BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

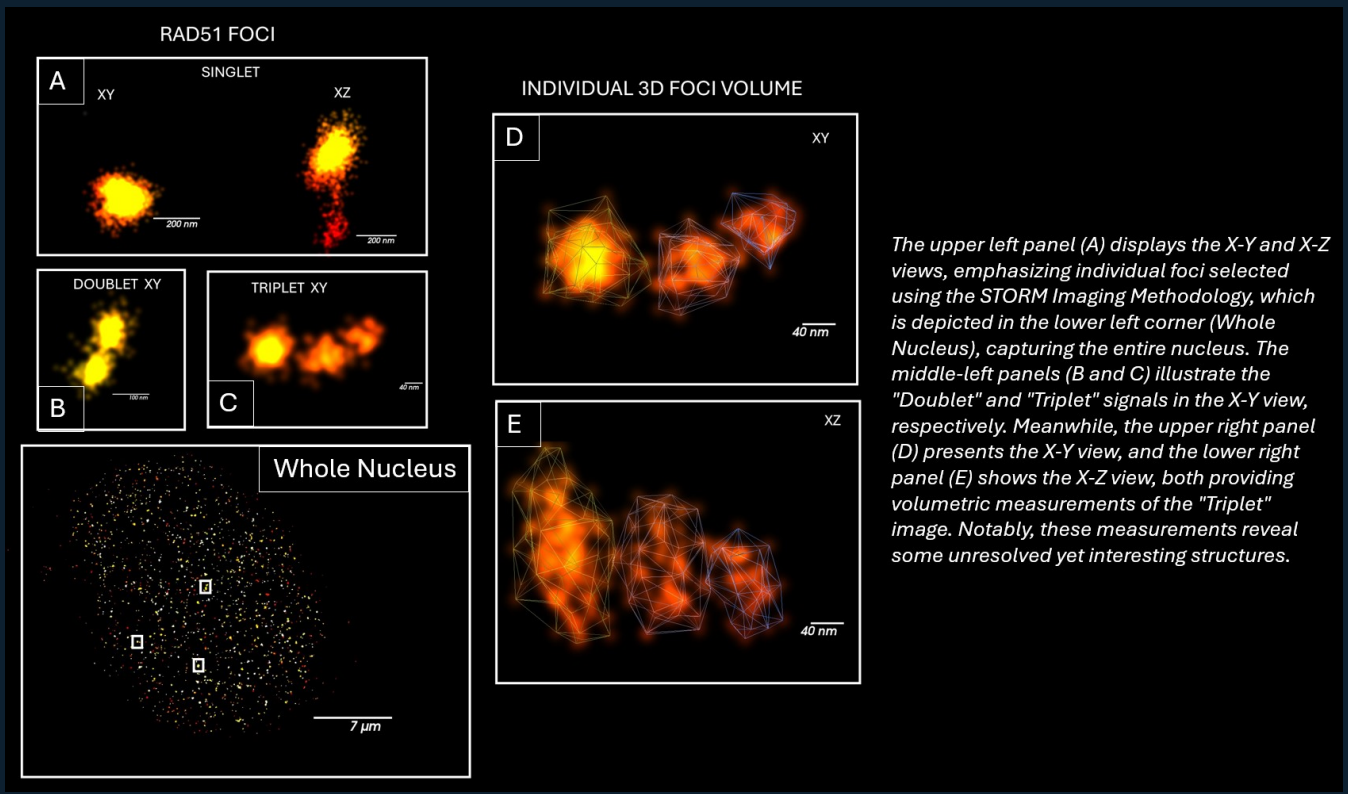
RAD51

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human RAD51 Recombinant Monoclonal

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

7	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



The upper left panel (A) displays the X-Y and X-Z views, emphasizing individual foci selected using the STORM Imaging Methodology, which is depicted in the lower left corner (Whole Nucleus), capturing the entire nucleus. The middle-left panels (B and C) illustrate the "Doublet" and "Triplet" signals in the X-Y view, respectively. Meanwhile, the upper right panel (D) presents the X-Y view, and the lower right panel (E) shows the X-Z view, both providing volumetric measurements of the "Triplet" image. Notably, these measurements reveal some unresolved yet interesting structures.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000002 | 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
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for RAD51, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

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

BENNETT ASSESSMENT

The preserved PA-RR-000002 record contains Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM²; 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 PA-RR-000002 record contains Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM²; Single-molecule STORM. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 7 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Widefield	405/DAPI; 488; 647	2; 50 Cy5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 555; 680	3; AF532-49014; 50 Cy5; 49 DAPI; 515 - 545; 625 - 655; 335 - 383; 555 - 595
Confocal	405/DAPI; 488; 647	3; None; 640nm @0.7%; 488nm @0.5%; 405nm @0.6%; 653; 493; 353
Airyscan	405/DAPI; 488; 647	3; None; 640nm @1.0%; 488nm @0.5%; 405nm @0.3%; 653; 493; 353
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820 - SR; T1-Axiocam 820 - SR; T3-Axiocam 820 #2-SR; 488
SIM²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820 - SR; T1-Axiocam 820 - SR; T3-Axiocam 820 #2-SR; 488
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	2; 38 HE eGFP; 50 Cy5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 -550

MODALITY ASSESSMENT / PART 1 OF 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 705 mono. Timing: Exposure Time: 150ms; 50ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 41.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 92 Slices (9.10µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 1024 X 1024; Image Size (Pixels): 1024 X 1024; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono; Axiocam 807. Timing: Exposure Time: 150ms; 350ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @8.0%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 49.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 555; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 127 Slices (3.78µm); Channels: 3; Scaling (Per Pixel): 0.030µm X 0.030µm X 0.030µm; Image size (Pixels): 1120 X 1120; Image Size (Pixels): 1120 X 1120; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt2; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.69µs; Frame Time: 8.15s. Illumination: Laser Wavelength: 640nm @0.7%; 488nm @0.5%. Optical/scan: Pinhole: 1.00 AU / 57µm; 1.00 AU / 43µm; Scan Zoom: 3.0; LSM Scan Speed: 8; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

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 900, 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 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 149 Slices (4.44µm); Channels: 3; Scaling (Per Pixel): 33.01nm X 33.01nm X 30.00nm; Image size (Pixels): 1000 X 1000; 151 X 140; Image Size (Pixels): 1000 X 1000; 151 X 140; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.52µs; Frame Time: 5.06s. Illumination: Laser Wavelength: 640nm @1.0%; 488nm @0.5%. Optical/scan: Pinhole: 5.00 AU / 280µm; 5.31 AU / 217µm; Scan Zoom: 3.0; LSM Scan Speed: 9; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 10.0 (3D, Auto); Super Resolution 8.0 (3D, Auto). Structured source metadata values: 64.

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 900, 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 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 148 Slices (4.41µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 1975 X 1489; Image Size (Pixels): 5056 X 5056; 1975 X 1489; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 1.33s; 1.59s. Illumination: Laser Wavelength: 488nm @1.00%; 640nm @2.50%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 70.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 148 Slices (4.41µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 106 X 98; Image Size (Pixels): 5056 X 5056; 106 X 98; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 1.33s; 1.59s. Illumination: Laser Wavelength: 488nm @1.00%; 640nm @2.50%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 69.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 8

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 8

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 950 X 832; Image Size (Pixels): 950 X 832; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 200ms; 60ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @6.0%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 41.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved PA-RR-000002 record contains Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM²; 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): 33.01nm X 33.01nm X 30.00nm -> 0.03301 µm X 0.03301 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)=1000 X 1000; Image Size (Scaled)=33.01µm X 33.01µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm -> 0.01320 µm X 0.01320 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)=5056 X 5056; Image Size (Scaled)=66.72µm X 66.72µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm -> 0.01320 µm X 0.01320 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)=5056 X 5056; Image Size (Scaled)=66.72µm X 66.72µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 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.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

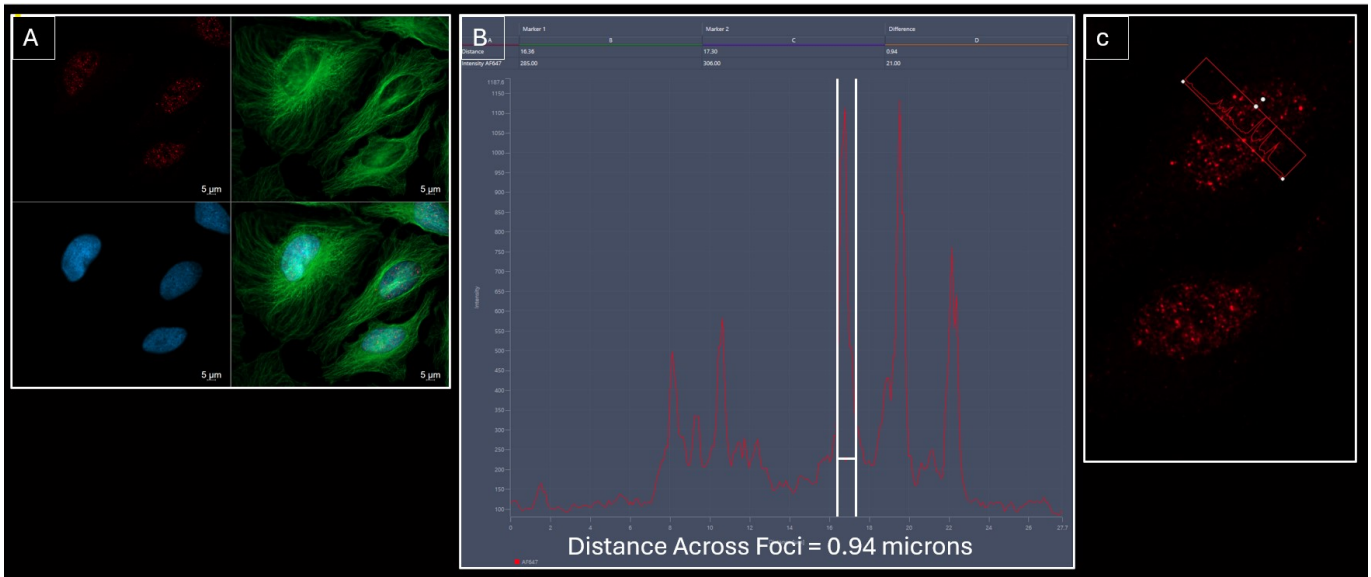
This dossier preserves the complete available evidence progression for PA-RR-000002. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A presents a split view of the three channels collected using widefield microscopy with an sCMOS camera: RAD51 (shown in red), Tubulin (in green), and DAPI (in blue). Panel B illustrates a line profile across a single nucleus, measuring the fluorescent intensity and distance of signal points, which appear as individual foci. However, Panel C highlights the significant limitations of using widefield microscopy to study foci or structural information. In this case, a single focus is observed to have a width of approximately 1 micron. In contrast, STORM data reveals that a single event has a Full Width at Half Maximum (FWHM) of approximately 40-100 nm. Despite these limitations, widefield microscopy is still useful for studying cellular sub-population protein expression levels and provides valuable population data. The term foci traditionally describes circular localized signal representing protein recruitment to the sight an event, or the minimum sub cellular structural information available.

A review of the entire RAD51 data set reveals that, even at STORM resolution (which allows for single-molecule imaging), there remains undiscovered structural detail. Additionally, the term "foci" should be regarded as a non-specific biological term, simply representing an observation that is defined by diffraction-limited acquisition methods.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	41

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 mono, 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)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	16 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	50 Cy5 | 38 HE eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @10% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms | 50ms | 40ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705 mono
Camera Adapter	1X
Depth of Focus	1.03µm | 0.80µm | 0.72µm
Binning Mode	2,2
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 27.7), Y (Min 80 and Max 1188)

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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

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

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D) - Split View Panel A

Channels = 2
Scaling (Per Pixel) = 0.110µm X 0.110µm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18µm X 112.59µm
Bit Depth = 16 Bit
Image Center Position = X: 0.00µm, Y: 0.00µm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 150ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 1.03µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10%
Exposure Time = 50ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.80µm
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%
Exposure Time = 40ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Post Processing

None

Profile View Display - Panels B and C

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 27.7), Y (Min 80 and Max 1188)

Summary

Panel A presents a split view of the three channels collected using widefield microscopy with an sCMOS camera: RAD51 (shown in red), Tubulin (in green), and DAPI (in blue). Panel B illustrates a line profile across a single nucleus, measuring signal points' fluorescent intensity and distance, which appear as individual foci. However, Panel C highlights the significant limitations of using widefield microscopy to study foci or structural information. In this case, a single focus is observed to have a width of approximately 1 micron. In contrast, STORM data reveals that a single event has a Full Width at Half Maximum (FWHM) of approximately 40-100 nm. Despite these limitations, widefield microscopy is still helpful for studying cellular sub-population protein expression levels and provides valuable population data. Foci traditionally describe a circular localized signal representing protein recruitment to the site of an event, or the minimum sub-cellular structural information available.

A review of the entire RAD51 data set reveals that undiscovered structural details remain, even at STORM resolution (which allows for single-molecule imaging). Additionally, "foci" should be regarded as a non-specific biological term representing an observation defined by diffraction-limited acquisition methods.

Image Correction

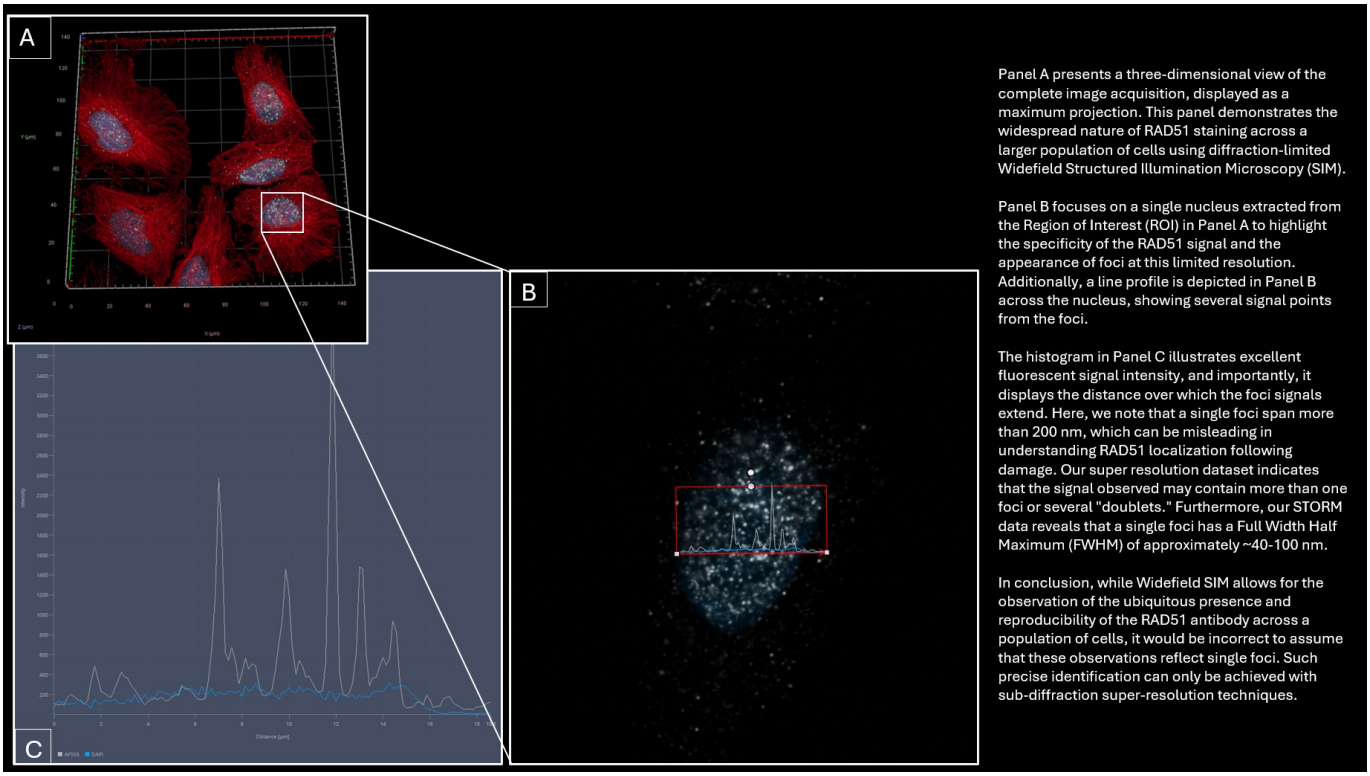
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000002
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 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	41
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3CA7DEA44BCCC33192B8F0341DA3824722FEE2C013B48A883EDBAC9D57EE6739
Method PDF SHA-256	54EDE1BC6427FB4397B7EFEE4E72C0C18BBA29146C394746D62FB929E009A584

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 680
METADATA VALUES	49

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	92 Slices (9.10µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	1024 X 1024
Image Size (Scaled)	146.29µm X 146.29µm
Bit Depth	14 Bit
Image Center Position	X: 89.99mm, Y: 51.11mm
Stage Position	X: 89.99mm, Y: 51.11mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF532-49014 | 50 Cy5 | 49 DAPI
Beam Splitter	550 | 660 | 395
Filter Excitation Wavelength	515 - 545 | 625 - 655 | 335 - 383
Filter Emission Wavelength	555 - 595 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	150ms | 350ms | 18ms
Excitation Wavelength	553 | 679 | 353
Emission Wavelength	568 | 702 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono | Axiocam 807
Camera Adapter	1X
Depth of Focus	1.10µm | 1.36µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1

FIELD	SOURCE-DOCUMENTED VALUE
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 18.6), Y (Min 2 and Max 4340)

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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000038

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000067

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 92 Slices (9.10µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.100µm
Image Size (Pixels) = 1024 X 1024
Image Size (Scaled) = 146.29µm X 146.29µm
Bit Depth = 14 Bit
Image Center Position = X: 89.99mm, Y: 51.11mm
Stage Position = X: 89.99mm, Y: 51.11mm
ROI Center Offset = X: 1.14, Y: 0.00µm

Single Plane (2D) - Panels B and C

Z- Plane 47 of 92

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 150ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.10µm
Binning Mode = 2,2

680 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 350ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.36µm
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 @8.0%
Exposure Time = 18ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing - Panel A

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

Profile View Display - Panels B and C

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 18.6), Y (Min 2 and Max 4340)

Summary

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. This panel demonstrates the widespread nature of RAD51 staining across a larger population of cells using diffraction-limited Widefield Structured Illumination Microscopy (SIM).

Panel B focuses on a single nucleus extracted from the Region of Interest (ROI) in Panel A to highlight the specificity of the RAD51 signal and the appearance of foci at this limited resolution. A line profile is also depicted in Panel B across the nucleus, showing several signal points from the foci.

The histogram in Panel C illustrates excellent fluorescent signal intensity, and importantly, it displays the distance over which the foci signals extend. Here, we note that the foci span more than 200 nm, which can be misleading in understanding RAD51 localization following damage. Our super-resolution dataset indicates that the signal observed may contain more than one focus or several "doublets." Furthermore, our STORM data reveals that a single event has a Full Width at Half Maximum (FWHM) of approximately 40-100 nm.

In conclusion, while Widefield SIM allows for the observation of the ubiquitous presence and reproducibility of the RAD51 antibody across a population of cells, it would be incorrect to assume that these observations reflect single events. Such precise identification can only be achieved with sub-diffraction super-resolution techniques.

Image Corrections

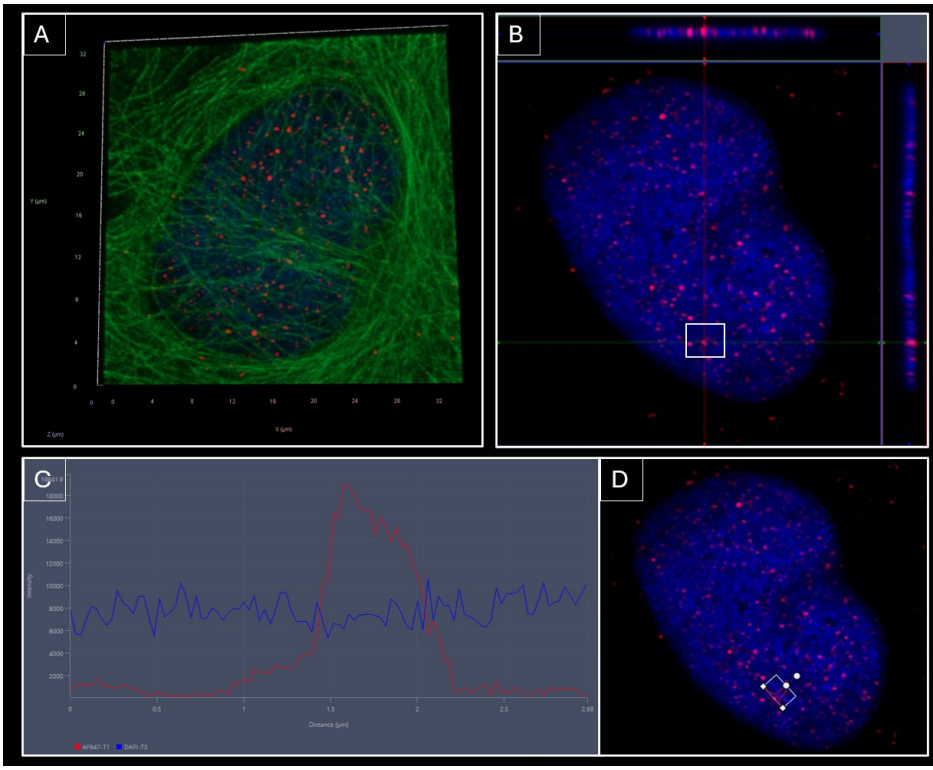
None

Image by E.F. Simo
Analysis and Presentation by B. T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6EAD5499146942F843987CF185B1C69759659D801F8153E1144EEBA819221465
Method PDF SHA-256	39D4D6567A7C040BD5635ACE5E5A319120123D71448C85B12F0DF9B7236C7EBC

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Panel B shows an orthogonal view of the same field, excluding the 488 (Tubulin) channel. This panel demonstrates the X-Y image of z-plane 51 out of 127, as well as the X-Z plane (top) and the Y-Z plane (right). The foci of interest are intersected by the green (Y) and red (X) cut lines. While the image may appear to show a "doublet" as we see in super-resolution, diffraction-limited confocal resolution (above 220nm X,Y) cannot resolve this, resulting in RAD51 being displayed as a single foci in the z-planes.

To further demonstrate the resolution of RAD51 foci in confocal imaging, panels C and D present a line profile across the same intersection shown in panel B. The intensity histogram reveals a single peak, indicating no separation and thus representing a single foci. In contrast, the super-resolution techniques—Airyscan, SIM, SIM^2, and STORM—provide further structural detail.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	56

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 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	127 Slices (3.78µm)
Channels	3
Scaling (Per Pixel)	0.030µm X 0.030µm X 0.030µm
Image Size (Pixels)	1120 X 1120
Image Size (Scaled)	33.80µm X 33.80µm
Bit Depth	16 Bit
Image Center Position	X: 78.88mm, Y: 46.61mm
Stage Position	X: 78.88mm, Y: 46.61mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 57µm | 1.00 AU / 43µm | 1.00 AU / 183µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @0.7% | 488nm @0.5% | 405nm @0.6%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.34
Pixel Time	0.69µs
Frame Time	8.15s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	656 - 700 | 478-539 | 410-470
Imaging Device	GaAsP-Pmt2 | Airyscan | GaAsp-Pmt1
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	650 V
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Cut Lines	X 607, Y 819, Z 51
Line Width	X 10, Y 10, Z 1
Cut Line Opacity	15
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 2.98), Y (Min 60 and Max 19842)

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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

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

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 127 Slices (3.78µm)

Channels = 3
Scaling (Per Pixel) = 0.030µm X 0.030µm X 0.030µm
Image Size (Pixels) = 1120 X 1120
Image Size (Scaled) = 33.80µm X 33.80µm
Bit Depth = 16 Bit
Image Center Position = X: 78.88mm, Y: 46.61mm
Stage Position = X: 78.88mm, Y: 46.61mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel B and D (Orthogonal and Cut Plane)

Z- Plane 51 of 127

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 57µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.7%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 478-539
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 183µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.6%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 8.15s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsp-Pmt1
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Orthogonal View - Uses Image 51 of the Z-Stack 127 Images - Panel B

Cut Lines = X 607, Y 819, Z 51
Line Width = X 10, Y 10, Z 1
Cut Line Opacity = 15
Maximum Intensity Opacity (MIP) and Measure 3D Distance NOT selected

Profile View Display - Panels C and D

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 2.98), Y (Min 60 and Max 19842)

Summary -

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Panel B shows an orthogonal view of the same field, excluding the 488 (Tubulin) channel. This panel demonstrates the X-Y image of z-plane 51 out of 127, the X-Z plane (top), and the Y-Z plane (right). The foci of interest are intersected by the green (Y) and red (X) cut lines. While the image may appear to show a "doublet," as we see in super-resolution, diffraction-limited confocal resolution (above 220nm X, Y) cannot resolve this, resulting in RAD51 being displayed as a single foci in the z-planes.

To further demonstrate the resolution of RAD51 foci in confocal imaging, panels C and D present a line profile across the same intersection shown in panel B. The intensity histogram reveals a single peak, indicating no separation and thus representing a single foci. In contrast, the super-resolution techniques-Airyscan, SIM, SIM2, and STORM-provide further structural detail.

Image Corrections -

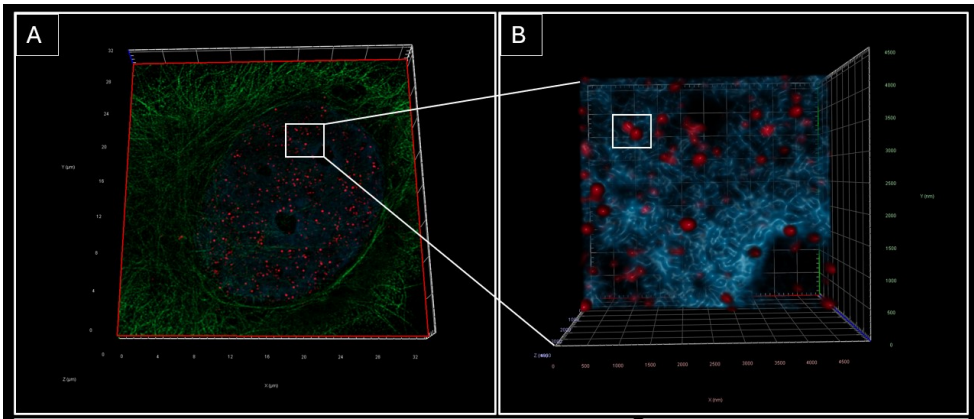
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6F46FB60A6B0BC386DE409BC841A83FBD4F3FD3C33457C01E6BD0952697A80DB
Method PDF SHA-256	66F05B1579F90313CC221D6627CD43DE576928C9094CE67DD32A81D9C35BFB68

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Additionally, Panel A highlights the Region of Interest (ROI) that is depicted in Panel B. Panel B provides a three-dimensional volume projection of the ROI from Panel A, showcasing RAD51 foci. Within this image, the selected ROI illustrates the "doublet" effect.

Panels C and D utilize line profiles to measure the signal intensity and spatial separation across the "doublet". The super-resolution Airyscan, with a resolution of approximately 120 nm (X,Y), reveals that what appears as a single focus in diffraction-limited images is actually the "doublet." In contrast, the SIM and SIM^2 images from the RAD51 dataset demonstrate a greater separation of this signal, along with elongation or extension of the single focus into a more thread-like structure, observed at the terminal end of the foci. Finally, STORM microscopy is capable of resolving structures that are limited in resolution by the previously mentioned super-resolution methods.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	64

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 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	149 Slices (4.44µm)
Channels	3
Scaling (Per Pixel)	0.03301 µm X 0.03301 µm X 0.03000 µm
Image Size (Pixels)	1000 X 1000 | 151 X 140
Image Size (Scaled)	33.01µm X 33.01µm | 4.98µm X 4.62µm
Bit Depth	16 Bit
Image Center Position	X: 50.31mm, Y: 47.96mm
Stage Position	X: 50.31mm, Y: 47.96mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 280µm | 5.31 AU / 217µm | 5.00 AU / 183µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @1.0% | 488nm @0.5% | 405nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.14
Pixel Time	0.52µs
Frame Time	5.06s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	630 - 700 | 480-550 | 400-469
Imaging Device	Airyscan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 10.0 (3D, Auto) | Super Resolution 8.0 (3D, Auto) | Super Resolution 8.4 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 2% (647), Threshold 2% (DAPI), Ramp 98% (647), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
Clipping Image Process	The Clipping plane is introduced to demonstrate signal specificity to the Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG (BLR017M) to the nuclear compartment following DNA damage.
X-Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment, RAD51 signal, and localization. A red Outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.
Position of Clip	-36%
Clip X	0°
Clip Z	0°
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 0.66), Y (Min 0.00 and Max 3067)

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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

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

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 149 Slices (4.44µm)

Channels = 3
Scaling (Per Pixel) = 33.01nm X 33.01nm X 30.00nm
Image Size (Pixels) = 1000 X 1000
Image Size (Scaled) = 33.01µm X 33.01µm
Bit Depth = 16 Bit
Image Center Position = X: 50.31mm, Y: 47.96mm
Stage Position = X: 50.31mm, Y: 47.96mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 149 Slices (4.44µm)

Channels = 3
Scaling (Per Pixel) = 33.01nm X 33.01nm X 30.00nm
Image Size (Pixels) = 151 X 140
Image Size (Scaled) = 4.98µm X 4.62µm
Bit Depth = 16 Bit
Image Center Position = X: 50.31mm, Y: 47.96mm
Stage Position = X: 50.31mm, Y: 47.96mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel D

Z- Plane 50 of 149

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 280µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.31 AU / 217µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480-550
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 183µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.14
Pixel Time = 0.52µs
Frame Time = 5.06s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-469
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode “SR”
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% (647), Threshold 2% (DAPI), Ramp 98% (647), Ramp 98% (DAPI), 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)

Cut Plane - Panel A

Clipping Image Process = The Clipping plane is introduced to demonstrate signal specificity to the Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG (BLR017M) to the nuclear compartment following DNA damage.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment, RAD51 signal, and localization. A red Outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = -36%
Clip X = 0°
Clip Z =0°

Profile View Display - Panels C and D

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 0.66), Y (Min 0.00 and Max 3067)

Summary -

Panel A presents a three-dimensional view of the complete image acquisition, displayed as a maximum projection. Additionally, Panel A highlights the Region of Interest (ROI) depicted in Panel B. Panel B provides a three-dimensional volume projection of the ROI from Panel A, showcasing RAD51 foci. Within this image, the selected ROI illustrates the "doublet" effect.

Panels C and D utilize line profiles to measure the signal intensity and spatial separation across the "doublet". The super-resolution Airyscan, with a resolution of approximately 120 nm (X, Y), reveals that what appears as a single focus in diffraction-limited images is the "doublet." In contrast, the SIM and SIM2 images from the RAD51 dataset demonstrate a greater separation of this signal, along with elongation or extension of the single focus into a more thread-like structure, observed at the terminal end of the foci. Finally, STORM microscopy can resolve structures that are limited in resolution by the previously mentioned super-resolution methods.

Image Corrections -

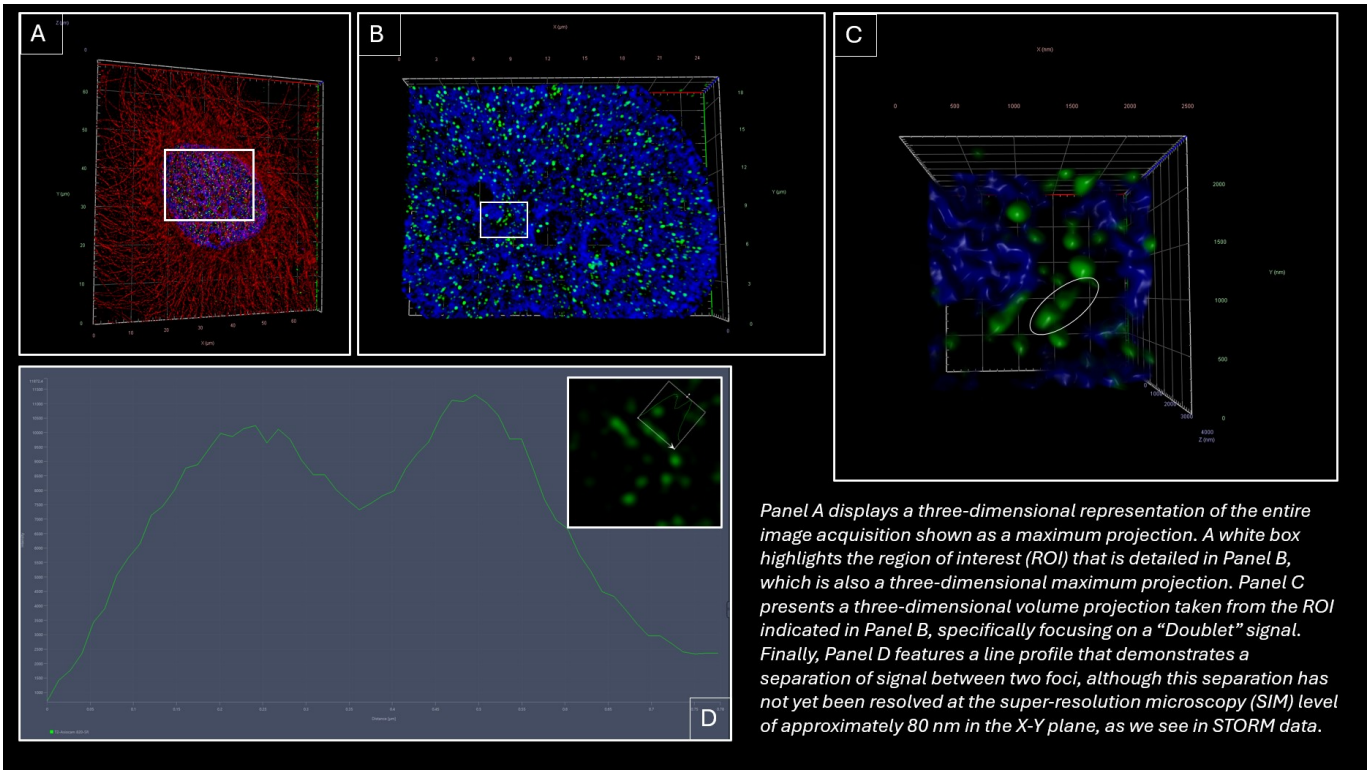
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A4F0BE84774D8987676362760327DF654526231CAE1C5B7EA95555975B2B5480
Method PDF SHA-256	25390CE2A6827A946BD0FA4F8721B520D02A4FA678BD4B440A1039AD5EA595FA

ORIGINAL IMAGE EVIDENCE / SIM



Panel A displays a three-dimensional representation of the entire image acquisition shown as a maximum projection. A white box highlights the region of interest (ROI) that is detailed in Panel B, which is also a three-dimensional maximum projection. Panel C presents a three-dimensional volume projection taken from the ROI indicated in Panel B, specifically focusing on a "Doublet" signal. Finally, Panel D features a line profile that demonstrates a separation of signal between two foci, although this separation has not yet been resolved at the super-resolution microscopy (SIM) level of approximately 80 nm in the X-Y plane, as we see in STORM data.

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

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	148 Slices (4.41µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1975 X 1489 | 192 X 186
Image Size (Scaled)	66.72µm X 66.72µm | 26.06µm X 19.65µm | 2.53µm X 2.45µm
Bit Depth	16 Bit
Image Center Position	X: 63.56mm, Y: 36.15mm
Stage Position	X: 63.56mm, Y: 36.15mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820 - SR | T1-Axiocam 820 - SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms | 120ms | 50ms
Depth of Focus	0.81µm | 1.13µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @1.00% | 640nm @2.50% | 405nm @5.0%
Frame Time	1.33s | 1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.9962 (647), 9.5688 (488), 8.4869 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Maximum Projection	Precise
Appearance	Threshold 4% (488), Threshold 3% (647), Threshold 3% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 4% (488), Threshold 2% (DAPI), Ramp 96% (488), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 100%, Azimuth 66°, Elongation -126°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 0.78), Y (Min 0.00 and Max 11872)

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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000020

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 148 Slices (4.41µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 63.56mm, Y: 36.15mm
Stage Position = X: 63.56mm, Y: 36.15mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 148 Slices (4.41µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 1975 X 1489
Image Size (Scaled) = 26.06µm X 19.65µm
Bit Depth = 16 Bit
Image Center Position = X: 63.56mm, Y: 36.15mm
Stage Position = X: 63.56mm, Y: 36.15mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel C

Z-Stack = 148 Slices (4.41µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 192 X 186
Image Size (Scaled) = 2.53µm X 2.45µm
Bit Depth = 16 Bit
Image Center Position = X: 63.56mm, Y: 36.15mm
Stage Position = X: 63.56mm, Y: 36.15mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820 - SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.00%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 - SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @2.50%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 32.0µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @5.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.9962 (647), 9.5688 (488), 8.4869 (DAPI)
Fitting = Fast Fit
Histogram = Scale to Min/Max
Baseline = Cut

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 4% (488), Threshold 3% (647), Threshold 3% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 4% (488), Threshold 2% (DAPI), Ramp 96% (488), Ramp 98% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 66°, Elongation -126°, Enabled Directional Light and Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Profile View Display

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 0.78), Y (Min 0.00 and Max 11872)

Summary

Panel A displays a three-dimensional representation of the entire image acquisition, which is shown as a maximum projection. A white box highlights the region of interest (ROI) detailed in Panel B, also a three-dimensional maximum projection. Panel C presents a three-dimensional volume projection from the ROI indicated in Panel B, specifically focusing on a “Doublet” signal. Finally, Panel D features a line profile demonstrating a signal separation between two foci. However, as we see in STORM data, this separation has not yet been resolved at the super-resolution microscopy (SIM) level of approximately 80 nm in the X-Y plane.

Image Corrections

None

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

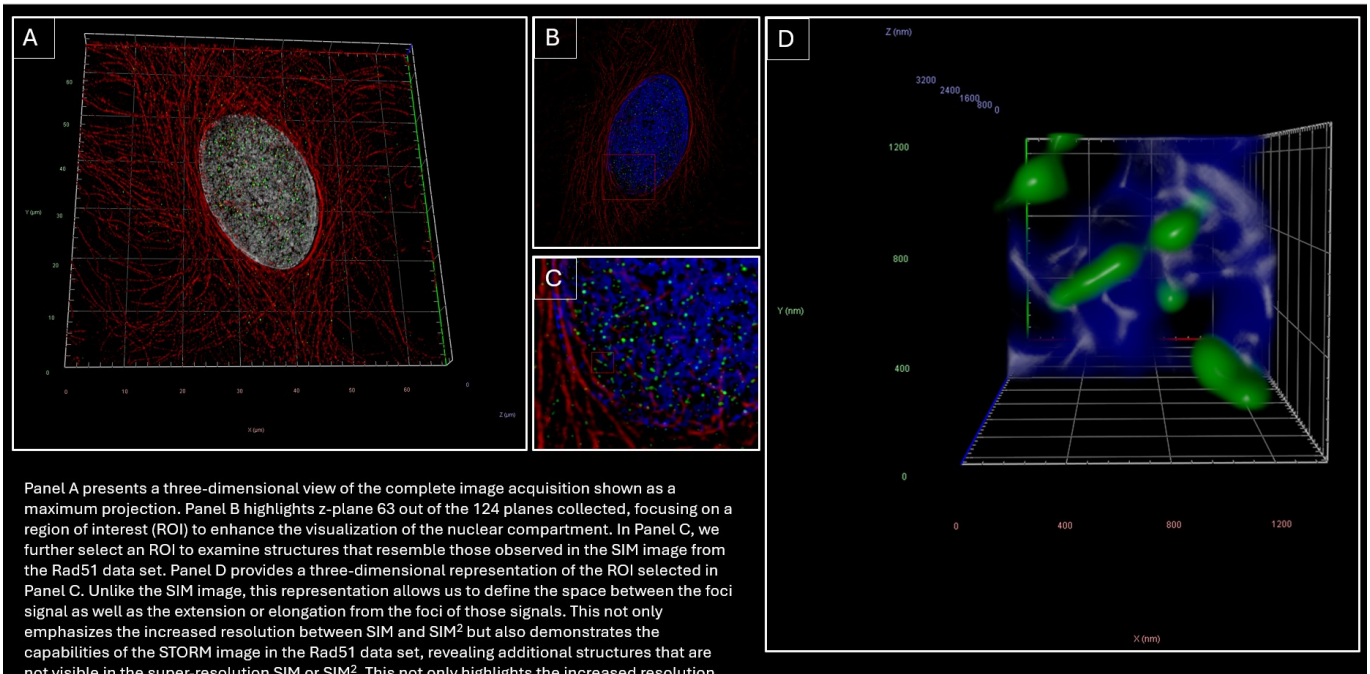
SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1F07A075D334355EE6A3D416D8CD13FC1426D26C486BDB44AD7C762B3924C71D
Method PDF SHA-256	D6EA15B0750FBDB64D0A608D6E5AE84826BFB65CC76A8CBB011BED9E91443C06

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A presents a three-dimensional view of the complete image acquisition shown as a maximum projection. Panel B highlights z-plane 63 out of the 124 planes collected, focusing on a region of interest (ROI) to enhance the visualization of the nuclear compartment. In Panel C, we further select an ROI to examine structures that resemble those observed in the SIM image from the Rad51 data set. Panel D provides a three-dimensional representation of the ROI selected in Panel C. Unlike the SIM image, this representation allows us to define the space between the foci signal as well as the extension or elongation from the foci of those signals. This not only emphasizes the increased resolution between SIM and SIM² but also demonstrates the capabilities of the STORM image in the Rad51 data set, revealing additional structures that are not visible in the super-resolution SIM or SIM². This not only highlights the increased resolution between SIM and SIM² but also illustrates the capabilities of STORM imaging to reveal additional structures that are not visible in super-resolution SIM or SIM².

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	69

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	148 Slices (4.41µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 106 X 98
Image Size (Scaled)	66.72µm X 66.72µm | 1.40µm X 1.29µm
Bit Depth	16 Bit
Image Center Position	X: 61.74mm, Y: 36.59mm
Stage Position	X: 61.74mm, Y: 36.59mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 | 640 | 405
Channel Name	T2-Axiocam 820 - SR | T1-Axiocam 820 - SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	488 | 640 | 405
Emission Wavelength	525 | 729 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms | 120ms | 50ms
Depth of Focus	0.81µm | 1.13µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @1.00% | 640nm @2.50% | 405nm @5.0%
Frame Time	1.33s | 1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.3429 (647), 8.7727 (488), 7.6827 (DAPI)
Order Combination	Wiener Filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Maximum Projection	Precise
Appearance	Threshold 4% (488), Threshold 4% (647), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels | Threshold 2% (488), Threshold 2% (DAPI), Ramp 98% (488), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000020

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000056

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 148 Slices (4.41µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 61.74mm, Y: 36.59mm
Stage Position = X: 61.74mm, Y: 36.59mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Single Plane (2D) - Panel B and C

Z- Plane 63 of 124

Z Stack - (3D) - Panel D

Z-Stack = 148 Slices (4.41µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 106 X 98
Image Size (Scaled) = 1.40µm X 1.29µm
Bit Depth = 16 Bit
Image Center Position = X: 61.74mm, Y: 36.59mm
Stage Position = X: 61.74mm, Y: 36.59mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820 - SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.00%
Frame Time = 1.33s
Scan Direction = Unidirectional
Grating 32.0µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820 - SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @2.50%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 32.0µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @5.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.3429 (647), 8.7727 (488), 7.6827 (DAPI)
Order Combination = Wiener Filter
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 4% (488), Threshold 4% (647), Threshold 4% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2% (488), Threshold 2% (DAPI), Ramp 98% (488), Ramp 98% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light and Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A presents a three-dimensional view of the complete image acquisition, which is shown as a maximum projection. Panel B highlights z-plane 63 out of the 124 planes collected, focusing on a region of interest (ROI) to enhance the visualization of the nuclear compartment. In Panel C, we further select an ROI to examine structures that resemble those observed in the SIM image from the Rad51 data set. Panel D provides a three-dimensional representation of the chosen ROI in Panel C. Unlike the SIM image, this representation allows us to define the space between the foci signal and the extension or elongation from the foci of those signals. This not only emphasizes the increased resolution between SIM and SIM2 but also demonstrates the capabilities of the STORM image in the Rad51 data set, revealing additional structures that are not visible in the super-resolution SIM or SIM2. This highlights the increased resolution between SIM and SIM2 and illustrates the capabilities of STORM imaging to reveal additional structures that are not visible in super-resolution SIM or SIM2.

Image Corrections

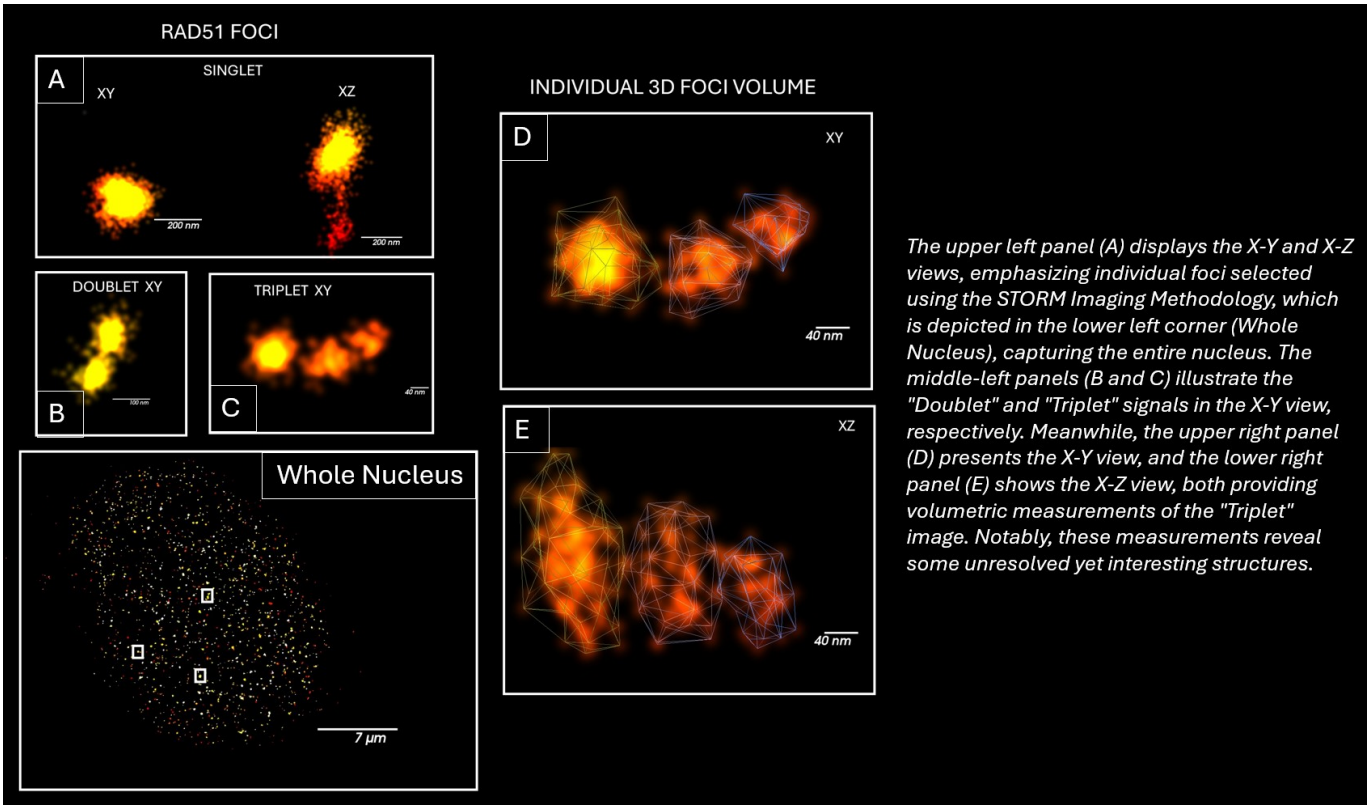
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7868239CD5341A007EAF2EABA845D9CEE1F1D0EC0E53D8115EBE3FEC6DAD0F1C
Method PDF SHA-256	C3E957112945EBDC85D8846F689A06BB03D28CE9D5D4FA64AF967754F6987935

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



The upper left panel (A) displays the X-Y and X-Z views, emphasizing individual foci selected using the STORM Imaging Methodology, which is depicted in the lower left corner (Whole Nucleus), capturing the entire nucleus. The middle-left panels (B and C) illustrate the "Doublet" and "Triplet" signals in the X-Y view, respectively. Meanwhile, the upper right panel (D) presents the X-Y view, and the lower right panel (E) shows the X-Z view, both providing volumetric measurements of the "Triplet" image. Notably, these measurements reveal some unresolved yet interesting structures.

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

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	182.3 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 179.0; End: 181.1
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 500
Z Positions	6 using Steps: 0.2µm (200nm)
Cycles	10
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	30%
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Constant
Particle size	20nm
Color by	Depth
Color Table	Single
Opacity Mode	Constant; Opacity 1: 0.3
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-450 to +450
Radial precision	25
Axial precision	60
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.05µm (500nm)
Maximum Particle Count to Form Cluster	20
Computer Hulls	Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	600nm
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 RAD51 Recombinant Monoclonal , IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

Hela cells were grown on a µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:50. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 182.3 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 (640 nm Dichroic): Not Selected

Advanced Tools Option

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

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

Autofocus - Calibration Values

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

View Mode

SuperRes: 50µm is selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 179.0; End: 181.1

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

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

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

Not Selected

Advanced Tools

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

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 500
Z Positions: 6 using Steps: 0.2µm (200nm)
Cycles: 10
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 30%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 15

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 20nm
Color by: Depth
Color Table: Single
Opacity Mode: Constant; Opacity 1: 0.3
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 10 Intervals
Pixel Size: 50nm
Smoothing: 8.00

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

PSF Z offset: -450 to +450
Radial precision: 25
Axial precision: 60

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: 0.05µm (500nm)
Maximum Particle Count to Form Cluster: 20
Computer Hulls: Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Hull Alpha shape Radius: 600nm
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Summary

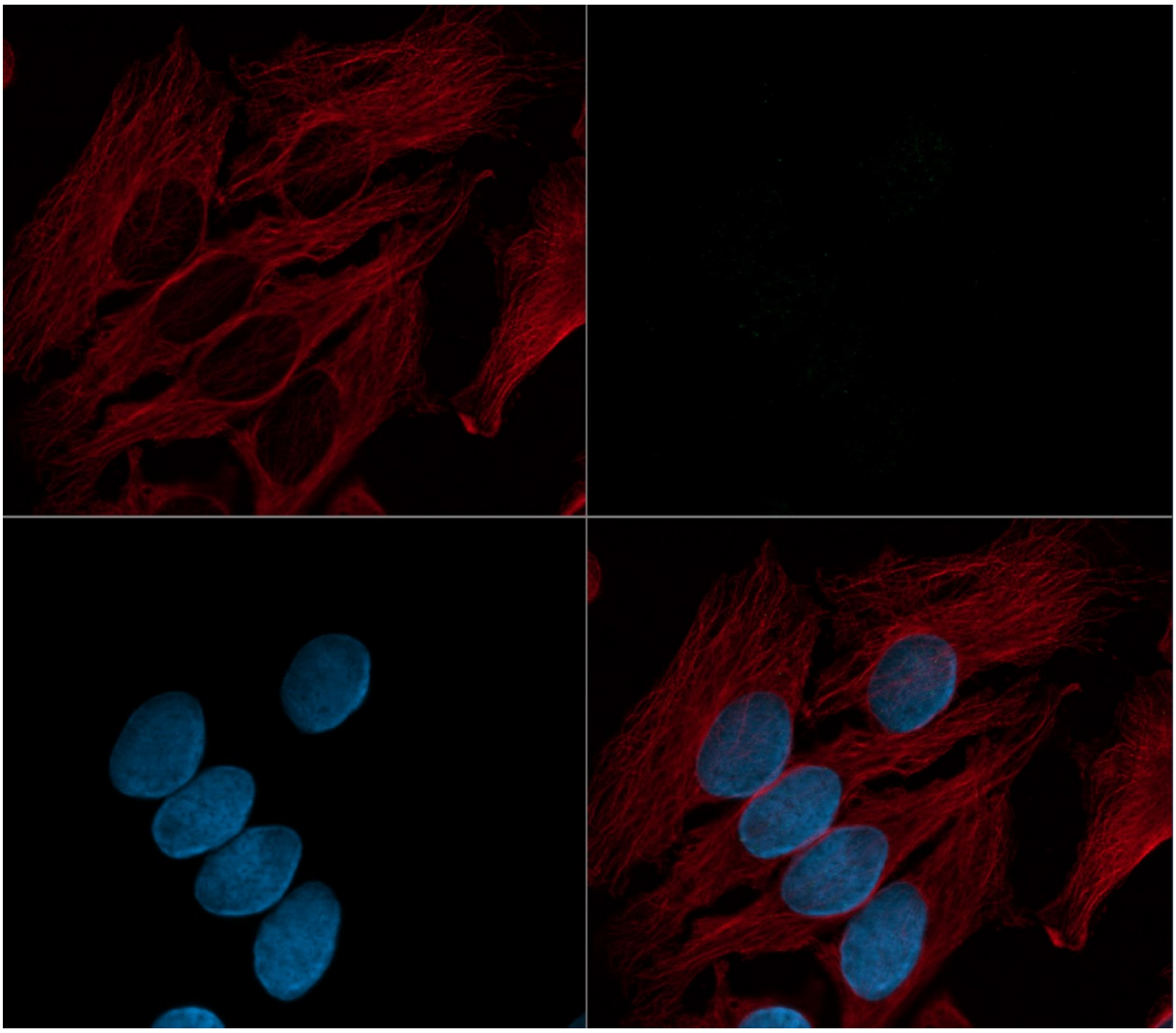
The upper left panel (A) displays the X-Y and X-Z views, emphasizing individual foci selected using the STORM Imaging Methodology, which is depicted in the lower left corner (Whole Nucleus), capturing the entire nucleus. The middle left panels (B and C) illustrate the "Doublet" and "Triplet" signals in the X-Y view, respectively. Meanwhile, the upper right panel (D) presents the X-Y view, and the lower right panel (E) shows the X-Z view, both providing volumetric measurements of the "Triplet" image. Notably, these measurements reveal some unresolved yet interesting structures.

Image by S. Thakur

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	80
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0021781FB8B968468CE709A743603C435B4F3761F861C110AC0E75FCCBE5D055
Method PDF SHA-256	00F373ABE0B235540110E90900E6686A3479AEA38DD98378B7C922B947D5500C

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	41

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	950 X 832
Image Size (Scaled)	135.71µm X 118.86µm
Bit Depth	14 Bit
Image Center Position	X: 95.43mm, Y: 54.48mm
Stage Position	X: 95.43mm, Y: 54.48mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	38 HE eGFP | 50 Cy5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 655 | 335 - 383
Filter Emission Wavelength	500 -550 | 665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @6.0% | X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	200ms | 60ms | 20ms
Excitation Wavelength	493 | 663 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.00µm | 1.30µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction 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 RAD51 Recombinant Monoclonal, IgG (BLR017M)
Cat ID# - PA-RR 000002

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

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

Rad51 is a critical protein in the homologous recombination (HR) process, which is a crucial mechanism for repairing DNA double-strand breaks (DSBs) and maintaining genomic stability. Rad51 foci formation in response to DNA damage is a hallmark of the cellular DNA repair process, particularly Homologous Recombination (HR). When cells encounter DNA Double-Strand Breaks (DSBs) or other forms of DNA damage, Rad51 proteins assemble into discrete nuclear foci at the damage sites. Localization and visualization of Rad51 aggregate via immunofluorescent applications allow for the study of Rad51 response kinetics in response to damage, thereby making the study of Rad51 foci formation critical to the study of the cellular response to DNA damage and how it plays a crucial role in the repair process. Rad51 is essential to recognizing DNA damage, activating the Homologous Recombination (HR) pathway, Rad51 filament formation, and DNA repair and resolution...

Method

DNA damage was NOT induced.

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human RAD51 Recombinant Monoclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 950 X 832
Image Size (Scaled) = 135.71µm X 118.86µm
Bit Depth = 14 Bit
Image Center Position = X: 95.43mm, Y: 54.48mm
Stage Position = X: 95.43mm, Y: 54.48mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 -550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 200ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.0%
Exposure Time = 60ms
Excitation Wavelength = 663
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30µm
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 @8.0%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode “Optical Sectioning” - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

Summary

The control images indicate that, in the absence of Etoposide-induced DNA damage, there is a significant reduction in the presence of RAD51 in both the nucleus and the extranuclear space. In contrast, the complete dataset for RAD51 demonstrates a strong nuclear aggregate signal after Etoposide induces DNA damage.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RR-000002
Stage	Control
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	41
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	C96D126564E57160AAE3D0800FC04AF3D2C619BA59209BC57A33D471152C2482
Method PDF SHA-256	B17A5155E8F14536F3B23A169BA47315D03BF520D2813CD53544F97CB23AD541