

IDENTIFYN RESOLUTION STEPDOWN

BRCA1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555

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

8

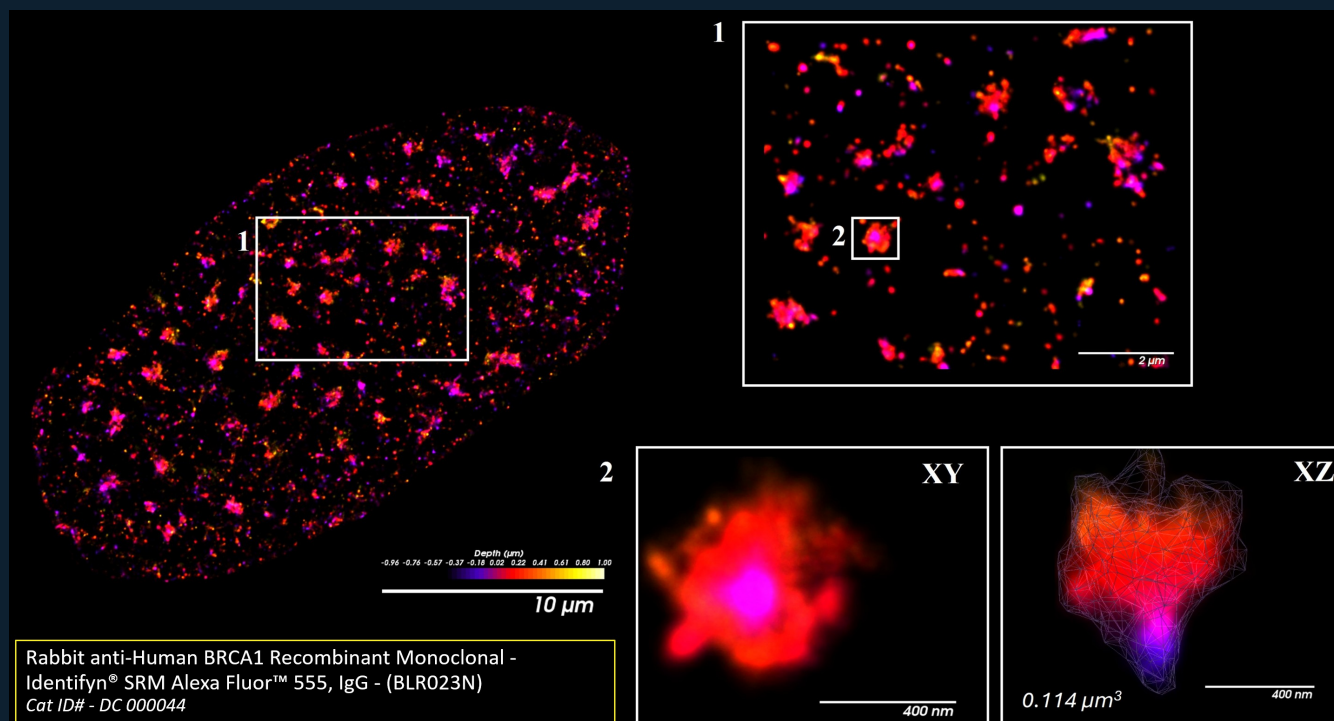
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000044 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for BRCA1, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

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

BENNETT ASSESSMENT

The preserved DC-000044 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000044 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 750	2; AF532-49014; AF405-49027; 515 - 545; 372 - 408; 555 - 595; 417 - 445; 553
Widefield SIM — Apotome	405/DAPI; 555	2; AF532-49014; AF405-49027; 515 - 545; 372 - 408; 555 - 595; 417 - 445; 553
Confocal	405/DAPI; 488; 555	2; None; 561 nm @0.07%; 405 nm @ 0.9%; 553; 401; 568; 422
Airyscan	405/DAPI; 488; 555	2; None; 561 nm @0.3%; 405 nm @ 1.0%; 553; 401; 568; 422
SIM	405/DAPI; 488; 555	2; 3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820 #2-SR; 561
SIM ²	405/DAPI; 488; 555	2; 3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820 #2-SR; 561
Single-molecule STORM	555	Both Channels

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 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 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 μm X 0.110 μm ; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150ms; 300ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%; X-Cite Xylis Lamp Intensity @30%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 32.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 555; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Z-Stack: 46 Slices (4.5 μm); Channels: 2; Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm ; Image size (Pixels): 625 X 593; 343 X 263; Image Size (Pixels): 625 X 593; 343 X 263; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 Mono. Timing: Exposure Time: 100ms; 300ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; X-Cite Xylis Lamp Intensity @30%. Optical/scan: Effective NA: 1.25; 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 43.

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.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 161 Slices (4.8 μm); Channels: 2; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 1121 X 1121; Image Size (Pixels): 1121 X 1121; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.30 μs ; Frame Time: 3.69 s. Illumination: Laser Wavelength: 561 nm @0.07%; 405 nm @ 0.9%. Optical/scan: Pinhole: 1.00 AU / 55 μm ; 1.00 AU / 41 μm ; Scan Zoom: 2.0; LSM Scan Speed: 12; 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; LSM Plus Mode: 7.8 (3D, Auto); 6.6 (3D, Auto). Structured source metadata values: 49.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 141 Slices (4.2 μm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1566 X 1566; 542 X 489; Image Size (Pixels): 1566 X 1566; 542 X 489; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66 μs ; Frame Time: 7.82 s. Illumination: Laser Wavelength: 561 nm @0.3%; 405 nm @ 1.0%. Optical/scan: Pinhole: 5.00 AU / 272 μm ; 5.00 AU / 214 μm ; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 8.4 (3D, Auto). Structured source metadata values: 53.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye: Acquisition: Z-Stack: 104 Slices (3.09 μ m); Channels: 2; 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1924 X 1468; 449 X 420; Image Size (Pixels): 1924 X 1468; 449 X 420; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @2.00%; 405 nm @4.00%; Illumination Wavelength: 561; 405. 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); View Angle 35°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 61.

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; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 86 Slices (2.64 μ m); Channels: 2; 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 120 X 123; Image Size (Pixels): 120 X 123; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.33 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @2.00%; 405 nm @3.00%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 54.

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; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 8

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected.

Structured source metadata values: 81.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000044 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.
- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 μ m X 0.03530 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1566 X 1566; Image Size (Scaled)=55.27 μ m X 55.27 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=449 X 420; Image Size (Scaled)=9.48 μ m X 8.87 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=120 X 123; Image Size (Scaled)=2.53 μ m X 2.60 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.13%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "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 EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

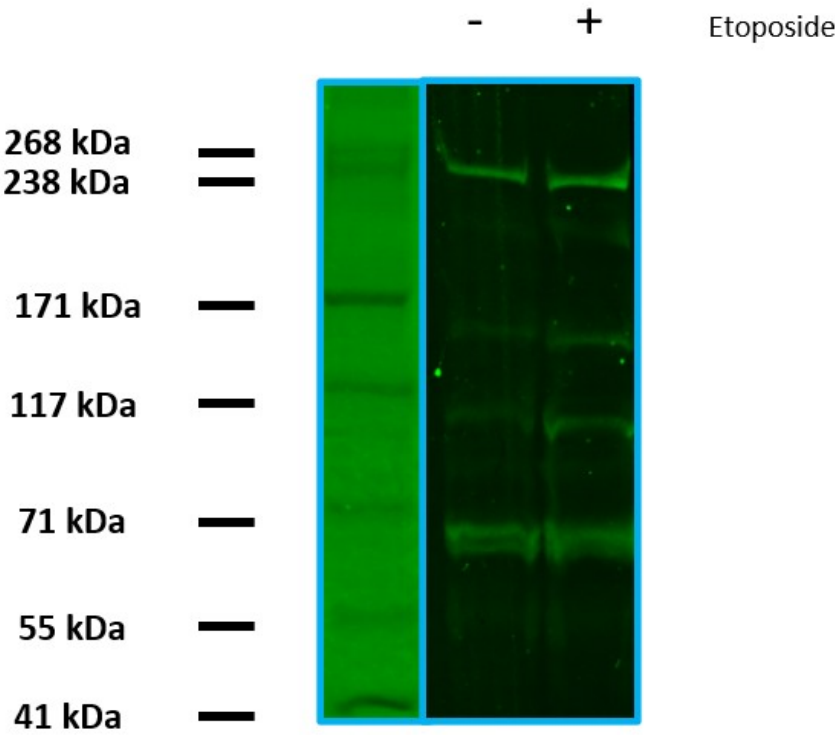
This dossier preserves the complete available evidence progression for DC-000044. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HEK293
DETECTED DYES	555
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	572±28nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Expected Molecular Weight: 220 kDa

HEK293 cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer and then centrifuged to pellet down the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 3 to 8% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. The Thermo Fisher's, HiMark™ Pre-stained Protein Standard, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG (BLR023N) for 2 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence

Laser = 532nm

Emission Filter = 572±28nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

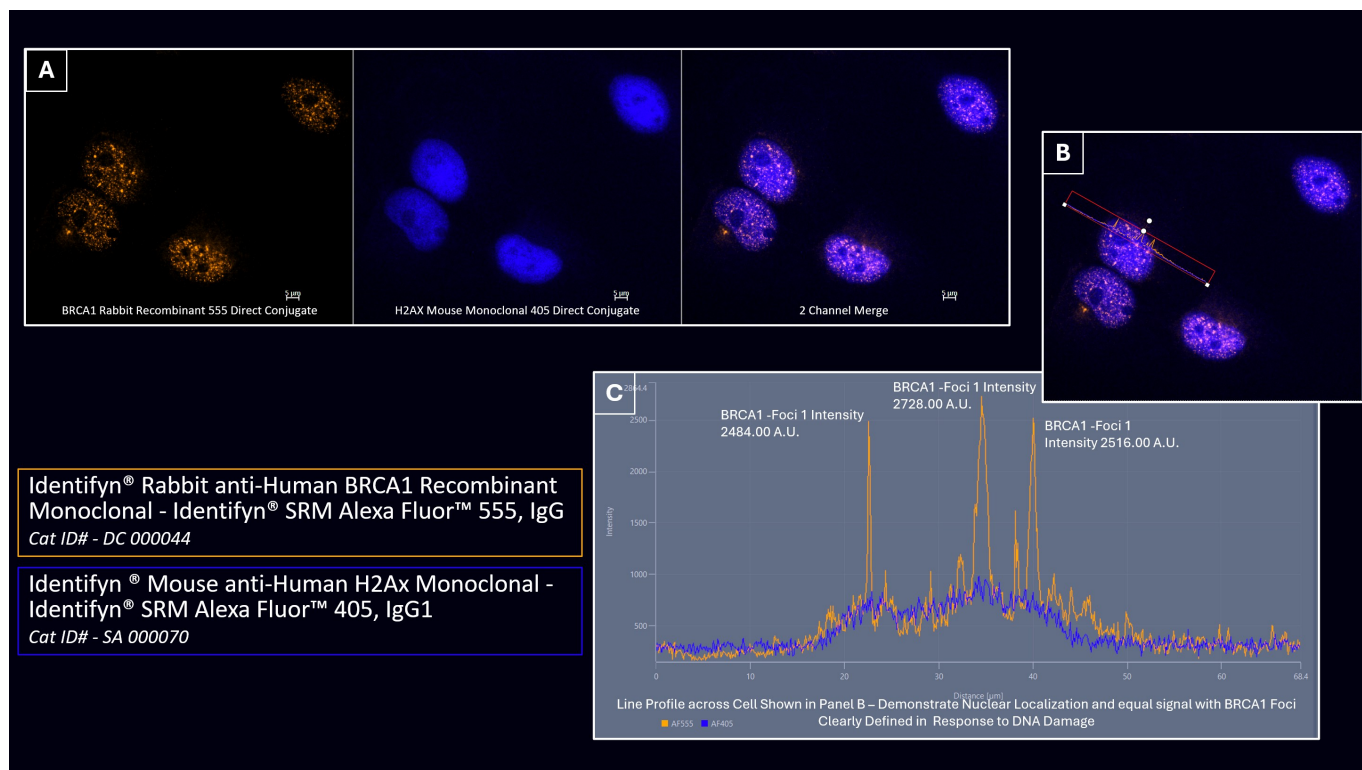
Image: K. Small

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SOURCE PROVENANCE

Catalog	DC-000044
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HEK293
Image SHA-256	8503D4BDEBA097BA6DC8923AB086FA4C01B06F0388F07A52ED27E1DFD31C3AB8
Method PDF SHA-256	FF546EC84B092EDE0392D16DAE874A16EAF43A06978B206FF550412F6B766738

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 750
METADATA VALUES	32

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 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 μm X 0.110 μm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 μm X 112.59 μm
Bit Depth	14 Bit
Image Center Position	X: 0.00 μm , Y: 0.00 μm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	AF532-49014 AF405-49027
Beam Splitter	550 412
Filter Excitation Wavelength	515 - 545 372 - 408
Filter Emission Wavelength	555 - 595 417 - 445
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15% X-Cite Xylis Lamp Intensity @30%
Exposure Time	150ms 300ms
Excitation Wavelength	553 401
Emission Wavelength	568 422
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.88 μm 0.65 μm
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 0.0 and Max 68.4), Y (Min 151 and Max 2864)

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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7)
Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 -(1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110 µm X 0.110 µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18 µm X 112.59 µm

Bit Depth = 14 Bit

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

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

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 @15%

Exposure Time = 150ms

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 0.88µm

Binning Mode = 2,2

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @30%
 Exposure Time = 300ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.65µm
 Binning Mode = 2,2

Split View - Panel A

The left panel features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N); the middle panel demonstrates the Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), and the right panel shows the merged image, including both channels. Noting the strong colocalization within the nuclei by both protein conjugates in response to DNA damage.

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1
 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 68.4), Y (Min 151 and Max 2864)

This panel also measures two BRCA1 DNA-damage-induced foci/aggregates, demonstrating signal across the three chosen aggregate or foci signals.

Image Correction -

None

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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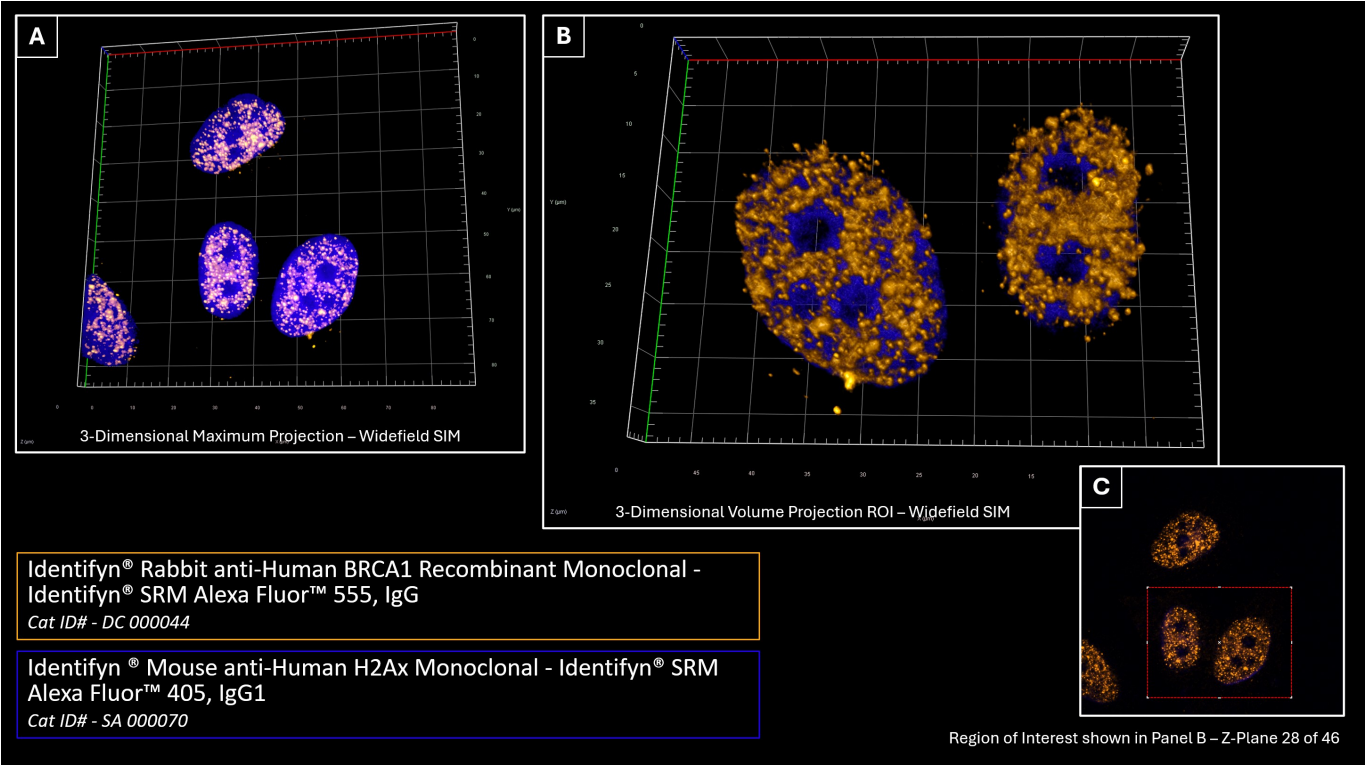
SOURCE PROVENANCE

Catalog	DC-000044
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	32

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2425456DE81D5BBBBB273AE5E5222ADC6C0671ACA4381D56A76BAE9F81C31ED0A
Method PDF SHA-256	B8037CF42FC7BD711EB051FC5445DEB7BA74EA6D86F326A0349A21A959C2B819

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
METADATA VALUES	43

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	46 Slices (4.5 µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100µm
Image size (Pixels)	625 X 593 343 X 263
Image Size (Scaled)	89.29 µm X 84.71 µm 49.00 µm X 37.57 µm
Bit Depth	14 Bit
Image Center Position	X: 91.99 µm, Y: 50.55 µm
ROI Center Offset	X: 91.99 µm, Y: 50.55 µm
Reflector	AF532-49014 AF405-49027
Beam Splitter	550 412
Filter Excitation Wavelength	515 - 545 372 - 408
Filter Emission Wavelength	555 - 595 417 - 445
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @30%
Exposure Time	100ms 300ms
Excitation Wavelength	553 401
Emission Wavelength	568 422
Effective NA	1.25 1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.10µm 0.82µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 12% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7)
Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 46 Slices (4.5 μ m)

Channels = 2

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m

Image size (Pixels) = 625 X 593

Image Size (Scaled) = 89.29 μ m X 84.71 μ m

Bit Depth = 14 Bit

Image Center Position = X: 91.99 μ m, Y: 50.55 μ m

ROI Center Offset = X: 91.99 μ m, Y: 50.55 μ m

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

Z-Stack = 46 Slices (4.5 μ m)

Channels = 2

Scaling (Per Pixel) = 0.143 μ m X 0.143 μ m X 0.100 μ m

Image size (Pixels) = 343 X 263

Image Size (Scaled) = 49.00 μ m X 37.57 μ m

Bit Depth = 14 Bit

Image Center Position = X: 91.99 μ m, Y: 50.55 μ m

ROI Center Offset = X: 91.99 μ m, Y: 50.55 μ m

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 @10%
 Exposure Time = 100ms
 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

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @30%
 Exposure Time = 300ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 0.82µm
 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 12% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Volume - Panel B

3D Volume Projection = Precise

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

Background = Black

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

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

Separation 5% (deselected), Parallax Shift 0% (deselected)

Region of Interest (ROI) - Panel C

Z-Plane 28 of 46, noted by red outline clipped from Zeiss Zen

Image Correction -

None

Image by E.F.Simon

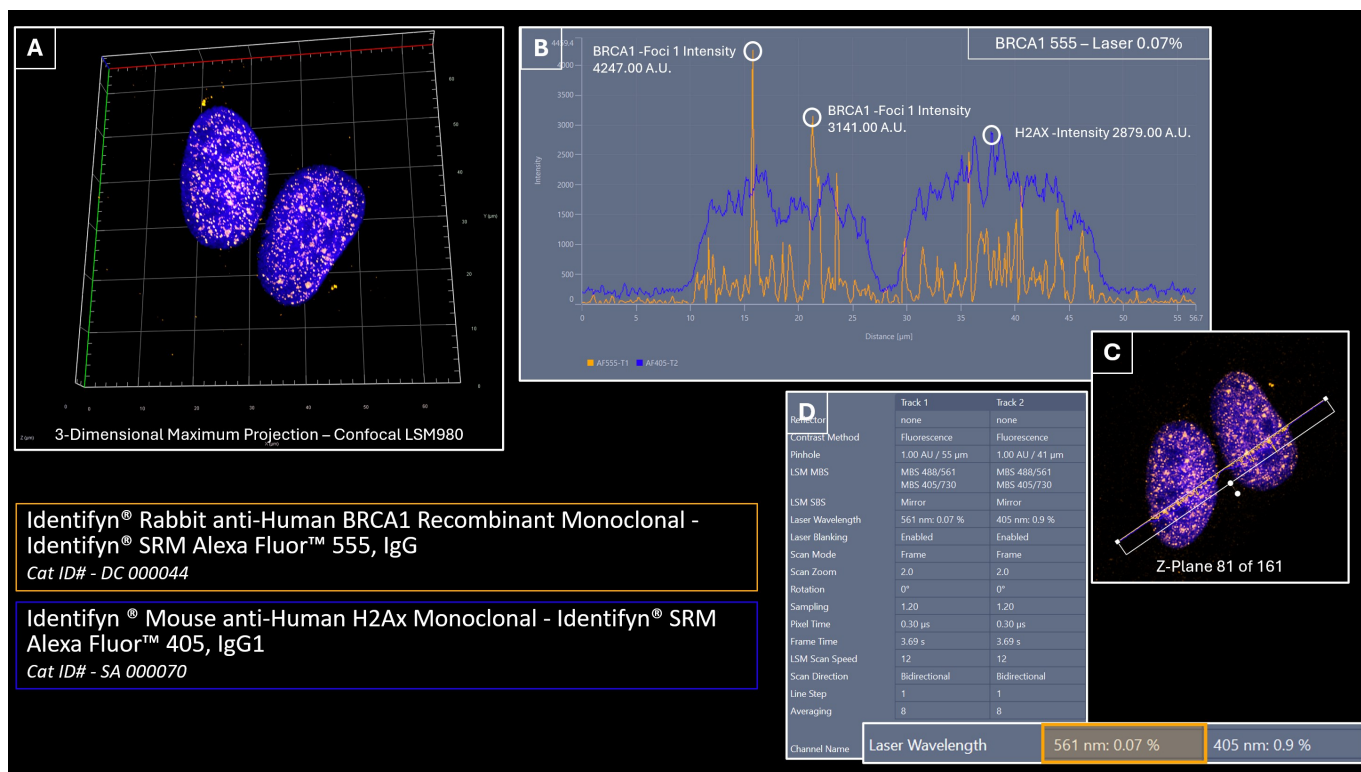
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000044
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	43
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2EC9798A96FF5D0B2A417CE42BEE2B5BCEE7907F726976D8EE168AF30CF4091E
Method PDF SHA-256	12D8FFE843B810A24581805E6232E867C98F72BB74ECD689EDC3DCD99B475C57

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	49

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	161 Slices (4.8 μm)
Channels	2
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	1121 X 1121
Image Size (Scaled)	65.93 μm X 65.93 μm
Bit Depth	16 Bit
Image Center Position	X: 74.02 mm, Y: 50.95 mm
Stage Position	X: 74.02 mm, Y: 50.95 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 55 μm 1.00 AU / 41 μm
LSM MBS	MBS 488/561, 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @0.07% 405 nm @ 0.9%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	1.20
Pixel Time	0.30 μs
Frame Time	3.69 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	553 401
Emission Wavelength	568 422
Effective NA	1.4
Detection Wavelength	550 - 600 395 - 460
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V 550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.8 (3D, Auto) 6.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness100%, 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 1.0, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 56.7), Y (Min 0 and Max 4459)

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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

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Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 161 Slices (4.8 µm)

Channels = 2

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

Image Size (Pixels) = 1121 X 1121

Image Size (Scaled) = 65.93 µm X 65.93 µm

Bit Depth = 16 Bit

Image Center Position = X: 74.02 mm, Y: 50.95 mm

Stage Position = X: 74.02 mm, Y: 50.95 mm

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

Single Plane (2D) - Z-plane 81 of 161 total - Panels B and C (Line Profile Measurements)

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 55 µm
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 561 nm @0.07%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.30 µs
 Frame Time = 3.69 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 550 - 600
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 7.8 (3D, Auto)

405 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 41 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @ 0.9%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.30 μ s
 Frame Time = 3.69 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 395 - 460
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.6 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness100%, 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 - Panel B

Profile Definition = Stroke Thickness 1.0, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 56.7), Y (Min 0 and Max 4459)

This line-profile analysis measures the intensity of the BRCA1 555 Direct Conjugate at two defined DNA-damage foci and simultaneously quantifies the H2AX 405 Direct Conjugate signal. Notably, the BRCA1 555 Direct Conjugate was imaged at only 0.07% power on the 561-nm laser, underscoring the reagent's exceptionally high brightness. The line profiles for both BRCA1 and H2AX clearly delineate nuclear localization, further confirming the accurate intranuclear positioning of the DNA-damage response signals.

Zeiss Zen Settings - Panel D, Laser Power Input

A clipped image from the Zeiss ZEN interface is included to provide clear documentation of the image acquisition parameters used for this confocal experiment. These settings demonstrate both the rigor of our acquisition workflow and the exceptionally low laser power required to achieve high-signal images with Identifyn's fluorescent direct conjugates. The recorded excitation powers, detector settings, and channel configurations underscore the superior brightness and efficiency of Identifyn reagents under standard confocal imaging conditions.

Image Corrections -

None

Image by J.K. Bennett

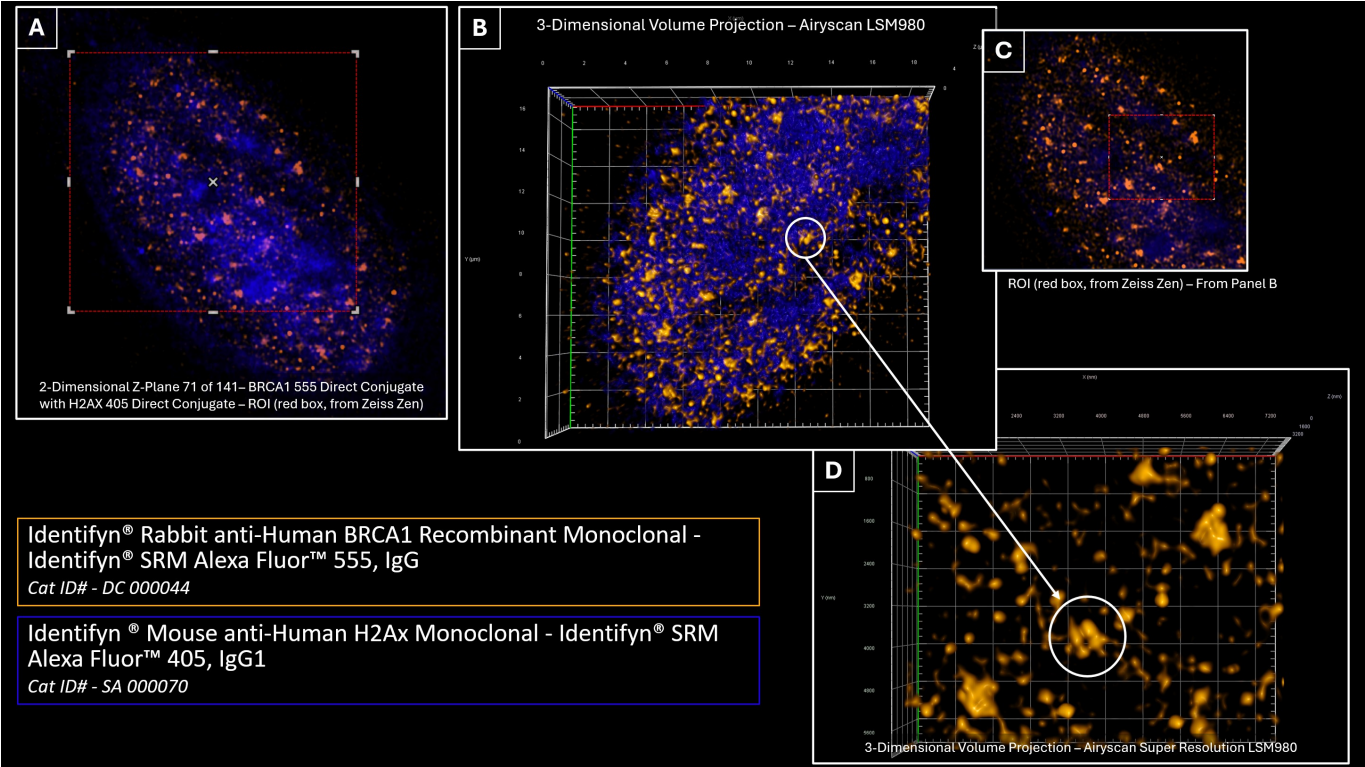
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000044
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	47F997D9A0E9621105C51CDA2F4D9EF628552DA6BA65ECEDCC86C660DF3D055F
Method PDF SHA-256	5AA6630883B6115426F8EB931D75AF0D852043ED2E9D8E820B444BCEE02DD12D

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	53

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	141 Slices (4.2 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1566 X 1566 542 X 489 216 X 173
Image Size (Scaled)	55.27 µm X 55.27 µm 19.13 µm X 17.26 µm 7.62 µm X 6.11 µm
Bit Depth	16 Bit
Image Center Position	X: 74.07 mm, Y: 50.23 mm
Stage Position	X: 74.07 mm, Y: 50.23 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 272 µm 5.00 AU / 214 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 525 SBS SP 505
Laser Wavelength	561 nm @0.3% 405 nm @ 1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66 µs
Frame Time	7.82 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	553 401
Emission Wavelength	568 422
Effective NA	1.4
Detection Wavelength	530 - 582 424 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	9.6 (3D, Auto)
LSM Plus Mode	8.4 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7)
Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A - Z-plane 71 of 141

Z-Stack = 141 Slices (4.2 µm)

Channels = 2

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

Image Size (Pixels) = 1566 X 1566

Image Size (Scaled) = 55.27 µm X 55.27 µm

Bit Depth = 16 Bit

Image Center Position = X: 74.07 mm, Y: 50.23 mm

Stage Position = X: 74.07 mm, Y: 50.23 mm

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

Z Stack - (3D) - Panel B

Z-Stack = 141 Slices (4.2 µm)

Channels = 2

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

Image Size (Pixels) = 542 X 489

Image Size (Scaled) = 19.13 µm X 17.26 µm

Bit Depth = 16 Bit

Image Center Position = X: 74.07 mm, Y: 50.23 mm

Stage Position = X: 74.07 mm, Y: 50.23 mm

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

Z Stack - (3D) - Panel D

Z-Stack = 141 Slices (4.2 µm)

Channels = 2

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

Image Size (Pixels) = 216 X 173

Image Size (Scaled) = 7.62 µm X 6.11 µm

Bit Depth = 16 Bit

Image Center Position = X: 74.07 mm, Y: 50.23 mm

Stage Position = X: 74.07 mm, Y: 50.23 mm

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

555 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 272 µm

LSM MBS = MBS 488/561, 405/730

LSM SBS = SBS LP 525

Laser Wavelength = 561 nm @0.3%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.4

Rotation = 0°

Sampling = 2.00

Pixel Time = 0.66 µs

Frame Time = 7.82 s

LSM Scan Speed = 7

Scan Direction = Bidirectional

Line Step = 1

Averaging = 4

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Detection Wavelength = 530 - 582

Imaging Device = Airyscan

Detector Type = GaAsP-PMT

Detector Gain = 800 V

Detector Offset = 0

Detector Digital Gain = 1.0

Super Resolution= 9.6 (3D, Auto)

405 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 214 µm
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = SBS SP 505
 Laser Wavelength = 405 nm @ 1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66 µs
 Frame Time = 7.82 s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 424 - 477
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 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 B

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

3D Image Processing - Panel C

3D Volume Projection = Precise

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

Background = Black

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

Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

None

Image by J.K. Bennett

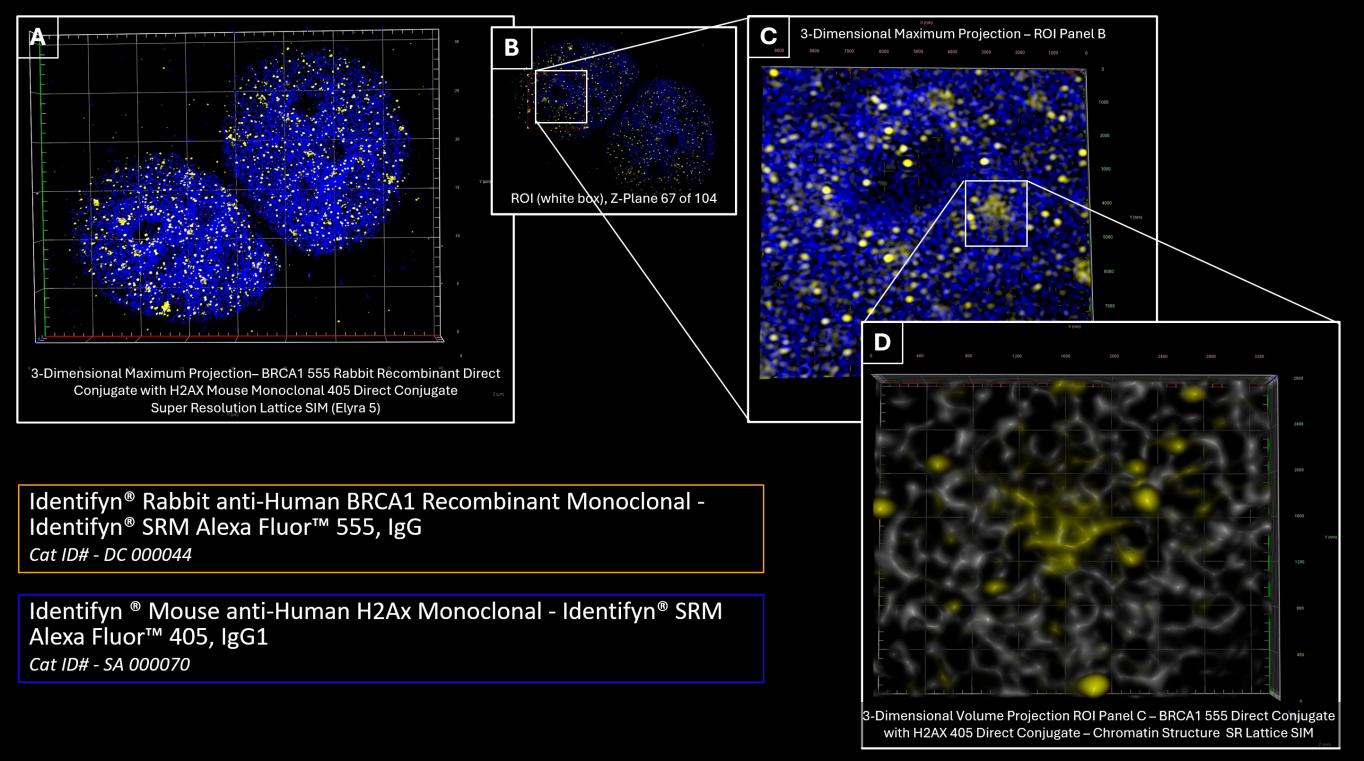
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000044
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	79F63BD5B3E7A86359161C2B32B499BA83DBF8208169D9BD328750615BA8802E
Method PDF SHA-256	9E0409D9E89F1F130E050E90E161B57BF94EE9A49D6114CF8F36954E930C9C54

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	61

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	104 Slices (3.09 μm)
Channels	2 3
Scaling (Per Pixel)	0.02111 μm X 0.02111 μm X 0.03000 μm
Image Size (Pixels)	1924 X 1468 449 X 420 159 X 134
Image Size (Scaled)	40.62 μm X 30.09 μm 9.48 μm X 8.87 μm 3.36 μm X 2.83 μm
Bit Depth	16 Bit
Image Center Position	X: 61.34 mm, Y: 36.40 mm
Stage Position	X: 61.34 mm, Y: 36.40 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms 50 ms
Depth of Focus	0.92 μm 0.69 μm
Binning Mode	2,2
Laser Wavelength	561 nm @2.00% 405 nm @4.00%
Frame Time	1.33 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 μm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.2694 (555), 10.1755 (405)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7)
Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 104 Slices (3.09 μ m)

Channels = 2

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

Image Size (Pixels) = 1924 X 1468

Image Size (Scaled) = 40.62 μ m X 30.09 μ m

Bit Depth = 16 Bit

Image Center Position = X: 61.34 mm, Y: 36.40 mm

Stage Position = X: 61.34 mm, Y: 36.40 mm

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

Single Plane (2D) - Z-Plane 67 of 104 - Panel B

Z Stack - (3D) - Panel C

Z-Stack = 104 Slices (3.09 μ m)

Channels = 2

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

Image Size (Pixels) = 449 X 420

Image Size (Scaled) = 9.48 μ m X 8.87 μ m

Bit Depth = 16 Bit

Image Center Position = X: 61.34 mm, Y: 36.40 mm

Stage Position = X: 61.34 mm, Y: 36.40 mm

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

Z Stack - (3D) - Panel D

Z-Stack = 104 Slices (3.09 µm)

Channels = 2

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

Image Size (Pixels) = 159 X 134

Image Size (Scaled) = 3.36 µm X 2.83 µm

Bit Depth = 16 Bit

Image Center Position = X: 61.34 mm, Y: 36.40 mm

Stage Position = X: 61.34 mm, Y: 36.40 mm

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

555 -

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 = 561

Channel Name = T1-Axiocam 820 #2-SR

Excitation Wavelength = 561

Emission Wavelength = 597

Effective NA = 1.4

Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800

Imaging Device = Axiocam 820 #2

Camera Adapter = 1X

Exposure Time = 120 ms

Depth of Focus = 0.92 µm

Binning Mode = 2,2

Laser Wavelength = 561 nm @2.00%

Frame Time = 1.33 s

Scan Direction = Unidirectional

Grating = 36.5 µm grating

405 -

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 = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @4.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = N/A
 Channels = 3
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 11.2694 (555), 10.1755 (405)
 Fitting = Fast Fit
 Normalization = Auto

3D Image Processing - Panel A

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

3D Image Processing - Panel C

3D Maximum Projection = Precise

Appearance = Threshold 4% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 20%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Volume Projection = Precise

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

Background = Black

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

Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

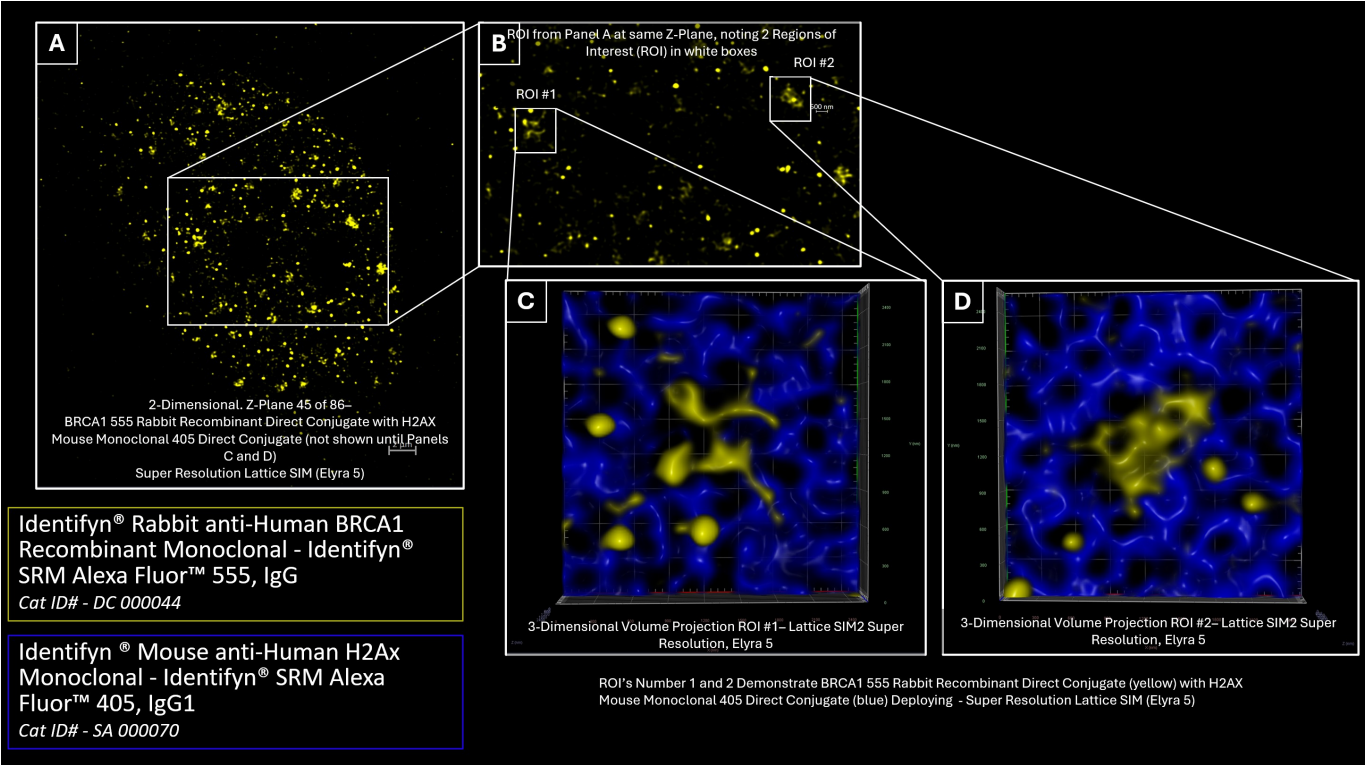
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000044
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	61
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0567B040FA564041AEDB6F7E9DDD17AB5850484FBF8693B184646588B9DBE131
Method PDF SHA-256	3FBD04E5B2D9C856CAA0C1343AB0FAE39FB332167F859B652B6F252A4DE253B5

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	54

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	86 Slices (2.64 µm)
Channels	2 3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	120 X 123
Image Size (Scaled)	2.53 µm X 2.60 µm
Bit Depth	16 Bit
Image Center Position	X: 63.34 mm, Y: 31.60 mm
Stage Position	X: 63.34 mm, Y: 31.60 mm
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	561 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120 ms 50 ms
Depth of Focus	0.92 µm 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @2.00% 405 nm @3.00%
Frame Time	1.33 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Algorithm	SIM ²
SIM ² Settings	Standard
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)
Cat ID# - DC 000044

Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7)
Cat ID# - SA 000070

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly double-strand breaks that provide function in the homologous recombination pathway.

BRCA1 also significantly regulates the cell cycle, ensuring cells progress through each phase appropriately. It interacts with various proteins involved in cell-cycle checkpoints, helping prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is important for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mouse anti-Human H2Ax Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG1 - (1H1D9/B7), was then incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-Plane 45 of 186 - Panels A and B

Z Stack - (3D) - Panel C

Z-Stack = 86 Slices (2.64 μ m)

Channels = 2

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

Image Size (Pixels) = 120 X 123

Image Size (Scaled) = 2.53 μ m X 2.60 μ m

Bit Depth = 16 Bit

Image Center Position = X: 63.34 mm, Y: 31.60 mm

Stage Position = X: 63.34 mm, Y: 31.60 mm

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

Z Stack - (3D) - Panel D

Z-Stack = 86 Slices (2.64 μ m)

Channels = 2

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

Image Size (Pixels) = 120 X 123

Image Size (Scaled) = 2.53 μ m X 2.60 μ m

Bit Depth = 16 Bit

Image Center Position = X: 63.34 mm, Y: 31.60 mm

Stage Position = X: 63.34 mm, Y: 31.60 mm

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

555 -

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 = 561
 Channel Name = T1-Axiocam 820 #2-SR
 Excitation Wavelength = 561
 Emission Wavelength = 597
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.92 μ m
 Binning Mode = 2,2
 Laser Wavelength = 561 nm @2.00%
 Frame Time = 1.33 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

405 -

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 = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @3.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM² Processing

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

3D Image Processing - Panel C

3D Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera
 Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera
 Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

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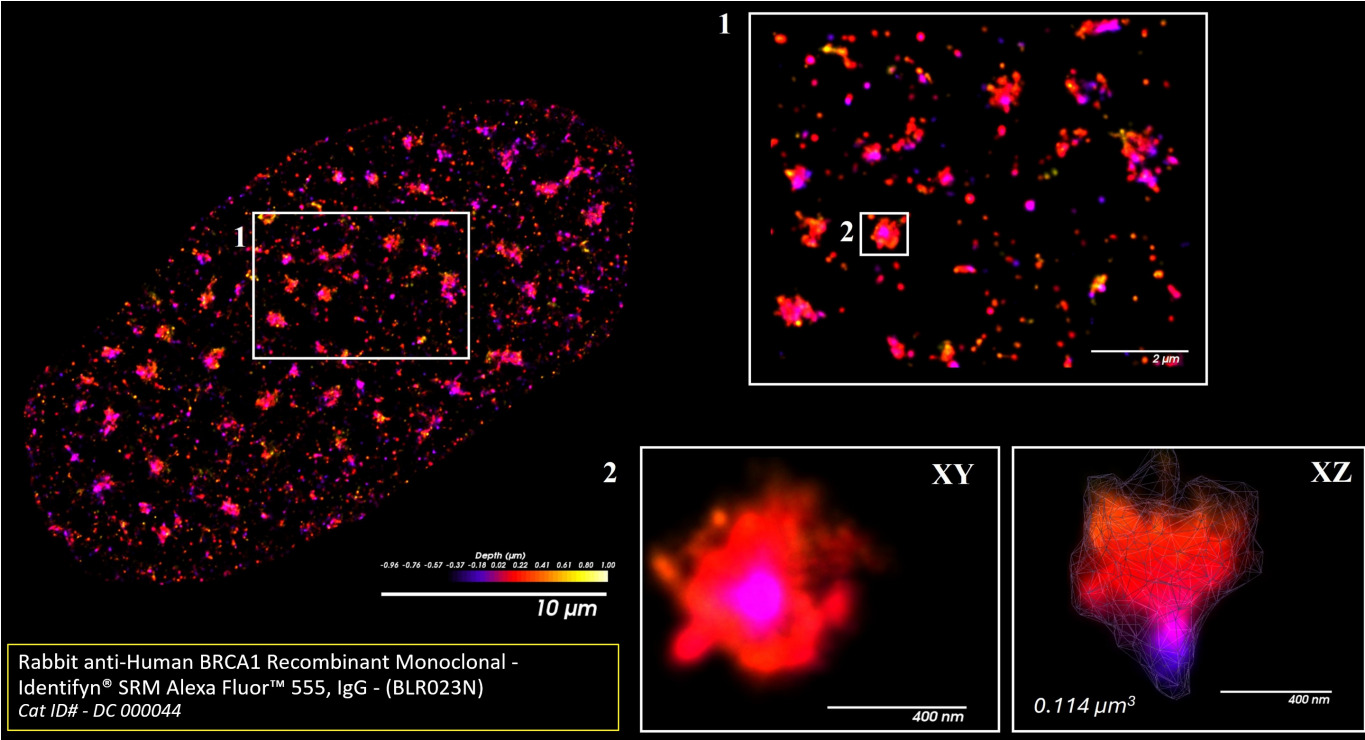
SOURCE PROVENANCE

Catalog	DC-000044
Stage	SIM ²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A8D47B55516251E13DF04318DD88AEE9F801DDDD3D8F9A12BA260AD454029E8A
Method PDF SHA-256	B9BEAD75785CD42CDA2A190905E8C1664C1E2DB87BDC9EBCA57FD2693DF60A3F

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	555
METADATA VALUES	81

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	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: 555nm (635nm Dichroic); First reference probe: 555nm (635nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	182.5µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 555nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 555nm (635nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 555nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 182.5; End: 182.5
SuperRes 555nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 19998
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	50%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 10
Sizing Mode	Radial Precision
Particle size	4nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.06
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	10 Intervals
Pixel Size	30nm
Smoothing	8.00
PSF Z offset	-999 to +999
Radial precision	60
Axial precision	120
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1µm (1000nm)
Maximum Particle Count to Form Cluster	10
Compute Hulls	Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	0.1µm
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.

Bennett Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR023N)

Cat ID# - DC 000044

BRCA1 (Breast Cancer 1) is a protein that plays a crucial role in maintaining the genome's stability and preventing cancer development. BRCA1's primary functions include DNA repair, particularly in response to double-strand breaks, which is a crucial step in the homologous recombination pathway.

BRCA1 also plays a significant role in regulating the cell cycle, ensuring that cells progress through each phase appropriately. It interacts with various proteins involved in cell cycle checkpoints, helping to prevent cells with damaged DNA from dividing. Additionally, BRCA1 acts as a transcriptional co-regulator, influencing the expression of genes involved in various cellular processes, including DNA repair, cell cycle control, and apoptosis. Finally, BRCA1 is involved in modifying the structure of chromatin (chromatin remodeling); this activity helps regulate gene expression and DNA repair processes.

BRCA1 interacts with numerous proteins, forming complexes involved in the aforementioned cellular processes (DNA repair, cell cycle regulation, transcriptional regulation, and chromatin remodeling). Some fundamental protein-protein interactions of BRCA1 include:

BRCA2: BRCA1 forms a complex with BRCA2, which plays a crucial role in homologous recombination-mediated DNA double-strand break repair.

PALB2 (Partner and Localizer of BRCA2): PALB2 binds to both BRCA1 and BRCA2, facilitating the recruitment of BRCA1-BRCA2 complexes to sites of DNA damage and promoting homologous recombination-mediated repair.

RAD51: BRCA1 interacts with RAD51, an essential protein involved in homologous recombination. This interaction promotes the loading of RAD51 onto single-stranded DNA at sites of DNA damage, facilitating the repair process.

BARD1 (BRCA1-Associated RING Domain protein 1): BARD1 forms a heterodimeric complex with BRCA1, and together they possess E3 ubiquitin ligase activity. This complex plays a role in DNA repair and cell cycle regulation.

CtIP (CtBP-interacting protein): BRCA1 interacts with CtIP, which is involved in the initial processing of DNA double-strand breaks to generate single-stranded DNA ends, a crucial step in homologous recombination-mediated repair.

ATM (Ataxia Telangiectasia Mutated): BRCA1 interacts with ATM, a kinase that plays a central role in the DNA damage response pathway. This interaction is essential for activating ATM-mediated signaling in response to DNA damage.

p53: BRCA1 interacts with the tumor suppressor protein p53 and participates in regulating p53-mediated transcriptional responses to DNA damage.

In summary, BRCA1 is essential for maintaining genomic stability and preventing the accumulation of mutations that can lead to cancer development. Mutations in the BRCA1 gene are associated with an increased risk of breast, ovarian, and other cancers, as they impair the protein's ability to carry out its normal functions.

Method

HeLa cells were grown in a μ -Slide 8 Well high Glass-Bottom Ibidi chamber. DNA damage was induced via treatment with 2 μ M 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 BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in Identifyn's® proprietary Wash Buffer followed by the addition of Identifyn's® proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Microscope

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

Calibration -

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

<https://identifyn.com/storm-calibration>

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope configuration

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

Camera Configuration

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

Probe Configuration

Predefined Probe: First probe: 555nm (635nm Dichroic); First reference probe: 555nm (635nm Dichroic)
 Photoactivation/Imaging Laser: 0% Selected
 Exposure Time: 20ms Selected

Experiment Configuration

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

Stage and Piezo Selection

Examination of the Sample

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

Piezo Current: 182.5µm

Probe 1 Laser control 555 nm (635 nm Dichroic)

Widefield 555nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
 Reference Probe 1 Laser control 555nm (635nm Dichroic): Not Selected

Advanced Tools Option

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

Widefield Laser: "Widefield 555nm" 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: 182.5; End: 182.5

Probe 1 Laser control 555 nm (635 nm Dichroic)

SuperRes 555nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control 555 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: 19998

Z Positions: 1 using Steps: 0.2µm (200nm)

Cycles:1

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 555 nm (635 nm Dichroic)

Laser Power: 50%

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16px

Max Frame Accumulation: 1 frame

Max Accumulation Offset: 1px

Fiducial at Max Accumulation: Not selected

Max Particles Per Frame: No Max

Background SLPE Removal: Not Selected

Cutout/Background Removal: Not Selected

Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 10

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Cloud

Sizing Mode: Radial Precision

Particle size: 4nm

Color by: Depth

Color Table: Single

Opacity Mode: Depth; Opacity 1: 0.06

Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)

Time Intervals: 10 Intervals

Pixel Size: 30nm

Smoothing: 8.00

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

PSF Z offset: -999 to +999

Radial precision: 60

Axial precision: 120

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: 0.1µm (1000nm)

Maximum Particle Count to Form Cluster: 10

Compute Hulls: Selected

Compute Density Isosurfaces: Not Selected

Edit Settings: All Probes

Hull Alpha shape Radius: 0.1um

Compute: Selected

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

Image by P. Raut

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SOURCE PROVENANCE

Catalog	DC-000044
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	47B58D1FA81B01AC3608EF539174705FB9E8C2A23A8E6A71F13831A1489CE607
Method PDF SHA-256	CA599B722B45B5EE1EFB4AA3A3549B77D5BDB00F057259A4FCDA75C6C7EC400C