. The RAD50 signal has been rendered in white to enhance structural visualization. Under etoposide-induced DNA damage conditions, RAD50 exhibits strong nuclear localization with pronounced signal enrichment consistent with recruitment to DNA-associated regions.

While distinct foci are observed at the confocal level, these features remain resolution-limited and represent an aggregated structural signal rather than a resolved subunit architecture. This is consistent with the Stepdown framework, in which higher-resolution modalities are required to define the underlying structural organization. Corresponding STORM datasets within this product further demonstrate that confocal imaging does not resolve subunit-level structures.

A line profile drawn across the image (Panel B) defines the region used for quantitative assessment. The profile demonstrates robust RAD50 signal intensity across the nuclear compartment, with a peak fluorescence of 22,528.00 A.U., indicating substantial protein recruitment and concentration following DNA damage.

Panel C provides acquisition parameters for the 647 channel, with the 639 nm laser operated at 1.80% input power, supporting transparent reporting of imaging conditions.

Collectively, these data demonstrate that the Identifyn® SRM Alexa Fluor™ 647 RAD50 direct conjugate delivers strong signal intensity, high detectability under controlled confocal conditions, and appropriate nuclear localization consistent with DNA damage response biology.

Image Correction

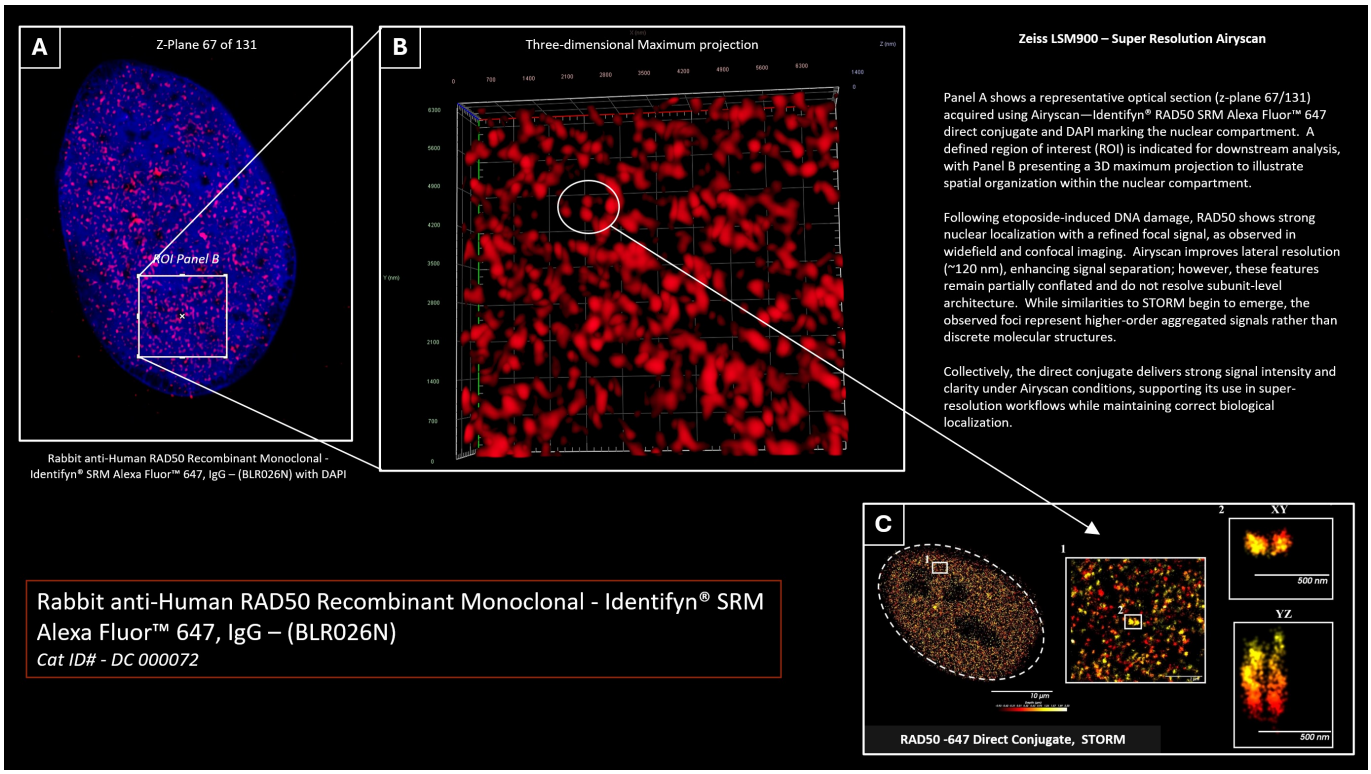
Identifyn® Rabbit anti-Human RAD50 Recombinant Monoclonal- Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) was pseudocolored in Zen Software from red, the default color, to white, to provide visual contrast in Panel C.

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

SOURCE PROVENANCE

Catalog	DC-000072
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	44
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F7B18AC17009E368BF8713BA3B2266258B66A58E54835DF869187384B96AE95E
Method PDF SHA-256	523E1DC6A1C84EDD61A9BC02A036A356CE5A3872068D1A97E1909CE159D6D1F6

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG – (BLR026N)
Cat ID# - DC 000072

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	49

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	131 Slices (3.90 µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	845 X 1016
Image Size (Scaled)	29.82 µm X 35.86 µm
Bit Depth	16 Bit
Image Center Position	X: 89.18 mm, Y: 52.00 mm
Stage Position	X: 89.18 mm, Y: 52.00 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 215 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 505
Laser Wavelength	639 nm @ 1.80% | 405 nm @ 0.2%
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	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Detection Wavelength	643 - 745 | 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	Super Resolution: 10.0 (3D, Auto) | Super Resolution: 8.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2%, Ramp 0%, Maximum 100%
Background	Black
Light	Brightness 90%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods®

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N)
Cat ID# - DC 000072

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. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth medium for 18 hours, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:250. 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 67 of 131, Line Profile Panel B

Z-Stack = 131 Slices (3.90 µm)

Channels = 2
Scaling (Per Pixel) = 0.035 nm X 0.035 nm X 0.030 nm
Image Size (Pixels) = 845 X 1016
Image Size (Scaled) = 29.82 µm X 35.86 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.18 mm, Y: 52.00 mm
Stage Position = X: 89.18 mm, Y: 52.00 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639 nm @ 1.80%
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 = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 745
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 215 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.2%
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 = Super Resolution: 8.3 (3D, Auto)

2-Dimensional View - Panel A

Shows Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) and DAPI. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Maximum Projection in Panel B.

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 2%, Ramp 0%, Maximum 100%
Background = Black
Light = Brightness 90%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 75%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product data set to demonstrate the resolution limitations of Airyscan, however, provides improved structural discrimination relative to confocal imaging, in determining localization and structural details.

Summary

Panel A shows a representative optical section (z-plane 67/131) acquired using Airyscan-Identifyn® RAD50 SRM Alexa Fluor™ 647 direct conjugate and DAPI marking the nuclear compartment. A defined region of interest (ROI) is indicated for downstream analysis, with Panel B presenting a 3D maximum projection to illustrate spatial organization within the nuclear compartment.

Following etoposide-induced DNA damage, RAD50 shows strong nuclear localization with a refined focal signal, as observed in widefield and confocal imaging. Airyscan improves lateral resolution (~120 nm), enhancing signal separation; however, these features remain partially conflated and do not resolve subunit-level architecture. While similarities to STORM begin to emerge, the observed foci represent higher-order aggregated signals rather than discrete molecular structures.

Collectively, the direct conjugate delivers strong signal intensity and clarity under Airyscan conditions, supporting its use in super-resolution workflows while maintaining correct biological localization.

Image Correction

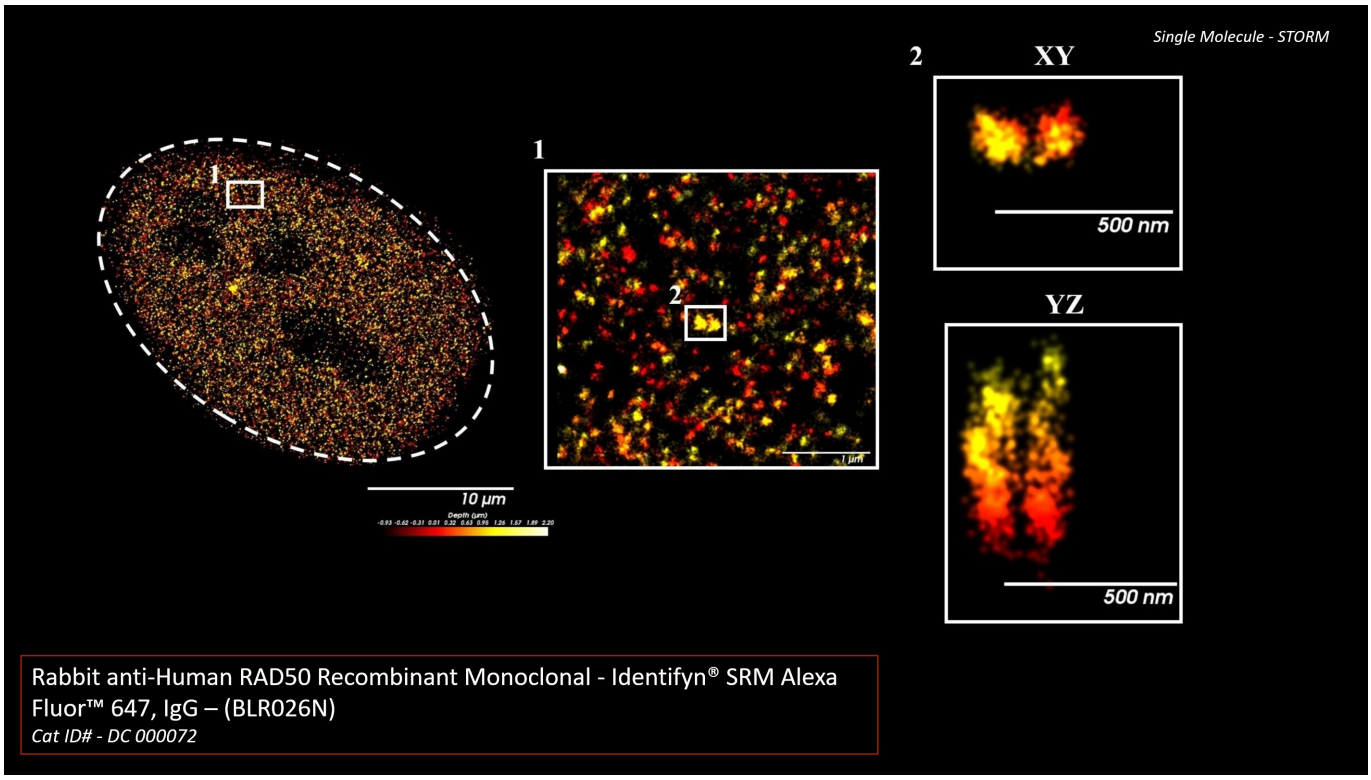
NONE

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

SOURCE PROVENANCE

Catalog	DC-000072
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	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B9759D2C24BE2DE2E72849BFD1AC6B649D05D5AF7F4CCB468C955EBA420D62A9
Method PDF SHA-256	2180040F3D97E36AC93BAEC1154D920F73D3CA663B169C99E61F991DE986C94E

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



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

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

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

FIELD	SOURCE-DOCUMENTED VALUE
Cell(s) or Cellular Structure Localization	The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused. | Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Current	184 µm
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	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50 µm is selected
Piezo Record	Begin: 184; End: 185.4
SuperRes 640 nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	7
Cycles	20
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	15
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Constant
Particle size	30
Color by	Depth
Color Table	Single

FIELD	SOURCE-DOCUMENTED VALUE
Opacity Mode	Depth; Opacity 1: 0.55
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	35
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Compute Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N)
Cat ID# - DC 000072

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 in a µ-Slide 8 Well High Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 18 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Control Data - Primary Unconjugated Antibody

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope Configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 184 µm

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: 100 ms
Default values are maintained unless otherwise noted.

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

Autofocus - Calibration Values

Current Position: -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50 µm is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 184; End: 185.4

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

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

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20 ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 7
Cycles: 20
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 15

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16 px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1 px
Fiducial at Max Accumulation: Not selected
Max Particles Per Frame: No Max
Background SLPE Removal: Not Selected
Cutout/Background Removal: Not Selected
Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 15

Region of Interest -

Not selected

Display -

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 30
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.55
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 8 Intervals
Pixel Size: 50 nm
Smoothing: 8.00

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

Radial precision: 35
Axial precision: 70

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1 µm
Maximum Particle Count to Form Cluster: 10
Compute Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Summary

Collectively, the STORM dataset demonstrates that Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) maintained strong fluorescence performance, localization fidelity, and nanoscale structural signal integrity under demanding single-molecule localization microscopy conditions. STORM imaging fundamentally depends on sufficient fluorophore brightness, stable photoswitching behavior, reproducible blinking kinetics, and resistance to photobleaching to generate accurate single-molecule localization events and reproducible nanoscale reconstructions. These imaging requirements frequently represent major limitations for conventional direct conjugates during prolonged super-resolution acquisition workflows.

Importantly, the Identifyn® direct conjugate maintained robust signal intensity, strong signal-to-noise characteristics, stable localization performance, and reproducible RAD50-associated nanoscale fluorescence organization throughout volumetric STORM acquisition, autofocus drift correction, advanced particle filtering, and DBSCAN cluster-analysis workflows. Despite extremely low SuperRes laser-input conditions, the direct conjugate preserved biologically coherent RAD50-associated fluorescence territories and structural organization highly consistent with the corresponding unconjugated Rabbit anti-Human RAD50 Recombinant Monoclonal, IgG - (PA-RR 000026) stepdown datasets generated across widefield, confocal, and Airyscan imaging modalities.

Unlike many conventional direct conjugates that exhibit diminished photon output, unstable blink kinetics, elevated background fluorescence, localization artifacts, or fluorophore instability under single-molecule acquisition conditions, Rabbit anti-Human RAD50 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR026N) demonstrated compatibility with demanding STORM localization microscopy workflows while preserving biologically coherent nuclear-associated RAD50 fluorescence organization. Collectively, these findings further support the Identifyn® direct conjugation platform's ability to maintain fluorophore efficiency, photoswitching compatibility, localization fidelity, and nanoscale structural signal integrity under advanced super-resolution imaging conditions, where conventional direct conjugates frequently fail.

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

SOURCE PROVENANCE

Catalog	DC-000072
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	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	609F31046EB6A6D95FE8078686D0E85CF2745BE6DBFE34C94D48891991A36918
Method PDF SHA-256	9A30A44012F6FA4E24DC8A3539CF277D3186B34F62E00DCB190A1244493A0B87