

IDENTIFYN RESOLUTION STEPDOWN

BRCA1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405

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

7

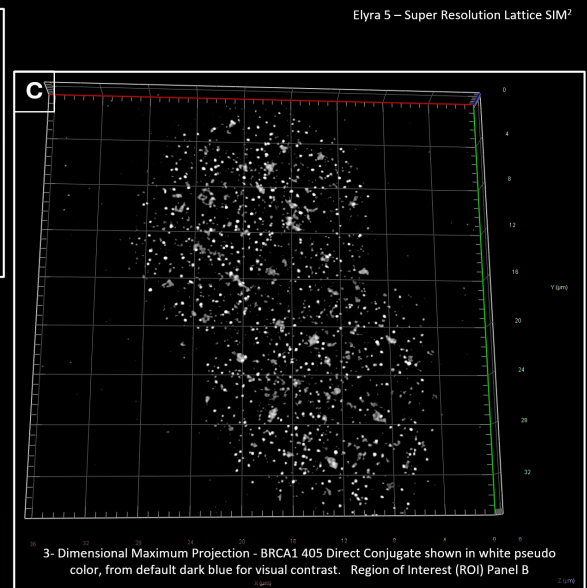
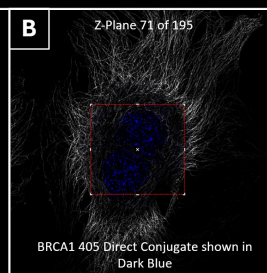
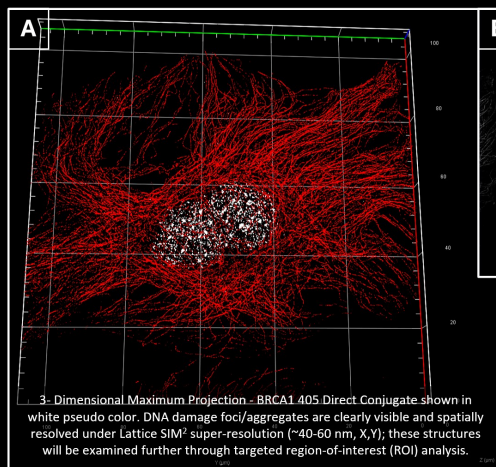
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR023N)
Cat ID# - DC 000042

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000041 | 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-000041 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000041 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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	405/DAPI	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 594	2; AF405-49027; 45 Texas Red; 372 - 408; 540 - 580; 517 - 445; 593 - 668; 401
Widefield SIM — Apotome	405/DAPI; 594	2; AF405-49027; 45 Texas Red; 372 - 408; 540 - 580; 517 - 445; 593 - 668; 401
Confocal	405/DAPI; 488; 594	2; None; 405 nm @0.4%; 561 nm @0.2%; 401; 590; 422; 618
Airyscan	405/DAPI; 488; 594	2; None; 405 nm @0.2%; 561 nm @0.3%; 401; 590; 422; 618
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; T2-Axiocam 820 #2-SR; T1-Axiocam 820 #2-SR; 405; 561
SIM ²	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; T2-Axiocam 820 #2-SR; T1-Axiocam 820 #2-SR; 405; 561

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 405/DAPI.

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: 2; Scaling (Per Pixel): 0.110 μm X 0.110 μm ; Image size (Pixels): 1118 X 847; Image Size (Pixels): 1118 X 847; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

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; 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 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: Z-Stack: 82 Slices (8.1µm); Channels: 2; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.200 µm; Image size (Pixels): 1185 X 961; 350 X 296; Image Size (Pixels): 1185 X 961; 350 X 296; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 Mono. Timing: Exposure Time: 250 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 35.

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 Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 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

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: 116 Slices (3.86 μm); Channels: 2; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 739 X 739; Image Size (Pixels): 739 X 739; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAs-Pmt3; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.34 μs ; Frame Time: 1.88 s. Illumination: Laser Wavelength: 405 nm @0.4%; 561 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 41 μm ; 1.00 AU / 65 μm ; Scan Zoom: 3.0; LSM Scan Speed: 13; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 6.1 (3D, Auto); 6.5 (3D, Auto). Structured source metadata values: 52.

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: 206 Slices (2.05 μm); Channels: 2; Scaling (Per Pixel): 35.00 nm X 35.00 nm X 10.00 nm; Image size (Pixels): 1428 X 1248; 552 X 454; Image Size (Pixels): 1428 X 1248; 552 X 454; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.82 μs ; Frame Time: 6.25 s. Illumination: Laser Wavelength: 405 nm @0.2%; 561 nm @0.3%. Optical/scan: Pinhole: 5.00 AU / 214 μm ; 5.00 AU / 286 μm ; Scan Zoom: 3.0; 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); Background: Black; LSM Plus Mode: Filter: 8.5 (3D, Auto); 10.0 (3D, Auto). Structured source metadata values: 61.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

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: 146 Slices (4.35 μ m); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 4128 X 3927; 677 X 674; Image Size (Pixels): 4128 X 3927; 677 X 674; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 676.00 ms; 1.33 s. Illumination: Laser Wavelength: 405 nm @2.00%; 561 nm @3.00%; Illumination Wavelength: 405; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 65.

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: 195 Slices (5.82 μ m); Channels: 2; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1608 X 1837; Image Size (Pixels): 5056 X 5056; 1608 X 1837; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 676.00 ms; 1.33 s. Illumination: Laser Wavelength: 405 nm @2.00%; 561 nm @3.00%; Illumination Wavelength: 405; 561. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

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-000041 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=4128 X 3927; Image Size (Scaled)=87.15 μ m X 82.91 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 μ m X 0.02111 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=106.75 μ m X 106.75 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

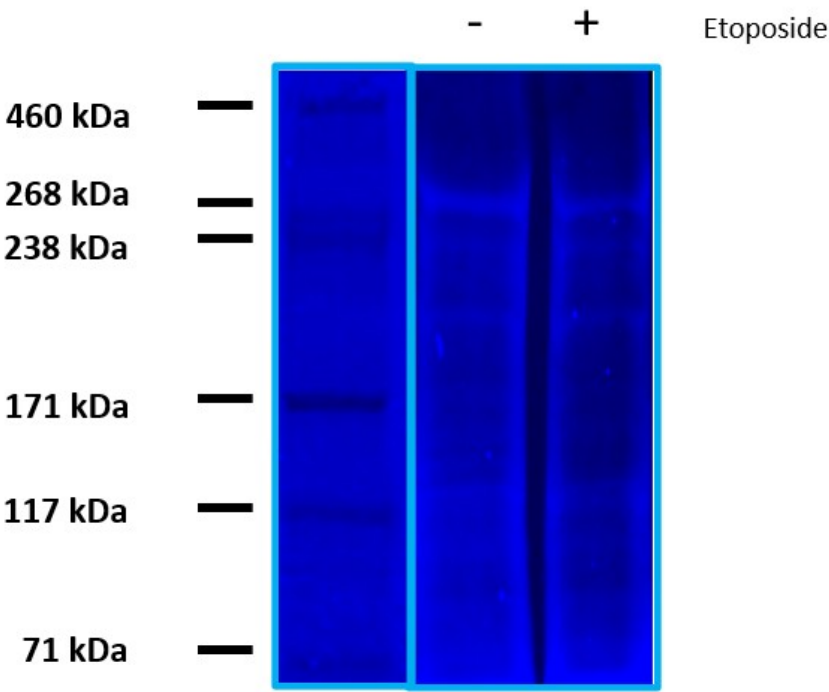
This dossier preserves the complete available evidence progression for DC-000041. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	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	405/DAPI
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	375nm
Emission Filter	452±45nm
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™ 405, IgG - (BLR023N)
Cat ID# - DC 000041

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 Blot™ 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™ 405, IgG (BLR023N) for 2 hours, followed by 5 washes with 5X 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 = 375nm

Emission Filter = 452±45nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

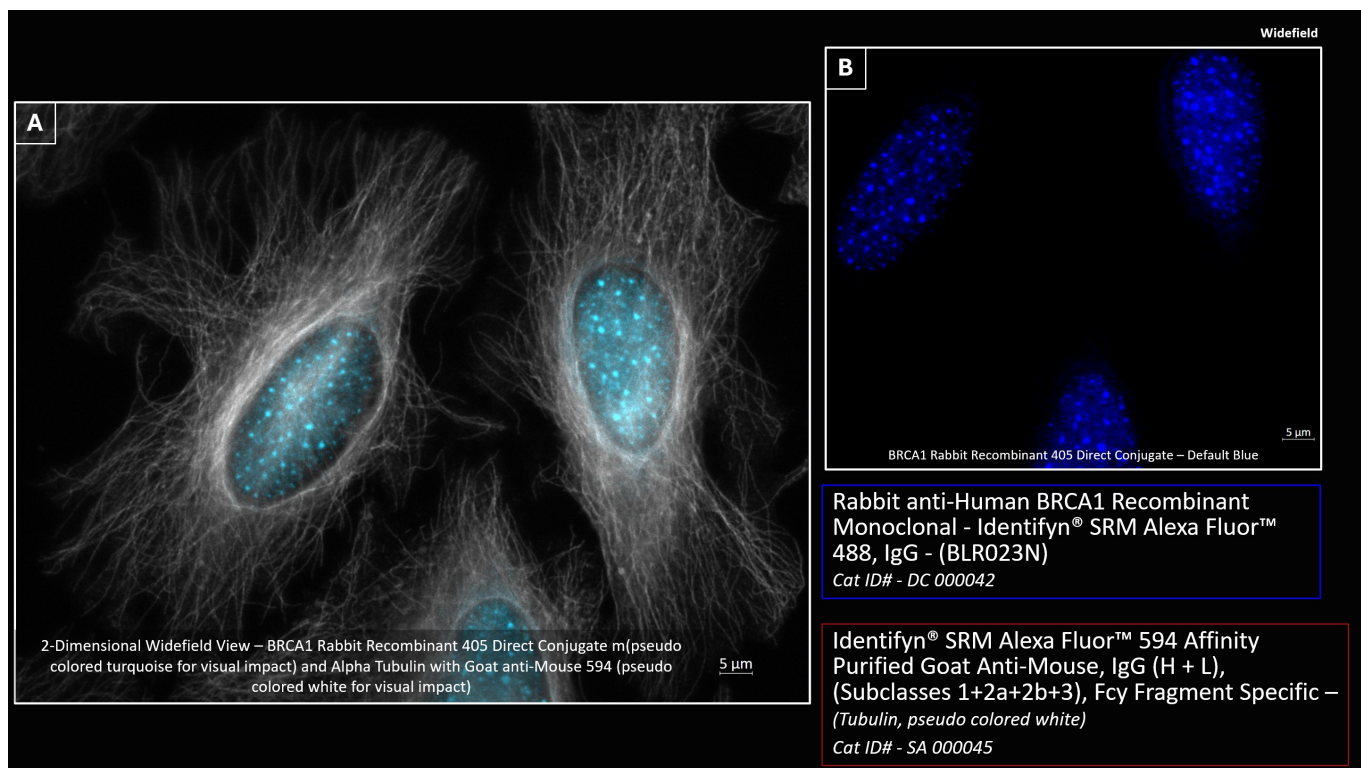
Image: K.Small

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SOURCE PROVENANCE

Catalog	DC-000041
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	438FDDE9BD8F839B3E17A60B14E35B7EE5EE94A2485AFB6F45D55E580EFF9C91
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ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
METADATA VALUES	30

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 μm X 0.110 μm
Image size (Pixels)	1118 X 847
Image Size (Scaled)	122.45 μm X 92.77 μm
Bit Depth	14 Bit
Image Center Position	X: 0.00 μm , Y: 0.00 μm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	AF405-49027 45 Texas Red
Beam Splitter	412 585
Filter Excitation Wavelength	372 - 408 540 - 580
Filter Emission Wavelength	517 - 445 593 - 668
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms 100 ms
Excitation Wavelength	401 590
Emission Wavelength	422 618
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.65 μm 0.96 μm
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™ 405, IgG - (BLR023N)
Cat ID# - DC 000041

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000045

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

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

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

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

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

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

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

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

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

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG, - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 1118 X 847
Image Size (Scaled) = 122.45 µm X 92.77 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

405-

Reflector = AF405-49027
Beam Splitter = 412
Filter Excitation Wavelength = 372 - 408
Filter Emission Wavelength = 517 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @30.00%
Exposure Time = 300 ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.65 µm
Binning Mode = 2,2

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 @10.00%
 Exposure Time = 100 ms
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.96 µm
 Binning Mode = 2,2

2- Dimensional View - Panel A

Features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG,- (BLR023N) and Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific.

Note that the 405 and 594 channels were changed to turquoise and white, respectively, to increase visual clarity.

Panel B - Demonstrates the BRCA1 405 Direct Congjuate in default dark blue, noting excellent signal at 30% and 300ms.

Image Correction

Pseudocoloring of panel A as described above.

Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

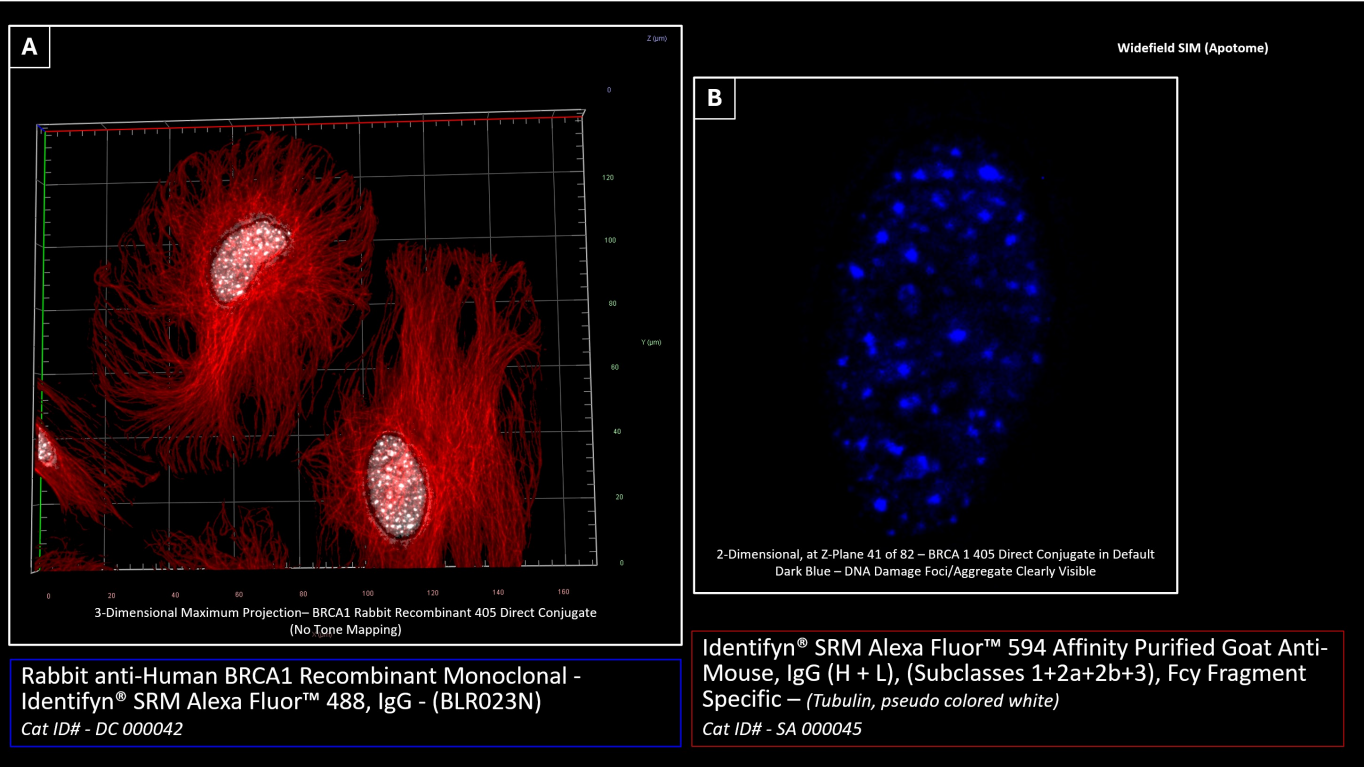
Catalog	DC-000041
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AF9340D98D5A943DF17C6F76C9F007D7693BA5C77B217ED428986F5065238C0C

SOURCE PROVENANCE

Method PDF SHA-256

59A246142C4B30B1A2583FE7A377B5E9F8671E5C8A2DCEB3F4937ADE3BC23259

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
METADATA VALUES	35

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 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
Z-Stack	82 Slices (8.1µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.200 µm
Image Size (Pixels)	1185 X 961 350 X 296
Image Size (Scaled)	169.21 µm X 137.29 µm 50.00 µm X 42.29 µm
Bit Depth	14 Bit
Image Center Position	X: 79.80 mm, Y: 47.78 mm
Stage Position	X: 79.80 mm, Y: 47.78 mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	AF405-49027 45 Texas Red
Beam Splitter	412 585
Filter Excitation Wavelength	372 - 408 540 - 580
Filter Emission Wavelength	517 - 445 593 - 668
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00% X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	250 ms 150 ms
Excitation Wavelength	401 590
Emission Wavelength	422 618
Effective NA	1.4
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	0.82 µm 1.20 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

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Cat ID# - DC 000041

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Fragment Specific
Cat ID# - SA 000045

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

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

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

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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™ 405, IgG, - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 82 Slices (8.1µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.200 µm
Image Size (Pixels) = 1185 X 961
Image Size (Scaled) = 169.21 µm X 137.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 79.80 mm, Y: 47.78 mm
Stage Position = X: 79.80 mm, Y: 47.78 mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

Single Plane (2D) - 41 of 82 - Panel B

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.200 µm
Image Size (Pixels) = 350 X 296
Image Size (Scaled) = 50.00 µm X 42.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 79.80 mm, Y: 47.78 mm
Stage Position = X: 79.80 mm, Y: 47.78 mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

405-

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 517 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25.00%
 Exposure Time = 250 ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 0.82 μ m
 Binning Mode = 2,2

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 @15.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Imaging Device = Axiocam 807 Mono
 Camera Adapter = 1X
 Depth of Focus = 1.20 μ m
 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.

2- Dimensional View - Panel A

Features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG, - (BLR023N) and Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific.

Note that the 405 channel was changed to turquoise to increase visual clarity against the 594 channel.

Panel B - Demonstrates the BRCA1 405 Direct Conjugate in default dark blue, DNA Damage Foci/Aggregate Clearly Visible.

Image Correction

Pseudocoloring of panel A as described above.

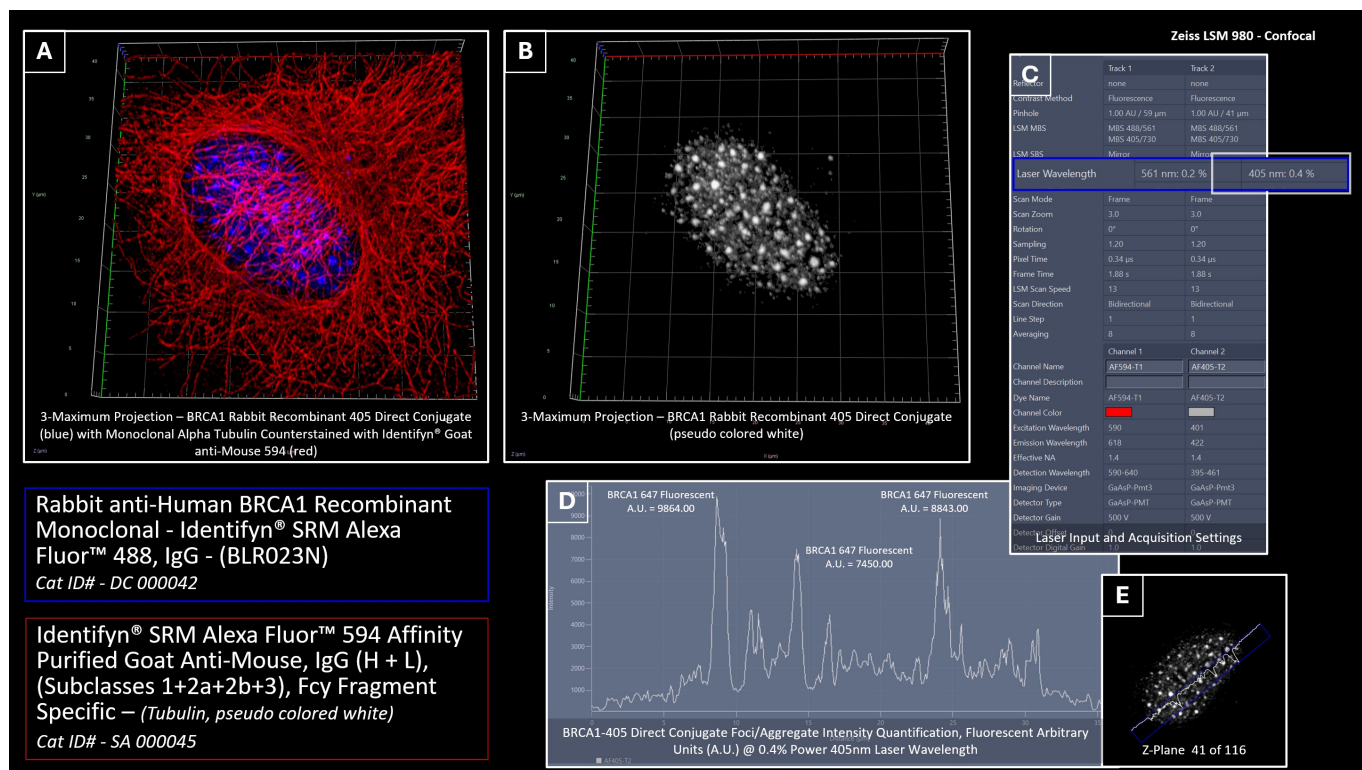
Image by E.F. Simon

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000041
Stage	Widefield SIM - Apotome
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 Xylys Lamp, was used with the following parameters for each dye:
Structured source metadata values	35
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5D3550271D47FE3B741EF42602C726D9C0977AA2010EE32E50BE80FBD924D353
Method PDF SHA-256	75CA3E17E5334E64A419A43704BC581D845487EFFDB929E758BCE7DE9F2E072B

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	52

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	116 Slices (3.86 μm)
Channels	2
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	739 X 739
Image Size (Scaled)	43.48 μm X 43.48 μm
Bit Depth	16 Bit
Image Center Position	X: 94.34 mm, Y: 52.26 mm
Stage Position	X: 94.34 mm, Y: 52.26 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Pinhole	1.00 AU / 41 μm 1.00 AU / 65 μm
LSM MBS	MBS 488/561, 405/730
LSM SBS	Mirror
Laser Wavelength	405 nm @0.4% 561 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	1.20
Pixel Time	0.34 μs
Frame Time	1.88 s
LSM Scan Speed	13
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	401 590
Emission Wavelength	422 618
Effective NA	1.4
Detection Wavelength	395 - 461 590-618
Imaging Device	GaAs-Pmt3 GaAsP-Pmt3
Detector Type	GaAs-PMT GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 6.1 (3D, Auto) 6.5 (3D, Auto)
Contrast Method	Fluorescence
3D Maximum Projection	Precise
Appearance	Threshold 1% (594), Threshold 2% (405), Ramp 0% all channels, Maximum 100% all channels Threshold 1% (405), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping was NOT 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)
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 35.7), Y (Min 38 and Max 10357)

SOURCE METHOD

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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 important for activating ATM-mediated signaling in response to DNA damage.

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The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

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

Z-Stack = 116 Slices (3.86 µm)

Channels = 2

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

Image Size (Pixels) = 739 X 739

Image Size (Scaled) = 43.48 µm X 43.48 µm

Bit Depth = 16 Bit

Image Center Position = X: 94.34 mm, Y: 52.26 mm

Stage Position = X: 94.34 mm, Y: 52.26 mm

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

Single Plane (2D) - Z-Plane 41 of 116 - Panel D (Same Image Dimensions)

405 -

Reflector = None
 Contrast Method - Fluorescence
 Pinhole = 1.00 AU / 41 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.4%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.34 μ s
 Frame Time = 1.88 s
 LSM Scan Speed = 13
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 395 - 461
 Imaging Device = GaAs-Pmt3
 Detector Type = GaAs-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.1 (3D, Auto)

594-

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 65 µm
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 561 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.34 µs
 Frame Time = 1.88 s
 LSM Scan Speed = 13
 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 = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.5 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 1% (594), Threshold 2% (405), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Tone Mapping was NOT 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 B

3D Maximum Projection = Precise
 Appearance = Threshold 1% (405), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Tone Mapping was NOT 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)

LSM 980 Zen Acquisition Info Tab - Panel C

Shown is the acquisition of the 405 channel (BRCA1 direct conjugate) using the 405nm laser at 0.4%, and the 594 channel (Alpha Tubulin with 594 Secondary Antibody) using the 561nm laser at 0.2%. Both channels at a sensitive input power of <1.0% display a significant fluorescent signal for the Identifyn® BRCA1 405 Direct Conjugate and the Identifyn® 594 Secondary Antibody.

Profile View Display - Panels D & E

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 35.7), Y (Min 38 and Max 10357)

This panel shows three BRCA1 DNA damage-induced foci/aggregates, with their fluorescent intensities under the image acquisition settings shown.

Measured at Z-plane 41 of 116

Image Corrections

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR023N) was pseudo-colored in Zen Software from blue, the default color, to light grey in Panels B and E.

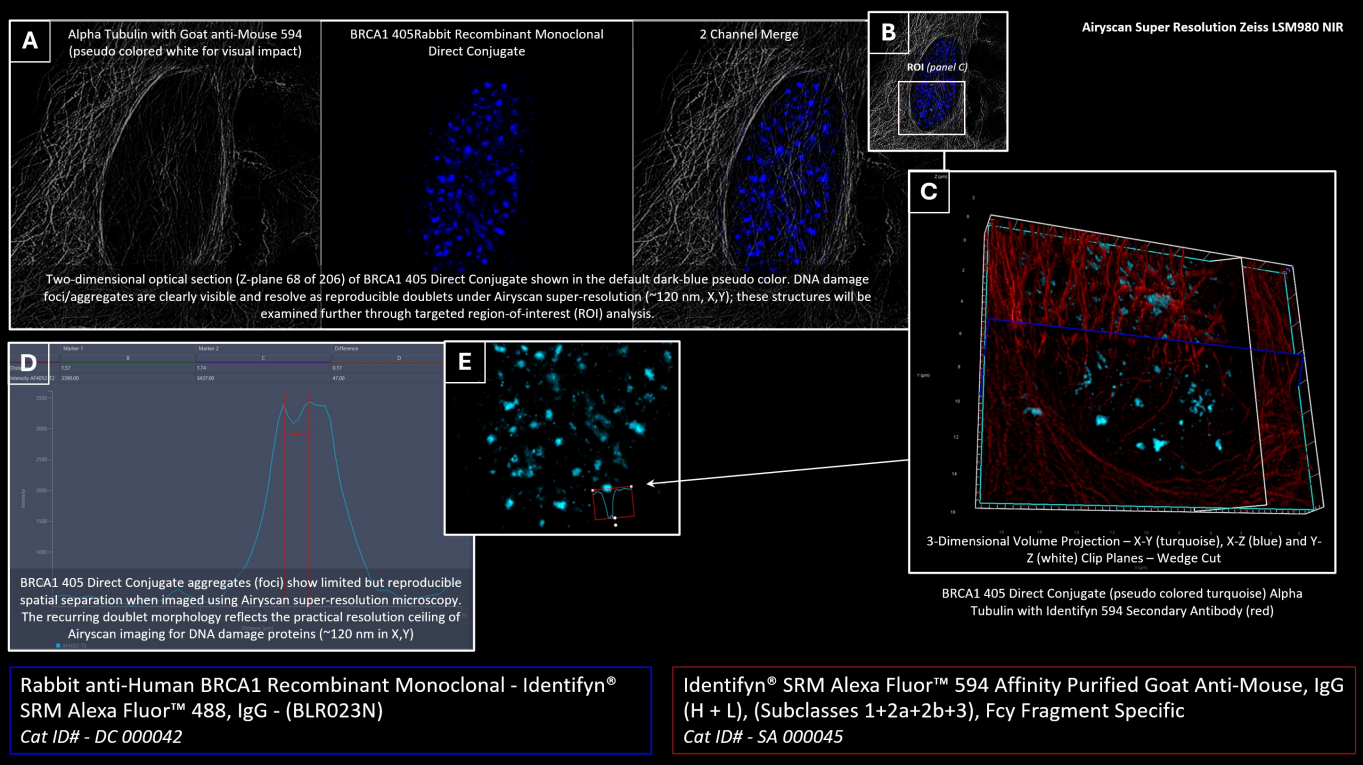
Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	DC-000041
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	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	842BF303A2CBCCD599CD05A4727D70E72AD80C541E1B101B90FB7626827B2279
Method PDF SHA-256	B64DC7C46C4FA1CEDC8EB41BB9F8BB5F3EC447938A8F19F584A9306F6330E007

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	61

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	2
Scaling (Per Pixel)	35.00 nm X 35.00 nm X 10.00 nm
Image Size (Pixels)	1428 X 1248 552 X 454
Image Size (Scaled)	44.05 µm X 44.05 µm 1948 µm X 16.02 µm
Bit Depth	16 Bit
Image Center Position	X: 94.75 mm, Y: 50.46 mm
Stage Position	X: 94.75 mm, Y: 50.46 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	206 Slices (2.05 µm)
Reflector	None
Pinhole	5.00 AU / 214 µm 5.00 AU / 286 µm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS SP 505 SBS LP 525
Laser Wavelength	405 nm @0.2% 561 nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.00
Pixel Time	0.82 µs
Frame Time	6.25 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	401 590
Emission Wavelength	422 618
Effective NA	1.4
Detection Wavelength	422 - 477 573-627
Imaging Device	Airyscan
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.5 (3D, Auto) 10.0 (3D, Auto)
Contrast Method	Fluorescence
3D Volume Projection	Precise
Appearance	Threshold 3% all channels, Ramp 97% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Activate was selected, textured opaque was chosen, and a turquoise outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	2% -36%
Clip X	0°
Clip Z	0°
X-Z	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.
Clip Y	60° -60°
Y-Z	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
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 2.77), Y (Min 0 and Max 3609)

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™ 405, IgG - (BLR023N)
Cat ID# - DC 000041

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000045

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

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

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

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

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

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

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

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

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

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG, - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-Plane 68 of 206 - Panels A, B, and E

Channels = 2
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 10.00 nm
Image Size (Pixels) = 1428 X 1248
Image Size (Scaled) = 44.05 μ m X 44.05 μ m
Bit Depth = 16 Bit
Image Center Position = X: 94.75 mm, Y: 50.46 mm
Stage Position = X: 94.75 mm, Y: 50.46 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

Z Stack - (3D) - Panel C

Z-Stack = 206 Slices (2.05 μ m)

Channels = 2
Scaling (Per Pixel) = 35.00 nm X 35.00 nm X 10.00 nm
Image Size (Pixels) = 552 X 454
Image Size (Scaled) = 1948 μ m X 16.02 μ m
Bit Depth = 16 Bit
Image Center Position = X: 94.75 mm, Y: 50.46 mm
Stage Position = X: 94.75 mm, Y: 50.46 mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

405 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 μ m
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82 μ s
Frame Time = 6.25 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.5 (3D, Auto)

594-

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 286 µm
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = SBS LP 525
 Laser Wavelength = 561 nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 3.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.82 µs
 Frame Time = 6.25 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
 LSM Plus Mode = 10.0 (3D, Auto)

Split View - Panel A

The left panel features Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The middle panel features Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR023N), and the right panel features the merged image of the two channels.

Two-dimensional optical section (Z-plane 68 of 206) of BRCA1 405 Direct Conjugate shown in the default dark-blue pseudo color. DNA damage foci/aggregates are clearly visible and resolve as reproducible doublets under Airyscan super-resolution (~120 nm, X, Y); these structures will be examined further through targeted region-of-interest (ROI) analysis.

Region of Interest - Panel B

Demonstrates the region of interest to be examined further in the 3-dimensional volume projection and line profile measurements in panels C, D, and E

3D Image Processing - Panel C

3D Volume Projection = Precise

Appearance = Threshold 3% all channels, Ramp 97% all channels, Maximum 100% all channels

Background = Black

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

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel C

In Panel B, clipping planes are applied to highlight BRCA1 405 Direct Conjugate localization within the nuclear compartment. A triple-wedge cut removes portions of the image volume in the X-Y, X-Z, and Y-Z planes, improving visualization of the recurrent doublet morphology characteristic of DNA damage foci at Airyscan resolution.

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

Position of Clip = 2%

Clip X = 0°

Clip Z = 0°

X-Z = 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 = 2%

Clip Y = 60°

Clip Z = 0°

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

Position of Clip = -36%

Clip Y = -60°

Clip Z = 0°

Panel B presents a top-down, slightly rotated wedge-cut view that emphasizes the region of wedge removal and reveals the full Z-stack signal depth, providing an alternative perspective on wedge geometry and the intranuclear localization of the BRCA1 405 Direct Conjugate under Airyscan super-resolution. These images reveal reproducible doublet structures, which may represent the opposing ends of resected DNA damage sites. At Airyscan resolution, this interpretation remains speculative; however, the repeated observation of this pattern supports its biological relevance. Further structural elucidation is achieved using higher-resolution modalities, including super-resolution SIM and SIM², and is most clearly resolved with STORM imaging, which allows extraction of three-dimensional structural information.

Profile View Display - Panels D & E

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 2.77), Y (Min 0 and Max 3609)

This panel employs the Caliper X tool to quantify the reproducible BRCA1 405 Direct Conjugate doublet formation observed under Airyscan super-resolution imaging. Although the structures remain unresolved at this resolution, the measured inter-peak separation is approximately ~170 nm, consistent with the effective Airyscan lateral resolution and closely matching separations measured using higher-power super-resolution modalities, including SIM and SIM².

Image Corrections

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR023N) was pseudo-colored in Zen Software from blue, the default color, to turquoise in Panels C and E.

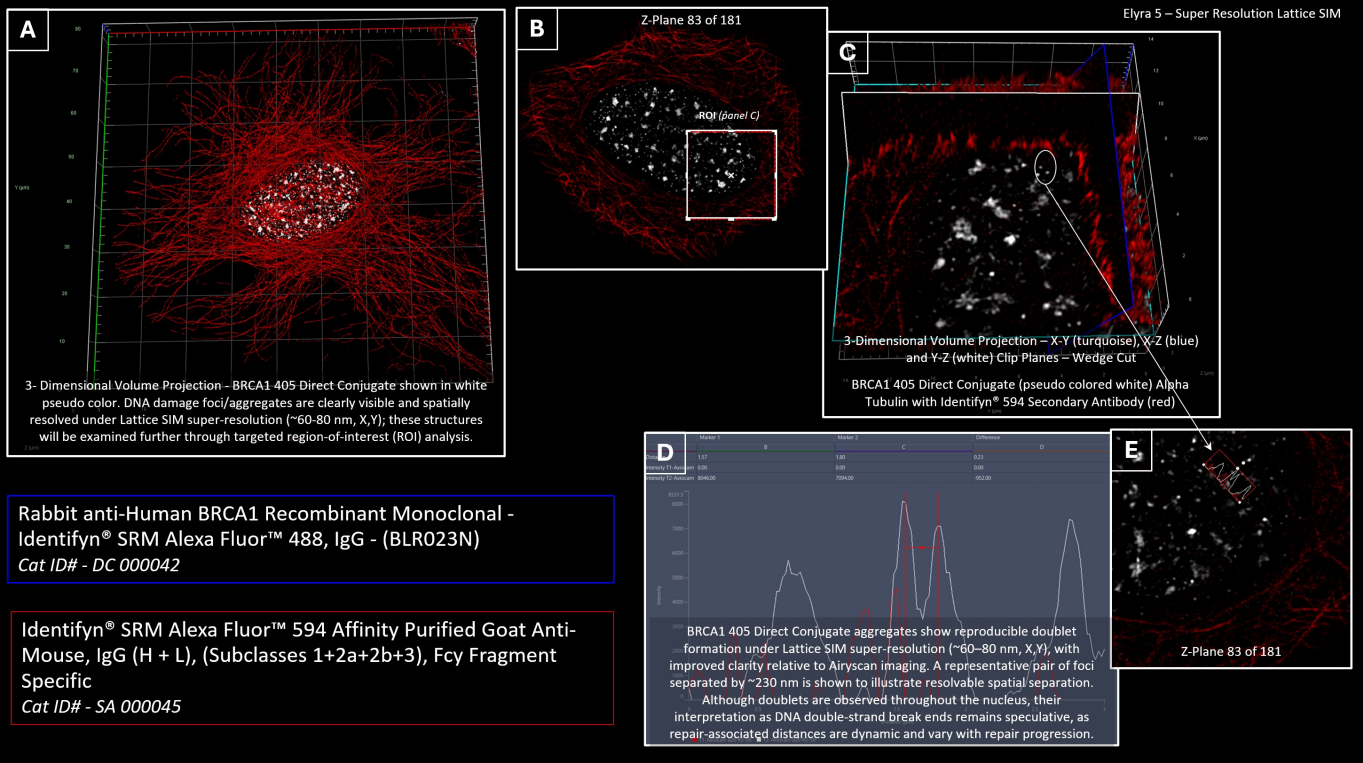
Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	DC-000041
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	61
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	754BC6D64E82D325A048C93FE62ADDB248DF5F36A59DC4A5E5A511C51A901F27
Method PDF SHA-256	23704522949E068F422174F9D421ACC1C9C7FE9735B39F9B4C2D32E5A5CAD5EC

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	65

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	146 Slices (4.35 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	4128 X 3927 677 X 674
Image Size (Scaled)	87.15 µm X 82.91 µm 14.29 µm X 14.23 µm
Bit Depth	16 Bit
Image Center Position	X: 60.77 mm, Y: 39.06 mm
Stage Position	X: 60.77 mm, Y: 39.06 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	405 561
Channel Name	T2-Axiocam 820 #2-SR T1-Axiocam 820 #2-SR
Excitation Wavelength	405 561
Emission Wavelength	445 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100 ms 120 ms
Depth of Focus	0.69 µm 0.92 µm
Binning Mode	2,2
Laser Wavelength	405 nm @2.00% 561 nm @3.00%
Frame Time	676.00 ms 1.33 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	10.2809 (405), 11.3626 (594)
Fitting	Fast Fit
Normalization	Auto
3D Volume 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%, 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)
X-Y	Activate was selected, textured opaque was chosen, and a turquoise outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	19% 47% 28%
Clip X	0°
Clip Z	0°
X-Z	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.
Clip Y	30°
Y-Z	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
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 3.02), Y (Min 0 and Max 9745)

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™ 405, IgG - (BLR023N)
Cat ID# - DC 000041

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000045

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

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

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

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

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

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

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

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

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

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG, - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#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

Z-Stack = 146 Slices (4.35 µm)

Channels = 2

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

Image Size (Pixels) = 4128 X 3927

Image Size (Scaled) = 87.15 µm X 82.91 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.77 mm, Y: 39.06 mm

Stage Position = X: 60.77 mm, Y: 39.06 mm

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

Single Plane (2D) - Z-Plane 68 of 206 - Panels B, D, and E

Z Stack - (3D) - Panels C

Z-Stack = 146 Slices (4.35 µm)

Channels = 2

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

Image Size (Pixels) = 677 X 674

Image Size (Scaled) = 14.29 µm X 14.23 µm

Bit Depth = 16 Bit

Image Center Position = X: 60.77 mm, Y: 39.06 mm

Stage Position = X: 60.77 mm, Y: 39.06 mm

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

405 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 100 ms
 Depth of Focus = 0.69 μm
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @2.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

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 = 120 ms
 Depth of Focus = 0.92 μm
 Binning Mode = 2,2
 Laser Wavelength = 561 nm @3.00%
 Frame Time = 1.33 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 = 10.2809 (405), 11.3626 (594)

Fitting = Fast Fit

Normalization = Auto

3D Image Processing - Panel A

3D Volume Projection = Precise

Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

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

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

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

Region of Interest - Panel B

Demonstrates the region of interest to be examined further in the 3-dimensional volume projection and line profile measurements in panels C, D, and E

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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

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

Clipping Image Process - Panel C

In Panel B, clipping planes are applied to highlight BRCA1 405 Direct Conjugate localization within the nuclear compartment. A triple-wedge cut removes portions of the image volume in the X-Y, X-Z, and Y-Z planes, improving visualization of the recurrent doublet morphology characteristic of DNA damage foci at Lattice SIM resolution.

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

Position of Clip = 19%

Clip X = 0°

Clip Z = 0°

X-Z = 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 = 47%**Clip Y = 30°****Clip Z = 0°**

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

Position of Clip = 28%**Clip Y = 30°****Clip Z = 0°**

Panel B shows a top-down, slightly rotated wedge-cut Lattice SIM view revealing full Z-stack depth and intranuclear localization of the BRCA1 405 Direct Conjugate. Reproducible doublet structures are observed and may reflect opposing ends of resected DNA damage sites; however, this interpretation remains speculative at SIM resolution. Notably, the pattern is consistently reproduced and becomes increasingly resolved using SIM² and STORM imaging.

Profile View Display - Panels D & E

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 3.02), Y (Min 0 and Max 9745)

This panel employs the Caliper X tool to quantify the reproducible BRCA1 405 Direct Conjugate doublet formation observed under Lattice SIM super-resolution imaging. Although the structures remain unresolved at this resolution, the measured inter-peak separation is approximately ~230 nm. BRCA1 405 Direct Conjugate aggregates show reproducible doublet formation under Lattice SIM super-resolution (~60-80 nm, X, Y), with improved clarity relative to Confocal and Airyscan imaging. A representative pair of foci separated by ~230 nm is shown to illustrate resolvable spatial separation. Although doublets are observed throughout the nucleus, their interpretation as DNA double-strand break ends remains speculative, as repair-associated distances are dynamic and vary with repair progression. Here we chose highly separated foci with clear delineation, focusing on the dynamics of the biology rather than the power of Lattice SIM to resolve to ~60-80nm.

Image Corrections

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR023N) was pseudo-colored in Zen Software from blue, the default color, to white throughout the analysis.

Image by J. H. Cheong

Image Analysis and Data Presentation by B.T. Bennett

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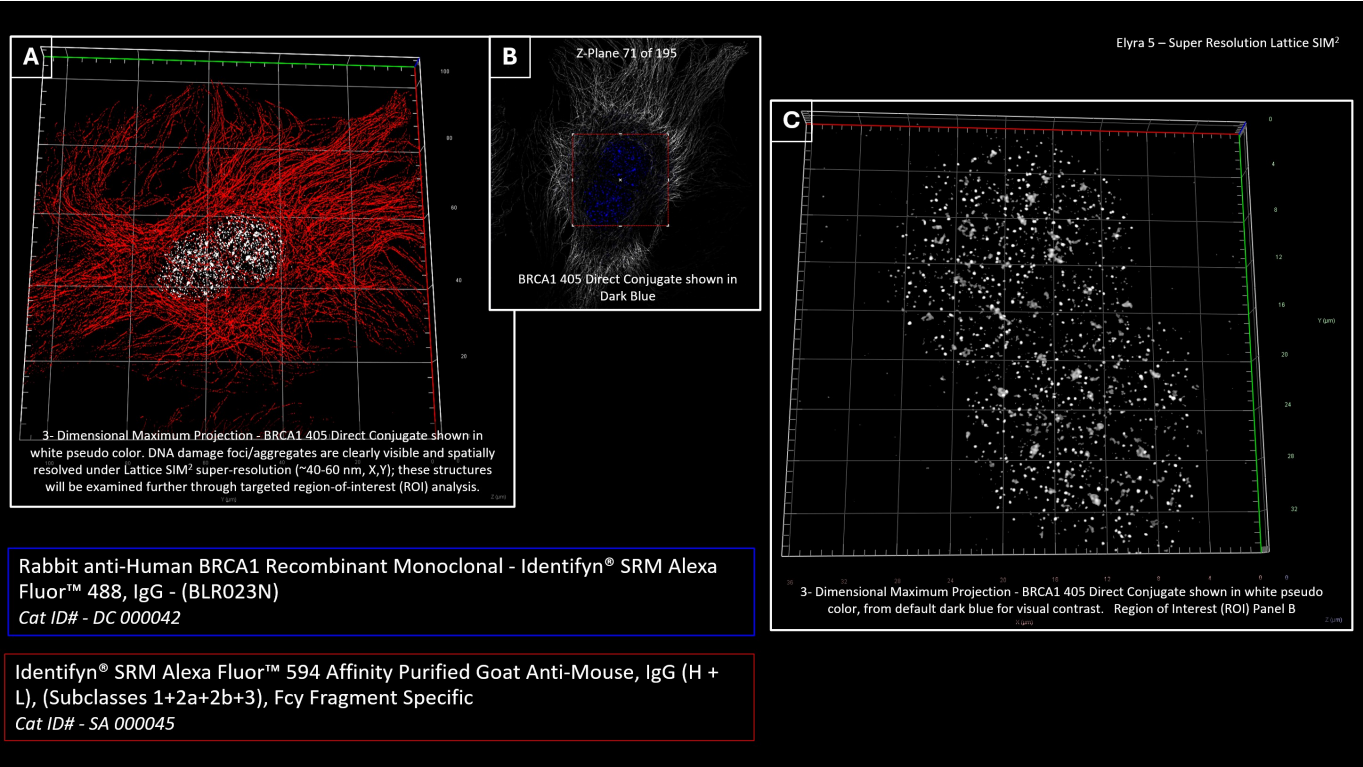
SOURCE PROVENANCE

Catalog	DC-000041
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	65

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C2875C13347AB132E32C531043F790BA38F8C7CFD8520A696BBCE734F241DC07
Method PDF SHA-256	1EBAD102B668F053C7F2E0DD909C0B7418A3398B0666C82FB97FB920AAE1F482

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	53

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	195 Slices (5.82 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 1608 X 1837
Image Size (Scaled)	106.75 µm X 106.75 µm 33.95 µm X 38.78 µm
Bit Depth	16 Bit
Image Center Position	X: 62.65 mm, Y: 37.44 mm
Stage Position	X: 62.65 mm, Y: 37.44 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	405 561
Channel Name	T2-Axiocam 820 #2-SR T1-Axiocam 820 #2-SR
Excitation Wavelength	405 561
Emission Wavelength	445 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100 ms 120 ms
Depth of Focus	0.69 µm 0.92 µm
Binning Mode	2,2
Laser Wavelength	405 nm @2.00% 561 nm @3.00%
Frame Time	676.00 ms 1.33 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
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)

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™ 405, IgG - (BLR023N)
Cat ID# - DC 000041

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000045

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

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

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

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

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

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

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

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

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

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

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG, - (BLR023N) was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 594 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, cat#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

Z-Stack = 195 Slices (5.82 μ m)

Channels = 2

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

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 106.75 μ m X 106.75 μ m

Bit Depth = 16 Bit

Image Center Position = X: 62.65 mm, Y: 37.44 mm

Stage Position = X: 62.65 mm, Y: 37.44 mm

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

Single Plane (2D) - Z-Plane 71 of 195 - Panel B

Z Stack - (3D) - Panels C

Z-Stack = 195 Slices (5.82 μ m)

Channels = 2

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

Image Size (Pixels) = 1608 X 1837

Image Size (Scaled) = 33.95 μ m X 38.78 μ m

Bit Depth = 16 Bit

Image Center Position = X: 62.65 mm, Y: 37.44 mm

Stage Position = X: 62.65 mm, Y: 37.44 mm

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

405 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 100 ms
 Depth of Focus = 0.69 μm
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @2.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

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 = 120 ms
 Depth of Focus = 0.92 μm
 Binning Mode = 2,2
 Laser Wavelength = 561 nm @3.00%
 Frame Time = 1.33 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
 Input SNR = Low
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Normalization = 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)

Region of Interest - Panel B

Demonstrates the region of interest to be shown in Panel C

3D Image Processing - Panel C

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)

Image Corrections

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR023N)
 was pseudo-colored in Zen Software from blue, the default color, to white throughout the analysis, except for Panel B.

Image by J. H. Cheong
 Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000041
Stage	SIM ²

SOURCE PROVENANCE

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	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	50AF5E92618E41102C223FE1B37CD616C05FE46ED10607C5BC9D4031F17764E2
Method PDF SHA-256	64D3D600D0052ACCB7DF0E869B68AC93AC04656DF5352E07813A75EE538F860F