BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

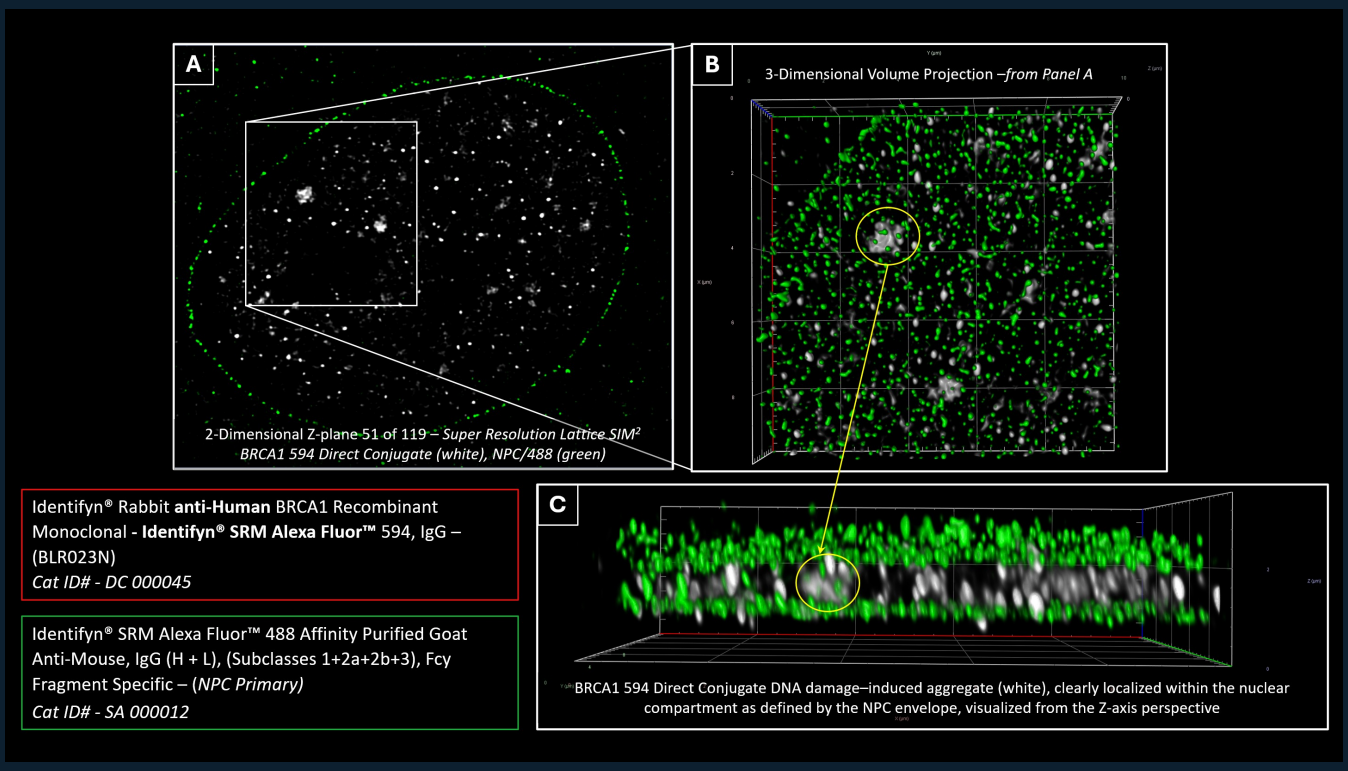
BRCA1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN SIM -> SIM²

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000045 | 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
- 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

BENNETT ASSESSMENT

The preserved DC-000045 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 / NIR-BOUNDED PARTIAL STEPDOWN

The preserved DC-000045 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 45 Texas Red; 38 eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594; 647	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561 nm @0.3%; 488 nm @0.2%; 405 nm @0.2%; 590; 493; 353
Airyscan	405/DAPI; 488; 594; 750	3; None; 561 nm @1.10%; 488 nm @0.10%; 405 nm @0.2%; 590; 493; 353
SIM	405/DAPI; 488; 594	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR; 561; 488
SIM²	405/DAPI; 488; 594	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR; 561; 488

MODALITY ASSESSMENT / PART 1 OF 7

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 594.

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 7

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110µm X 0.110µm; 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: 200 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

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 7

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 89 Slices (8.8 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 716 X 631; 335 X 299; Image Size (Pixels): 716 X 631; 335 X 299; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 200 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 20%; X-Cite Xylis Lamp Intensity @20%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 49.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 594; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 7

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 131 Slices (3.9 µm); Channels: 3; Scaling (Per Pixel): 0.052 µm X 0.052 µm X 0.030 µm; Image size (Pixels): 1101 X 1101; Image Size (Pixels): 1101 X 1101; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.31 µs; Frame Time: 3.63 s. Illumination: Laser Wavelength: 561 nm @0.3%; 488 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 59 µm; 1.00 AU / 50 µm; Scan Zoom: 2.3; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.5 (3D, Auto); 7.9 (3D, Auto). Structured source metadata values: 64.

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; 594.

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 7

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: 130 Slices (3.9 µm) - Panel A; 130 Slices (3.9 µm) - Panel C; Channels: 3; Scaling (Per Pixel): 35.28 nm X 35.28 nm X 30.00 nm; Image size (Pixels): 1503 X 1503; 389 X 337; Image Size (Pixels): 1503 X 1503; 389 X 337; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.69 µs; Frame Time: 7.51 s. Illumination: Laser Wavelength: 561 nm @1.10%; 488 nm @0.10%. Optical/scan: Pinhole: 5.00 AU / 286 µm; 5.00 AU / 250 µm; Scan Zoom: 2.5; 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 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 8.0 (3D, Auto). Structured source metadata values: 62.

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; 594; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 7

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: 119 Slices (3.54 µm); 131 Slices (3.90 µm); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1482 X 1414; 190 X 155; Image Size (Pixels): 1482 X 1414; 190 X 155; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 561 nm @5.00%; 488 nm @2.00%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 20°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 59.

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; 594.

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 7

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: 119 Slices (3.54 µm); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 466 X 474; Image Size (Pixels): 466 X 474; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2; Axiocam 820. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 561 nm @5.00%; 488 nm @2.00%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 13°, Scale Z 73%, 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; 594.

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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000045 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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): 35.28 nm X 35.28 nm X 30.00 nm -> 0.03861 µm X 0.03861 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1503 X 1503; Image Size (Scaled)=58.03 µm X 58.03 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1482 X 1414; Image Size (Scaled)=31.29 µm X 29.85 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=466 X 474; Image Size (Scaled)=9.84 µm X 10.01 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

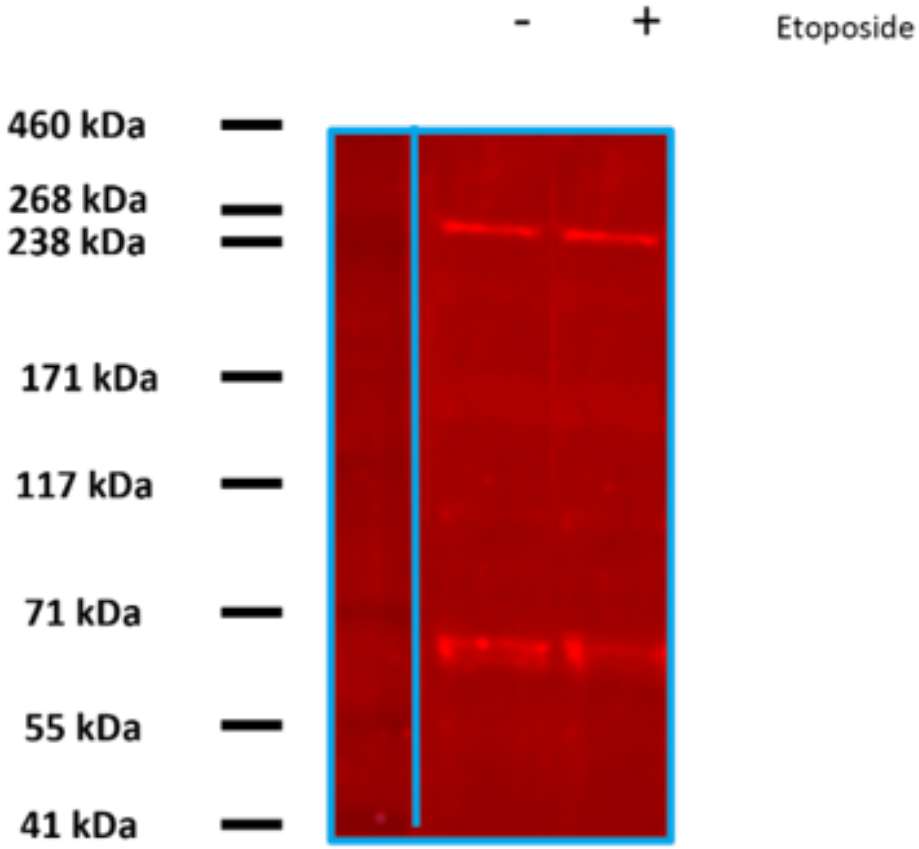
This dossier preserves the complete available evidence progression for DC-000045. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
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	SIM²	ZEISS Elyra	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	594
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	624±40nm
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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Expected Molecular Weight: 220 kDa

HEK293 cells were damaged with 200 µM etoposide for 24 hours. Cells were homogenized using an ultrasonication probe and RIPA lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 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 the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG (BLR023N) for 2 hours, followed by 5 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 = 624±40nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

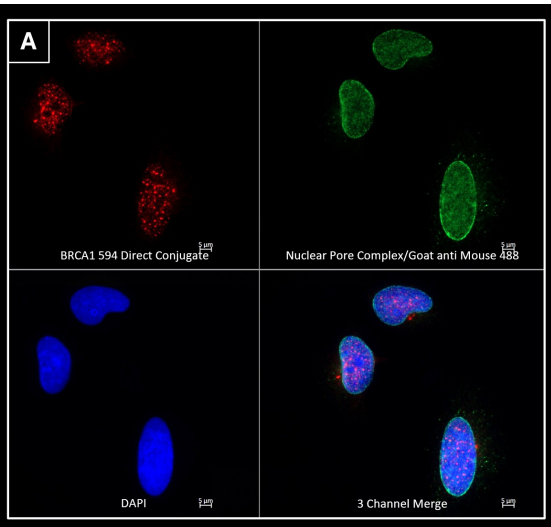
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000045
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	AE2C86C64EA8D88DD16C398C927036656AEE53FE7CB3FACEDDB834C231B3C621
Method PDF SHA-256	E1E5C0ABB060733D15E6169AA5AC123FD53D896FD583E750C651CA513F799BD9

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



B

	Channel 1	Channel 2	Channel 3
Reflector	45 Texas Red	38 eGFP	49 DAPI
Beam Splitter	585	495	395
Filter Ex. Wavelength	540 - 580	450 - 490	335 - 383
Filter Em. Wavelength	593 - 668	500 - 550	420 - 470
Contrast Method	Fluorescence	Fluorescence	Fluorescence
Light Source	X-Cite Xylis Lamp	X-Cite Xylis Lamp	X-Cite Xylis Lamp
Light Source Intensity	20.00 %	20.00 %	10.00 %
Channel Name	AF594	AF488	DAPI
Channel Description			
Dye Name	AF594	AF488	DAPI
Channel Color			
Excitation Wavelength	590	493	353
Emission Wavelength	618	517	465
Effective NA	1.4	1.4	1.4
Imaging Device	Axiocam 705	Axiocam 705	Axiocam 705
Camera Adapter	1x Camera Adapter	1x Camera Adapter	1x Camera Adapter
Exposure Time	200 ms	200 ms	20 ms
Depth of Focus	0.96 µm	0.80 µm	0.72 µm
Binning Mode	2,2	2,2	2,2

Identifyn® Rabbit **anti-Human** BRCA1 Recombinant Monoclonal - **Identifyn® SRM Alexa Fluor™** 594, IgG – (BLR023N)
Cat ID# - DC 000045

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

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	37

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	45 Texas Red | 38 eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	200 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96µm | 0.80µm | 0.72µm
Binning Mode	2,2

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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110µm X 0.110µm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18µm X 112.59µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00µm, Y: 0.00µm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 200 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.96µm
Binning Mode = 2,2

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Split View - Panel A

The upper left panel is Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR023N) after DNA damage, the top-right panel features Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, and the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

Zeiss Zen Acquisition Settings - Panel B

Displays the settings used for image acquisition within Zeiss Zen software. The Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR023N), Direct Conjugate was captured using 20% LED input with a 200 ms exposure time. In comparison, the Identifyn® SRM Alexa Fluor™ 488 was captured with 20% LED input with a 200 ms exposure time. The image settings indicate that the 594 direct conjugate is bright and requires low power and short exposure times, and that it was captured using the same settings as the NPC counterstained with a 488 secondary.

Post Processing

None

Image Correction

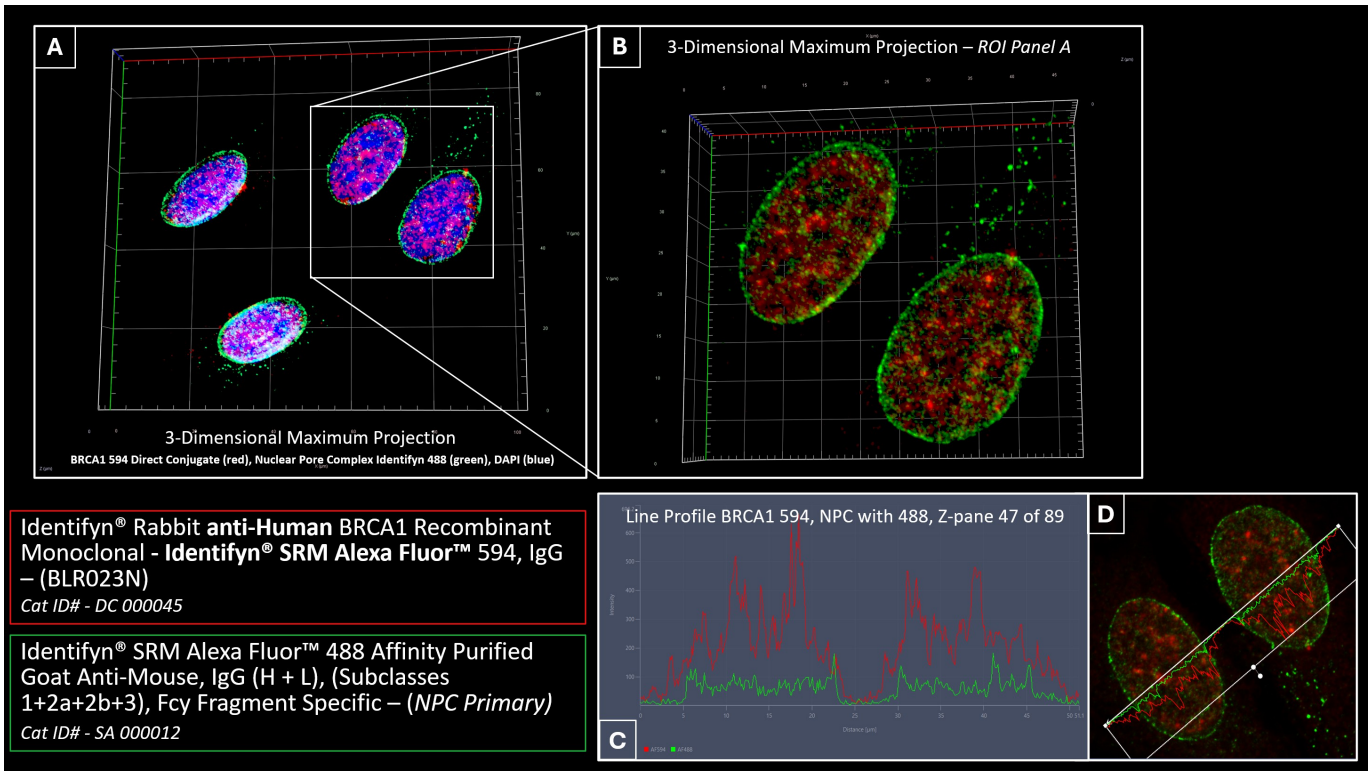
None

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

SOURCE PROVENANCE

Catalog	DC-000045
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	572A2489F9F5FC6E2C8124206282C69B11A6FA79735DF0354AC332A799F795FC
Method PDF SHA-256	5442E4B0D5581D3401A7B64E4C3C72D565802417A271B39FC18375ABFF43D74E

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647
METADATA VALUES	49

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	89 Slices (8.8 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	716 X 631 | 335 X 299
Image Size (Scaled)	102.29 µm X 90.14 µm | 47.86 µm X 42.71 µm
Bit Depth	14 Bit
Image Center Position	X: 84.02 mm, Y: 47.39 mm
Stage Position	X: 84.02mm, Y: 47.39 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	45 Texas Red | 38 HE eGFP | 49 DAPI
Beam Splitter	585 | 495 | 395
Filter Excitation Wavelength	540 - 580 | 450 - 490 | 335 - 383
Filter Emission Wavelength	593 - 668 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @ 20% | X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	200 ms | 20 ms
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.20 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
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)

FIELD	SOURCE-DOCUMENTED VALUE
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 51.1), Y (Min 1 and Max 696)

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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

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 = 89 Slices (8.8 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 716 X 631
Image Size (Scaled) = 102.29 µm X 90.14 µm
Bit Depth = 14 Bit
Image Center Position = X: 84.02 mm, Y: 47.39 mm
Stage Position = X: 84.02mm, Y: 47.39 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 89 Slices (8.8 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 335 X 299
Image Size (Scaled) = 47.86 µm X 42.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 84.02 mm, Y: 47.39 mm
Stage Position = X: 84.02mm, Y: 47.39 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 20%
Exposure Time = 200 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10%
Exposure Time = 20 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

3D Image Processing - Panel A

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

3D Image Processing - Panel B

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

Profile View Display - Panel C and D

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 51.1), Y (Min 1 and Max 696)

The line profile demonstrates the high brightness of the BRCA1 647 direct conjugate and shows that its signal overlaps with the nuclear pore complex (NPC) along the same spatial path. This is expected, as the NPC forms the outer boundary of the nucleus. The overlapping intensities confirm proper nuclear specificity for the BRCA1 direct conjugate.

Image Correction

None

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

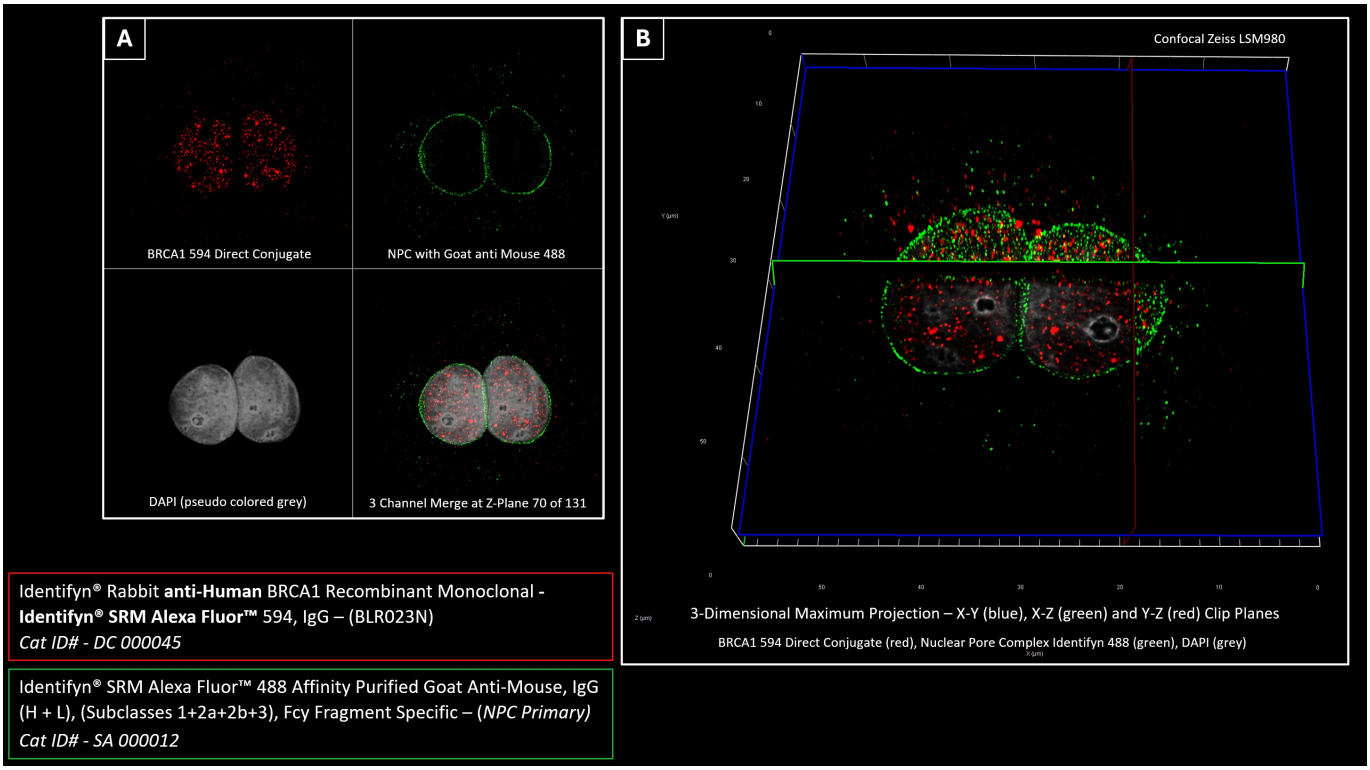
SOURCE PROVENANCE

Catalog	DC-000045
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	49

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CA9FC46C181EA2623EDFDC2E19348639775BBD42D098BF456874C57AAA63B437
Method PDF SHA-256	AB76A6504B3C83DD383F909F54E6E2DB854DD1A89A74A44CEDA95F2DBD7724E0

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	131 Slices (3.9 µm)
Channels	3
Scaling (Per Pixel)	0.052 µm X 0.052 µm X 0.030 µm
Image Size (Pixels)	1101 X 1101
Image Size (Scaled)	57.31 µm X 57.31 µm
Bit Depth	16 Bit
Image Center Position	X: 69.77 mm, Y: 47.61 mm
Stage Position	X: 69.77 mm, Y: 47.61 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 59 µm | 1.00 AU / 50 µm | 1.00 AU / 43 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @0.3% | 488 nm @0.2% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.3
Rotation	0°
Sampling	1.36
Pixel Time	0.31 µs
Frame Time	3.63 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	590-618 | 495-549 | 435-467
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.5 (3D, Auto) | 7.9 (3D, Auto) | 6.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	9% | 4% | -33%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	45° | -15°
Y-Z	Activate was selected, textured opaque was chosen, and a dark red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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.

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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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-Plane 70 of 131 - Panel A

Z Stack - (3D) - Panels B

Z-Stack = 131 Slices (3.9 µm)

Channels = 3
Scaling (Per Pixel) = 0.052 µm X 0.052 µm X 0.030 µm
Image Size (Pixels) = 1101 X 1101
Image Size (Scaled) = 57.31 µm X 57.31 µm
Bit Depth = 16 Bit
Image Center Position = X: 69.77 mm, Y: 47.61 mm
Stage Position = X: 69.77 mm, Y: 47.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 59 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 561 nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.36
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590-618
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 50 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.36
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 495-549
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 43 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.3
Rotation = 0°
Sampling = 1.36
Pixel Time = 0.31 µs
Frame Time = 3.63 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 435-467
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.3 (3D, Auto)

Split View - Panel A

The top left panel features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG (BLR023N)- Direct Conjugate, the upper right panel features a monoclonal Nuclear Pore Complex primary antibody with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the lower left panel features DAPI (psuedo colored grey for contrast), and the lower right is the merged image of all 3 channels.

Shown at Z-plane 70 of 131

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel B

Clipping planes are introduced in Panel B to highlight the BRCA1 594 Direct Conjugate within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

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

Position of Clip = 9%
Clip X = 0°
Clip Z = 0°

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

Position of Clip = 4%
Clip Y = 45°
Clip Z = 0°

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

Position of Clip = -33%
Clip Y = -15°
Clip Z = 0°

Panel B presents the wedge-cut view, shown from the top with a slight backward rotation to emphasize the area of wedge removal. This orientation reveals the full depth of the signal across the z-stack. It offers an alternative perspective on both the wedge geometry and the intranuclear localization of the BRCA1 594 Direct Conjugate, as indicated by the NPC perimeter signal (green) and DAPI (grey) for contrast.

Image Corrections

DAPI was pseudo-colored grey from its default blue in Zeiss ZEN to improve image contrast.

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

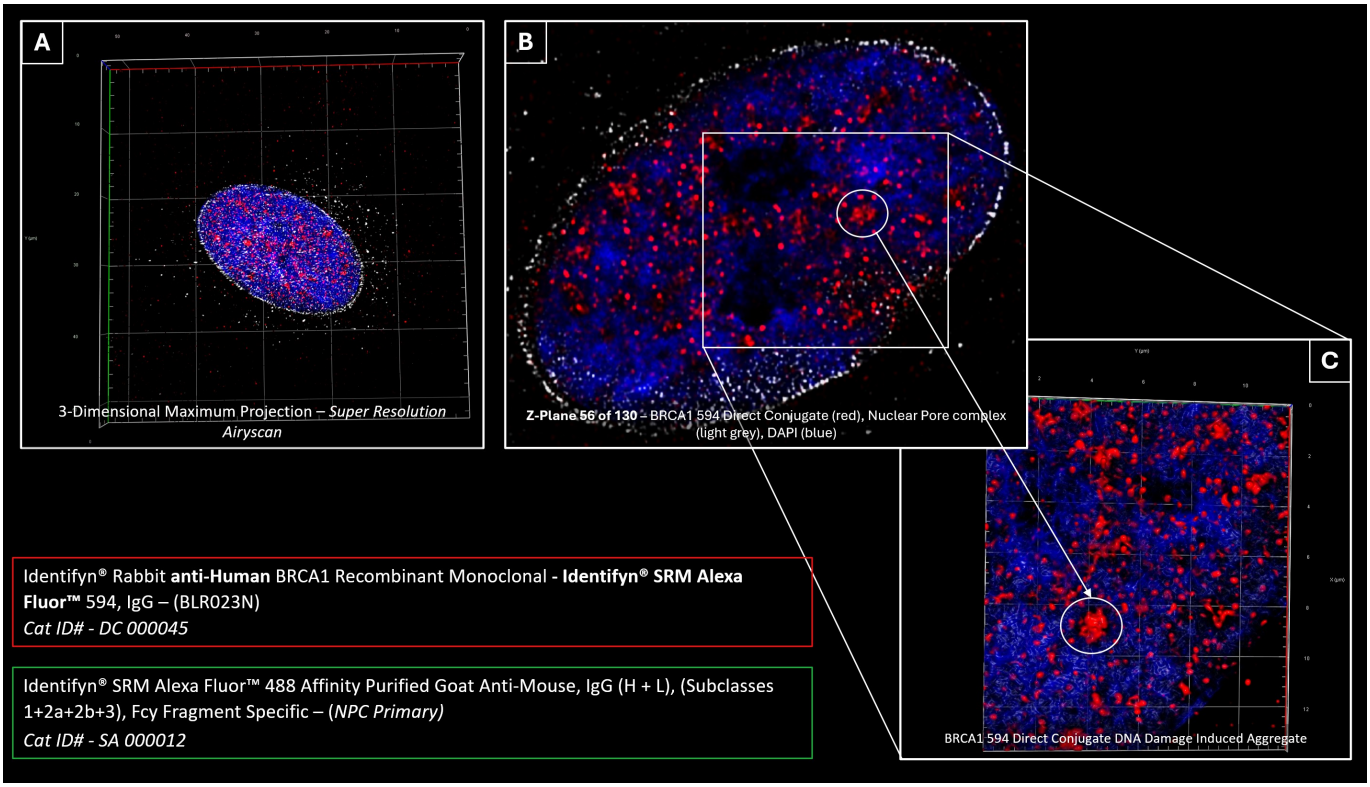
SOURCE PROVENANCE

Catalog	DC-000045
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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	944E4C53F7571D3D33A9C1AA54B945EAA7A3C5483B7264DCCBD407D0BF2C5D67
Method PDF SHA-256	7FA952DE4A1C45B3A78B07E7DAEBF94DF57D2D35B977013C079B27B14DF715EB

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 750
METADATA VALUES	62

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	130 Slices (3.9 µm) - Panel A | 130 Slices (3.9 µm) - Panel C
Channels	3
Scaling (Per Pixel)	0.03861 µm X 0.03861 µm X 0.03000 µm
Image Size (Pixels)	1503 X 1503 | 389 X 337
Image Size (Scaled)	58.03 µm X 58.03 µm | 13.72 µm X 11.89 µm
Bit Depth	16 Bit
Image Center Position	X: 71.09 mm, Y: 50.06 mm
Stage Position	X: 71.09 mm, Y: 50.06 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 286 µm | 5.00 AU / 250 µm | 5.00 AU / 235 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 525 | SBS SP 550 | SBS SP 505
Laser Wavelength	561 nm @1.10% | 488 nm @0.10% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.5
Rotation	0°
Sampling	2.00
Pixel Time	0.69 µs
Frame Time	7.51 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 493 | 353
Emission Wavelength	618 | 517 | 465
Effective NA	1.4
Detection Wavelength	573-627 | 495-548 | 420-480, 495-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Super Resolution	9.4 (3D, Auto)
LSM Plus Mode	8.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 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 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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 130 Slices (3.9 µm) - Panel A

Channels = 3
Scaling (Per Pixel) = 35.28 nm X 35.28 nm X 30.00 nm
Image Size (Pixels) = 1503 X 1503
Image Size (Scaled) = 58.03 µm X 58.03 µm
Bit Depth = 16 Bit
Image Center Position = X: 71.09 mm, Y: 50.06 mm
Stage Position = X: 71.09 mm, Y: 50.06 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-Plane 56 of 130 - Panel B

Z Stack - (3D)

Z-Stack = 130 Slices (3.9 µm) - Panel C

Channels = 3
Scaling (Per Pixel) = 35.28 nm X 35.28 nm X 30.00 nm
Image Size (Pixels) = 389 X 337
Image Size (Scaled) = 13.72 µm X 11.89 µm
Bit Depth = 16 Bit
Image Center Position = X: 71.09 mm, Y: 50.06 mm
Stage Position = X: 71.09 mm, Y: 50.06 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 286 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @1.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.69 µs
Frame Time = 7.51 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 573-627
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.4 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 550
Laser Wavelength = 488 nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.69 µs
Frame Time = 7.51 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 495-548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Super Resolution = 9.4 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 235 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.69 µs
Frame Time = 7.51 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 420-480, 495-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.0 (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 = 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)

2-Dimensional View - Panel B

Shown at Z-plane 56 of 130

Features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG (BLR023N) Direct Conjugate (red), Monoclonal Nuclear Pore Complex primary antibody detected with Identifyn® SRM Alexa Fluor™ 488 Affinity-Purified Goat Anti-Mouse IgG (H+L) (Subclasses 1+2a+2b+3), Fcγ Fragment-Specific (light grey), and DAPI. The image includes a defined Region of Interest (ROI) - a white box - used to generate the volume projection displayed in Panel C. A white circle highlights the Etoposide-induced DNA damage response, emphasizing the BRCA1 Alexa Fluor™ 594 Direct Conjugate aggregation at the damage site.

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)

Image Corrections

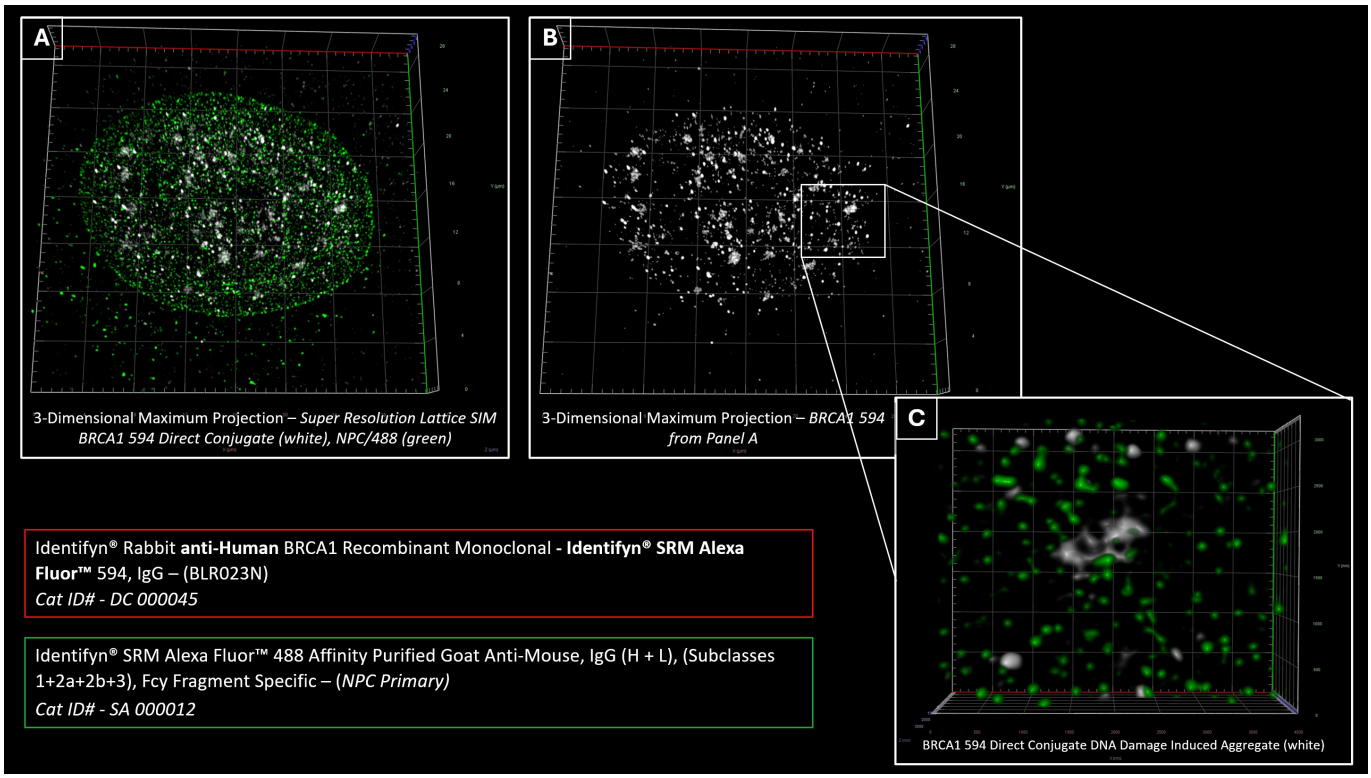
The Nuclear Pore Complex (NPC) signal was pseudo-colored in Zeiss ZEN, adjusted from the default Alexa Fluor™ 488 green to light grey to enhance overall image contrast and improve visualization of nuclear structure.

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

SOURCE PROVENANCE

Catalog	DC-000045
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	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	39B714B810F29F32A9E10A524074337C2AC8E5057ACAB9DF12D2EE36FC1870CB
Method PDF SHA-256	ABD5E7C35E85667F64BBCD4B6C477DECDB9860CD4C31EF2A3E6AD1BAAA77BD16

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	59

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	119 Slices (3.54 µm) | 131 Slices (3.90 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1482 X 1414 | 190 X 155
Image Size (Scaled)	31.29 µm X 29.85 µm | 4.01 µm X 3.27 µm
Bit Depth	16 Bit
Image Center Position	X: 62.73 mm, Y: 34.55 mm
Stage Position	X: 62.73 mm, Y: 34.55 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 | 488
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR
Excitation Wavelength	561 | 488
Emission Wavelength	597 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	150 ms | 120 ms
Depth of Focus	0.92 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	561 nm @5.00% | 488 nm @2.00%
Frame Time	1.59 s
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	11.0768 (594), 11.0662 (488)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | 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 20°, Scale Z 50%, 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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

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) - Panels A and B

Z-Stack = 119 Slices (3.54 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1482 X 1414
Image Size (Scaled) = 31.29 µm X 29.85 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.73 mm, Y: 34.55 mm
Stage Position = X: 62.73 mm, Y: 34.55 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 131 Slices (3.90 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 190 X 155
Image Size (Scaled) = 4.01 µm X 3.27 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.73 mm, Y: 34.55 mm
Stage Position = X: 62.73 mm, Y: 34.55 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @5.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.0768 (594), 11.0662 (488)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panels A and B

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping Enabled
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 20°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Image Correction

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR023N) was pseudo-colored in Zen Software from red (the default color) to white to provide visual contrast.

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

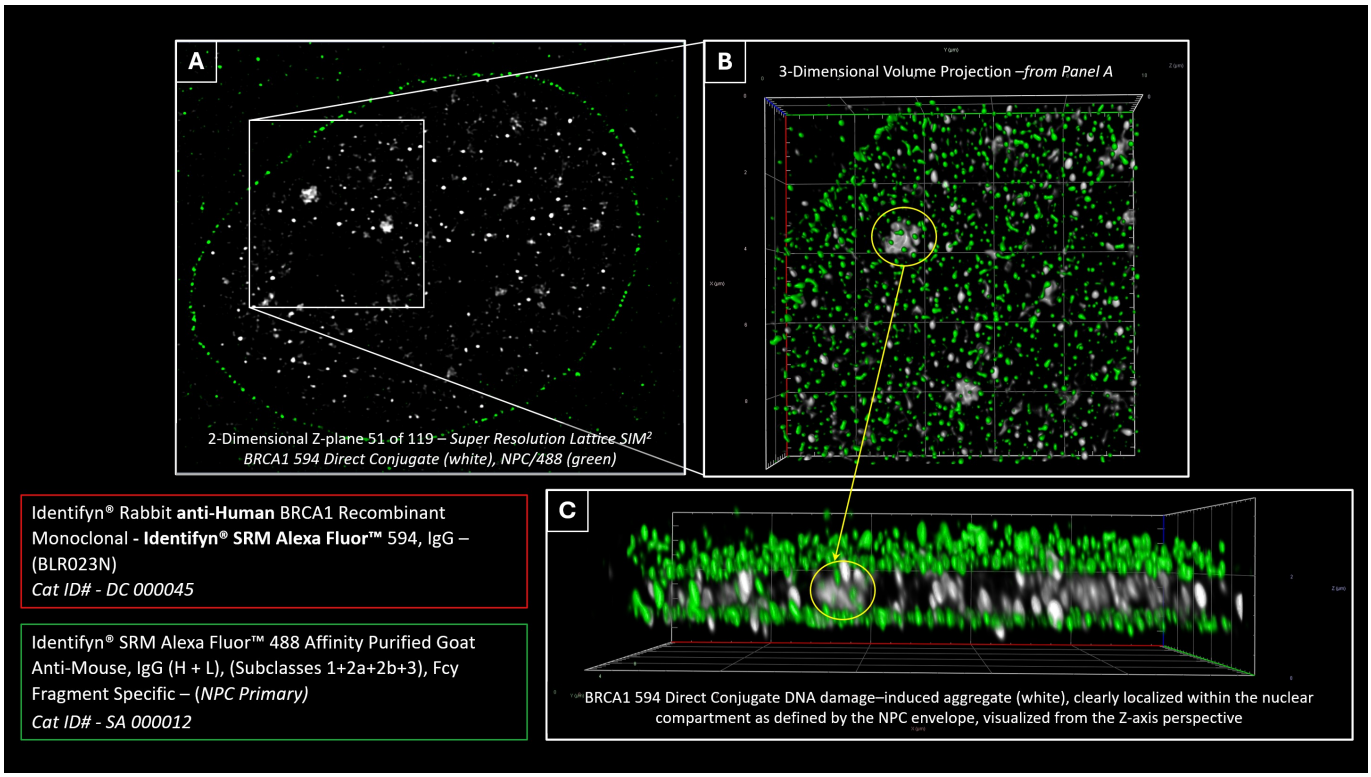
SOURCE PROVENANCE

Catalog	DC-000045
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:

SOURCE PROVENANCE

Structured source metadata values	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4122DC5BB2329F397D7EDD4BE09050E28C308A0725A59B02AF58A6A995E900D3
Method PDF SHA-256	2D3BD263243BCBCD9E84560D4F3A7A721F07FBD263FFFE5F4152A2FB75A92641

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
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	119 Slices (3.54 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	466 X 474
Image Size (Scaled)	9.84 µm X 10.01 µm
Bit Depth	16 Bit
Image Center Position	X: 62.73 mm, Y: 34.55 mm
Stage Position	X: 62.73 mm, Y: 34.55 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 | 488
Channel Name	T1-Axiocam 820 #2-SR | T2-Axiocam 820-SR
Excitation Wavelength	561 | 488
Emission Wavelength	597 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 | Axiocam 820
Camera Adapter	1X
Exposure Time	150 ms | 120 ms
Depth of Focus	0.92 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	561 nm @5.00% | 488 nm @2.00%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM²
SIM² Settings	Standard
Input SNR	Low
Regularization	None
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 13°, Scale Z 73%, 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™ 594, IgG - (BLR023N)
Cat ID# - DC 000045

Identifyn® Secondary Antibody

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

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 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 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™ 594, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, at#P36962.

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 51 of 119 - Panel A

Z Stack - (3D) - Panels B and C

Z-Stack = 119 Slices (3.54 µm)

Channels = 2
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 466 X 474
Image Size (Scaled) = 9.84 µm X 10.01 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.73 mm, Y: 34.55 mm
Stage Position = X: 62.73 mm, Y: 34.55 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

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 = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @5.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 503 - 546, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @2.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panels B and 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 13°, Scale Z 73%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR023N) was pseudo-colored in Zen Software from red (the default color) to white to provide visual contrast.

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

SOURCE PROVENANCE

Catalog	DC-000045
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C9173D5B040BA98C3BF2E3F780B575DC5087044557AEC728AD25FD3B14F785DF
Method PDF SHA-256	A6D21A12F2147AD3EC96CBF9D04FA600D4248D99C0D21CCAA75E425980725501