BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

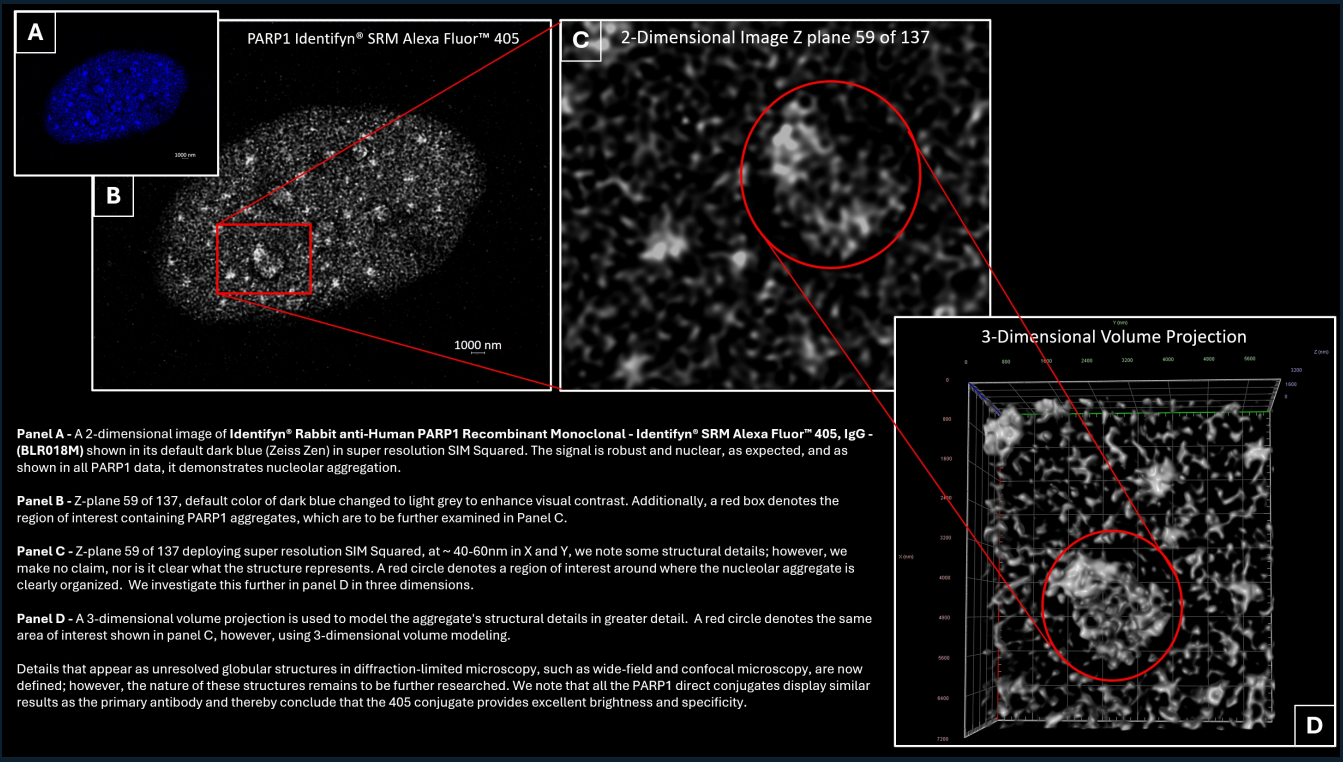
PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405

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

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A - A 2-dimensional image of **Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)** shown in its default dark blue (Zeiss Zen) in super resolution SIM Squared. The signal is robust and nuclear, as expected, and as shown in all PARP1 data, it demonstrates nucleolar aggregation.

Panel B - Z-plane 59 of 137, default color of dark blue changed to light grey to enhance visual contrast. Additionally, a red box denotes the region of interest containing PARP1 aggregates, which are to be further examined in Panel C.

Panel C - Z-plane 59 of 137 deploying super resolution SIM Squared, at ~ 40-60nm in X and Y, we note some structural details; however, we make no claim, nor is it clear what the structure represents. A red circle denotes a region of interest around where the nucleolar aggregate is clearly organized. We investigate this further in panel D in three dimensions.

Panel D - A 3-dimensional volume projection is used to model the aggregate's structural details in greater detail. A red circle denotes the same area of interest shown in panel C, however, using 3-dimensional volume modeling.

Details that appear as unresolved globular structures in diffraction-limited microscopy, such as wide-field and confocal microscopy, are now defined; however, the nature of these structures remains to be further researched. We note that all the PARP1 direct conjugates display similar results as the primary antibody and thereby conclude that the 405 conjugate provides excellent brightness and specificity.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000054 | 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 PARP1, 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-000054 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-000054 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	405/DAPI	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 647; 750	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401
Widefield SIM — Apotome	405/DAPI; 647	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401
Confocal	405/DAPI; 488; 647	2; None; 405 nm @ 1.0%; 639 nm @0.7%; 401; 653; 422; 668
Airyscan	405/DAPI; 488; 594; 647	2; None; 405nm @1.0%; 639nm @0.2%; 401; 653; 422; 668
SIM	405/DAPI; 488	1; 3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; 405; 445
SIM²	405/DAPI; 488	1; 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; 405; 445; 405 nm @5.00%

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 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 944 X 791; Image Size (Pixels): 944 X 791; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 250ms; 150ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @15%. 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; 647; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 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: 77 Slices (8.6 µm); Channels: 2; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 661 X 529; Image Size (Pixels): 661 X 529; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 350ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 25%; 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: 38.

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; 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: 141 Slices (4.2 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: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.34 µs; Frame Time: 1.88 s. Illumination: Laser Wavelength: 405 nm @ 1.0%; 639 nm @0.7%. Optical/scan: Pinhole: 1.00 AU / 41 µm; 1.00 AU / 66 µm; Scan Zoom: 2.5; 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: 6.1 (3D, Auto); 6.9 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 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: 164 Slices (1.63µm); Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 30.00nm; Image size (Pixels): 887 X 718; 283 X 247; Image Size (Pixels): 887 X 718; 283 X 247; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.82µs; Frame Time: 3.13s. Illumination: Laser Wavelength: 405nm @1.0%; 639nm @0.2%. Optical/scan: Pinhole: 5.00AU / 215µm; 5.00AU / 328µm; Scan Zoom: 3.0; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 2; 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: 8.1 (3D, Auto); Filter: 9.3 (3D, Auto). Structured source metadata values: 50.

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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 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: 62 Slices (1.83 µm); Channels: 1; 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 2656 X 2469; 595 X 492; Image Size (Pixels): 2656 X 2469; 595 X 492; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2. Timing: Exposure Time: 100 ms; Frame Time: 676.00 ms. Illumination: Laser Wavelength: 405 nm @5.00%; Illumination Wavelength: 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 54.

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.

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: 137 Slices (4.08 µm); Channels: 1; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; Image size (Pixels): 1658 X 1335; 342 X 295; Image Size (Pixels): 1658 X 1335; 342 X 295; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820 #2. Timing: Exposure Time: 100 ms; Frame Time: 676.00 ms. Illumination: Laser Wavelength: 405 nm @5.00%; Illumination Wavelength: 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 68%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 45.

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.

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-000054 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): 35.30nm X 35.30nm X 30.00nm -> 0.03530 µm X 0.03530 µm X 0.01000 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)=887 X 718; Image Size (Scaled)=31.31µm X 25.34µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm -> 0.02111 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)=2656 X 2469; Image Size (Scaled)=56.08 µm X 52.13 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)=1658 X 1335; Image Size (Scaled)=35.00 µm X 28.19 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

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.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

HIGH CONFIDENCE - SOURCE-DERIVED SEMANTIC CORRECTION

Product identity / target_name: P09874 used as canonical target name -> PARP1. The preserved product identity explicitly names PARP1. The archive does not explicitly define the semantic role of P09874, so it is retained separately as an unresolved identifier rather than treated as the biological target name. Supporting evidence: Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405

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

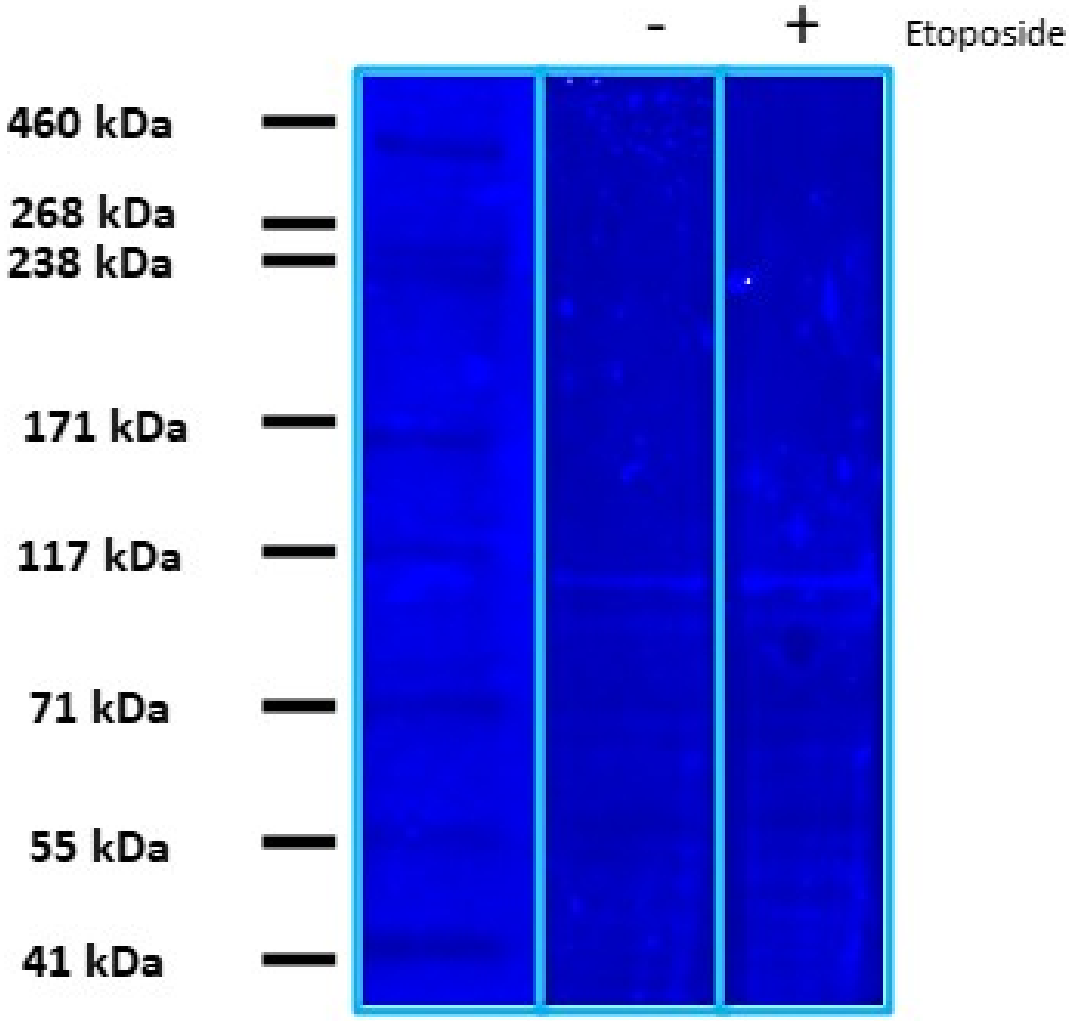
This dossier preserves the complete available evidence progression for DC-000054. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	HeLa
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	3
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.

Idenitfyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Expected MW: 116 kD

HeLa cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and 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™ Tris-Acetate Mini Protein Gels, 3-8%, 1.0 mm, 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 and then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 405 - Direct Conjugate for 2 hours, followed by five washes in 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence
Laser = 375nm
Emission Filter = 452±45nm
Detector = PMT
Intensity = 3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

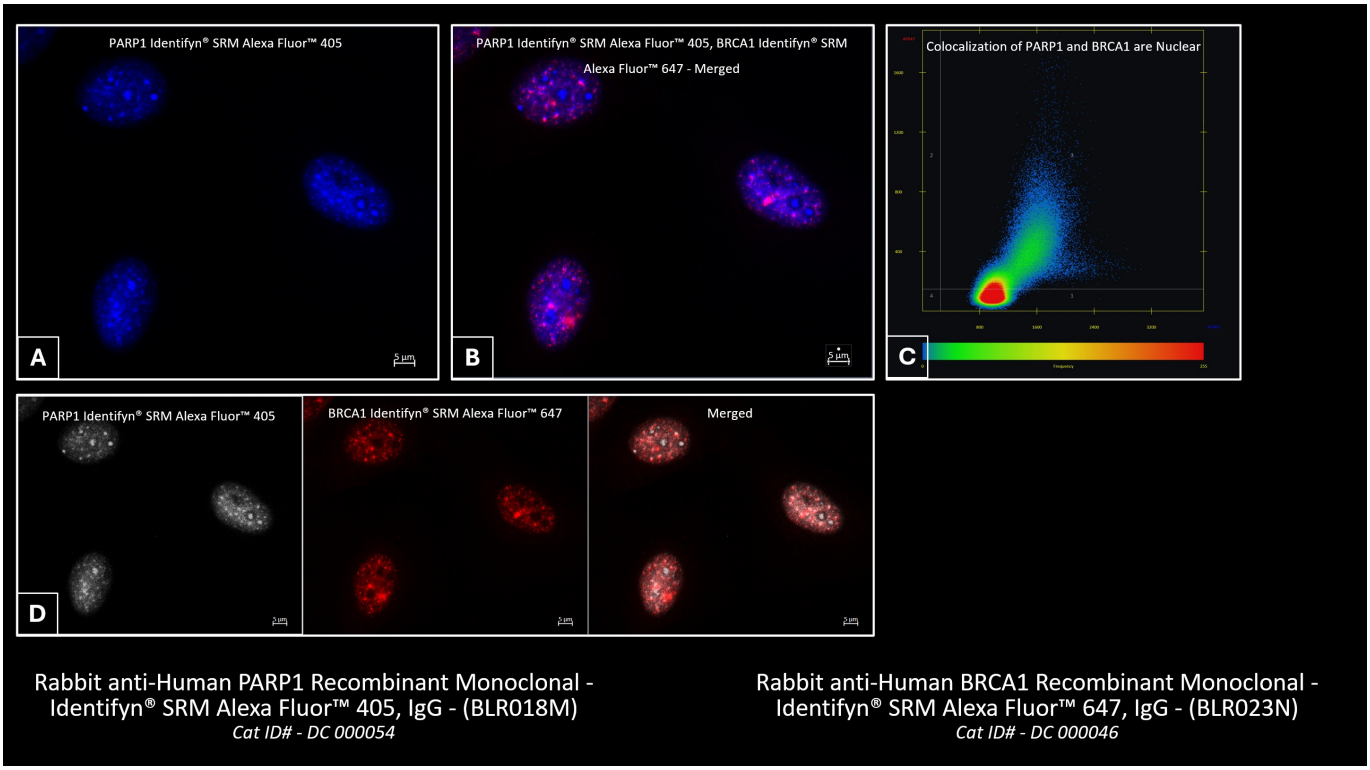
None

Image: K. Small

SOURCE PROVENANCE

Catalog	DC-000054
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	HeLa
Image SHA-256	B8B0D6457555DBA0BC007C90EE517282BD799DBEC2E900C03606797E082B055E
Method PDF SHA-256	5DECECD48FCFFC325F82089062DF202753A7491E216A61BBBB10219B83A8700D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N)
Cat ID# - DC 000046

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647; 750
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 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	944 X 791
Image Size (Scaled)	103.39 µm X 86.63 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 | 50 Cy5
Beam Splitter	412 | 660
Filter Excitation Wavelength	372 - 408 | 625 - 655
Filter Emission Wavelength	417 - 445 | 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	250ms | 150ms
Excitation Wavelength	401 | 653
Emission Wavelength	422 | 668
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.65µm | 1.03µm
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 PARP1 RecombinantMonoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N)
Cat ID# - DC 000046

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 μm X 0.110 μm
Image size (Pixels) = 944 X 791
Image Size (Scaled) = 103.39 μm X 86.63 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 = 417 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 250ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.65µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 150ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03µm
Binning Mode = 2,2

Post Processing

None

Summary -

Panel A - Demonstrates the single 405-channel, 2-dimensional view of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405 - Direct Conjugate - (BLR018M).

Panel B - Demonstrates both the 405 and 647 channels, 2-dimensional view of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405 - Direct Conjugate - (BLR018M) and Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N) in a merged image.

Panel C - Colocalization View

Horizontal Axis -BRCA1 Direct Conjugate 647 Threshold 146, Range Auto
Vertical Axis - PAPR1 Direct Conjugate 405, Threshold 255, Range Auto

Colocalization data show that both Identifyn® PARP1 SRM Alexa Fluor Direct Conjugate 405 and Identifyn® BRCA1 SRM Alexa Fluor 647 is strongly colocalized as expected, and both reside within the nuclear compartment following DNA damage.

Panel D - Split view, whereby the Identifyn® PARP1 SRM Alexa Fluor Direct Conjugate 405 default color of dark blue has been changed to light grey in Zeiss Zen to provide better visual contrast for and between the Identifyn® BRCA1 SRM Alexa Fluor 647.

Image Correction - Panel D

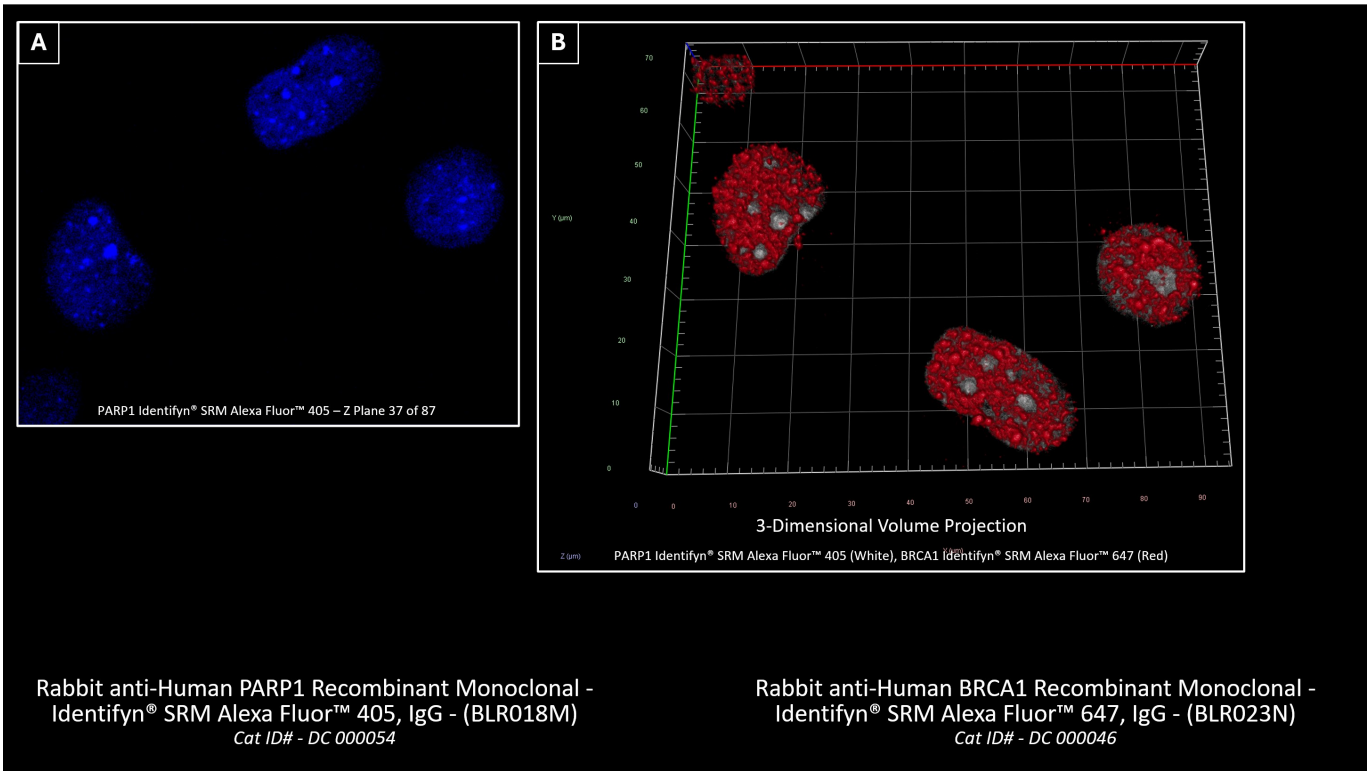
Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - BLR018M was represented as light grey instead of its default blue coloring to demonstrate contrast and show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405/647 PARP1 and BRCA1 Direct Conjugates.

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

SOURCE PROVENANCE

Catalog	DC-000054
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2D7D1C2404145338194245964 3D50FA85FF991436BFBDFB9D84DEC053432EF01
Method PDF SHA-256	1A19B554602B35E36CB1D3AAFD991A51F534E9C658A0EE5F63765509EB97A56C

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; 647
METADATA VALUES	38

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	77 Slices (8.6 µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	661 X 529
Image Size (Scaled)	94.43 µm X 75.57 µm
Bit Depth	14 Bit
Image Center Position	X: 82.52 mm, Y: 52.11 mm
Stage Position	X: 82.52 mm, Y: 52.11 mm
ROI Center Offset	X: 1.14mm, Y: 0.00mm
Reflector	AF405-49027 | 50 Cy5
Beam Splitter	412 | 660
Filter Excitation Wavelength	372 - 408 | 625 - 655
Filter Emission Wavelength	417 - 445 | 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @ 25% | X-Cite Xylis Lamp Intensity @ 20%
Exposure Time	350ms | 200 ms
Excitation Wavelength	401 | 653
Emission Wavelength	422 | 668
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	0.82µm | 1.30µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% (405), Threshold 13% (647), Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness100%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 PARP1 RecombinantMonoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N)
Cat ID# - DC 000046

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 77 Slices (8.6 μm)

Channels = 2
Scaling (Per Pixel) = 0.143 μm X 0.143 μm X 0.100 μm
Image Size (Pixels) = 661 X 529
Image Size (Scaled) = 94.43 μm X 75.57 μm
Bit Depth = 14 Bit
Image Center Position = X: 82.52 mm, Y: 52.11 mm
Stage Position = X: 82.52 mm, Y: 52.11 mm
ROI Center Offset = X: 1.14mm, Y: 0.00mm

405 -

Reflector = AF405-49027
Beam Splitter = 412
Filter Excitation Wavelength = 372 - 408
Filter Emission Wavelength = 417 - 445
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 25%
Exposure Time = 350ms
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.82µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 20%
Exposure Time = 200 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

2D Single Plane - Panel A

Z-plane 37 of 87 shown here, for the Identifyn® PARP1 SRM Alexa Fluor 405 in its default color of dark blue.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 13% (405), Threshold 13% (647), Ramp 50% all channels, Maximum 100% all channels
Background = Black
Light = Brightness100%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections - Panel B

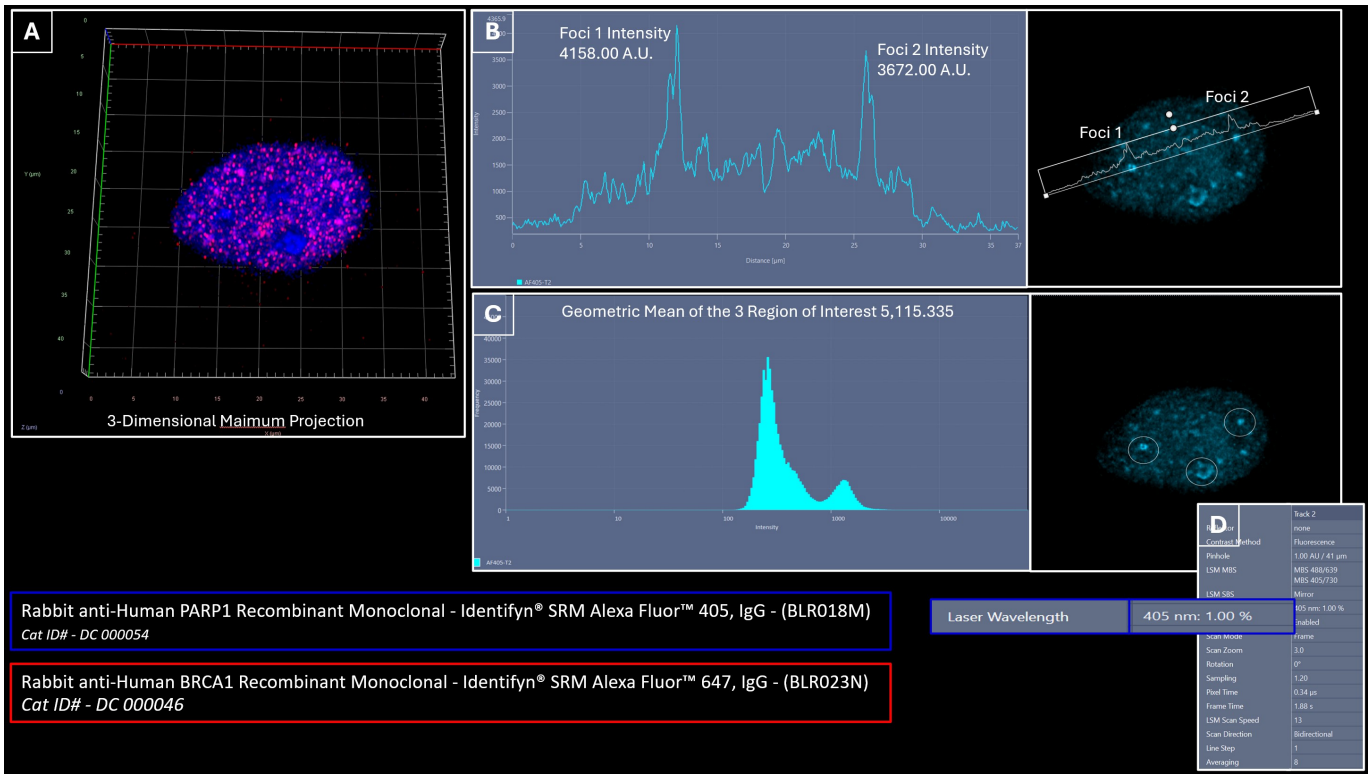
Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor 405, IgG - BLR018M was represented as light grey instead of its default blue coloring to demonstrate contrast and show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405/647 PARP1 and BRCA1 Direct Conjugates.

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

SOURCE PROVENANCE

Catalog	DC-000054
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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	14E6DF73C480CD2FEA750882A0F1700A9FC1BEC3B6C068491F07D2B0EA3A313F
Method PDF SHA-256	6F47AAE31F2D1EF0A85AF47ACC6A40084128F3C355F9A06AD4908EE569A37449

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	50

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	141 Slices (4.2 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: 64.70 mm, Y: 48.53 mm
Stage Position	X: 64.70 mm, Y: 48.53 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 41 µm | 1.00 AU / 66 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	Mirror
Laser Wavelength	405 nm @ 1.0% | 639 nm @0.7%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.5
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 | 653
Emission Wavelength	422 | 668
Effective NA	1.4
Detection Wavelength	395 - 460 | 635 - 745
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	6.1 (3D, Auto) | 6.9 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Profile Definition	Stroke Thickness 1.0, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 37.0), Y (Min 189 and Max 4366)
Geometric Mean	5,155.335

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 PARP1 RecombinantMonoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N)
Cat ID# - DC 000046

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 87 of 141 total - Panels B and C (Profile and Histogram Measurements)

Z Stack - (3D) - Panels A (Maximum Projection)

Z-Stack = 141 Slices (4.2 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: 64.70 mm, Y: 48.53 mm
Stage Position = X: 64.70 mm, Y: 48.53 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

405 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 41 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @ 1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
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
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 395 - 460
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.1 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 66 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 639 nm @0.7%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
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 = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 635 - 745
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.9 (3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Profile View Display - Panel B, Measuring 2 Specific DNA Damage Induced Foci

Profile Definition = Stroke Thickness 1.0, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 37.0), Y (Min 189 and Max 4366)

Histogram View - Panel C, 3 Regions of Interest

Histogram Definition - Normal, Histo Table was Frequency, Bin Count 256, Bin Size 256, Lower Threshold 0, Upper Threshold 65535, Showing Threshold, and deploying Logarithmic Binning

Histogram View - X and Y axis auto, Channel Transparency 100%, Statistic Table, Frequency Table, Internal Measurements, and Image were selected.

Geometric Mean = 5,155.335

Zeiss Zen Settings - Panel D, Laser Power Input

A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 141 planes. We note that the 405 nm laser power is set at 1.0% to obtain maximal imaging of the direct conjugate. Indicating high brightness and stability over the course of the z-stack comprised of 141 z-planes.

Image Corrections - Panels B and C

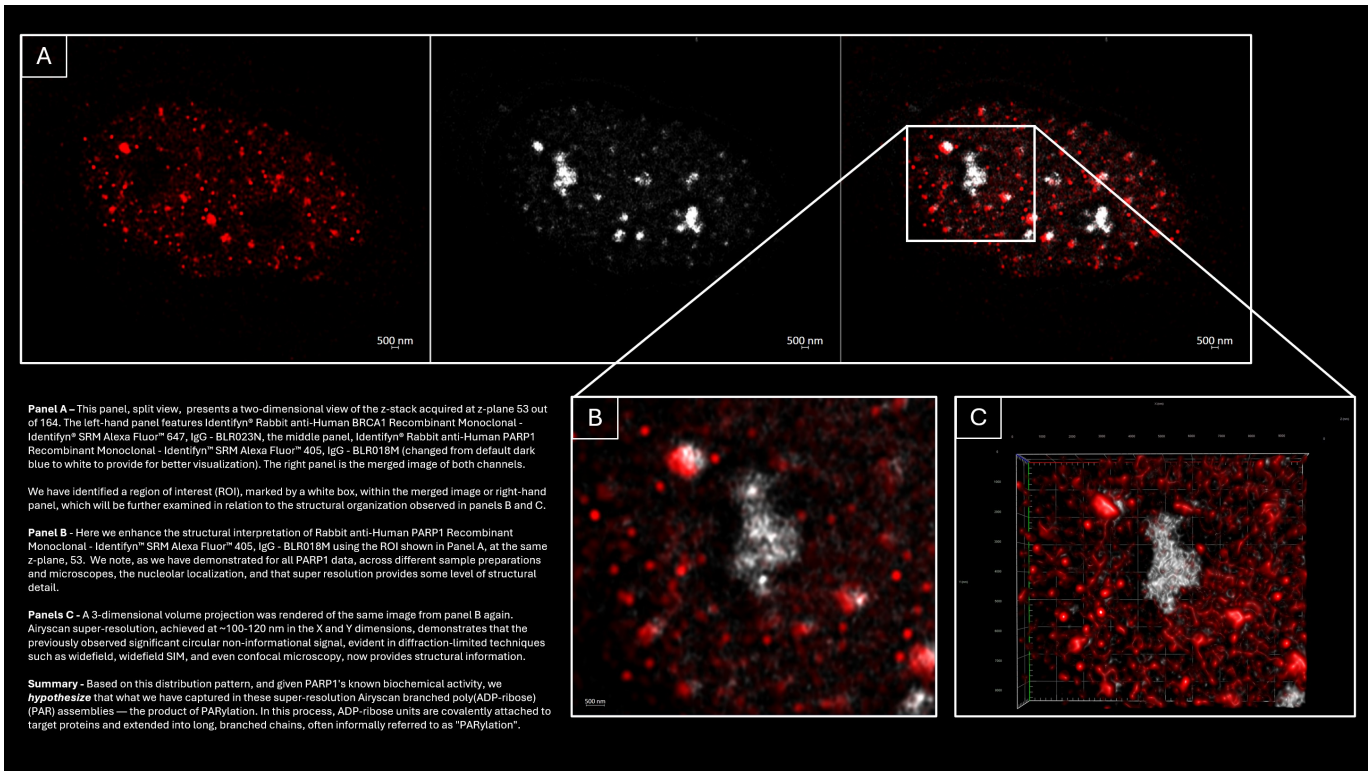
Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M) was pseudo-colored in Zen Software from dark blue, the default color, to light blue to enhance visual contrast.

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

SOURCE PROVENANCE

Catalog	DC-000054
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A884D685A7C64E63CA5A1EE7B6EC09E52A7E34F79ED14C2DDE0B911696A9C1A6
Method PDF SHA-256	BD1D9D88E3B6A46CEF0C92325E3EB523AC590225A051383897821169D9DE928E

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647
METADATA VALUES	50

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	164 Slices (1.63µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.01000 µm
Image Size (Pixels)	887 X 718 | 283 X 247
Image Size (Scaled)	31.31µm X 25.34µm | 9.99µm X 8.72µm
Bit Depth	16 Bit
Image Center Position	X: 64.24mm, Y: 47.43mm
Stage Position	X: 64.24mm, Y: 47.43mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 215µm | 5.00AU / 328µm
LMS MBS	Plate MBS 488/639 MBS 405/730
LMS SBS	SBS SP 505 | SBS LP 640
Laser Wavelength	405nm @1.0% | 639nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.00
Pixel Time	0.82µs
Frame Time	3.13s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	401 | 653
Emission Wavelength	422 | 668
Effective NA	1.4
Detection Wavelength	350 - 499 | 643 - 735
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsp-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.1 (3D, Auto) | Filter: 9.3 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% (405), Threshold 2% (647), Ramp98% (405), Ramp 98% (647), 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)

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 PARP1 RecombinantMonoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N)
Cat ID# - DC 000046

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR023N), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack =164 Slices (1.63μm)

Channels = 2
Scaling (Per Pixel) = 35.30nm X 35.30nm X 30.00nm
Image Size (Pixels) = 887 X 718
Image Size (Scaled) = 31.31μm X 25.34μm
Bit Depth = 16 Bit
Image Center Position = X: 64.24mm, Y: 47.43mm
Stage Position =X: 64.24mm, Y: 47.43mm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

Z Stack - (3D) - Panel B ROI

Z-Stack =164 Slices (1.63µm)

Channels = 2
Scaling (Per Pixel) = 35.30nm X 35.30nm X 30.00nm
Image Size (Pixels) = 283 X 247
Image Size (Scaled) = 9.99µm X 8.72µm
Bit Depth = 16 Bit
Image Center Position = X: 64.24mm, Y: 47.43mm
Stage Position =X: 64.24mm, Y: 47.43mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 215µm
LMS MBS = Plate MBS 488/639 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time =3.13s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.1 (3D, Auto)

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328µm
LMS MBS = Plate MBS 488/639 MBS 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 3.13s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 9.3 (3D, Auto)

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 2% (405), Threshold 2% (647), Ramp98% (405), Ramp 98% (647), 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)

Summary -

Panel A - This panel, split view, presents a two-dimensional view of the z-stack acquired at z-plane 53 out of 164. The left-hand panel features Identifyn® Rabbit anti-Human BRCA1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - BLR023N, the middle panel, Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - BLR018M (changed from default dark blue to white to provide for better visualization). The right panel is the merged image of both channels.

We have identified a region of interest (ROI), marked by a white box, within the merged image or right-hand panel, which will be further examined in relation to the structural organization observed in panels B and C.

Panel B - Here we enhance the structural interpretation of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 405, IgG - BLR018M using the ROI shown in Panel A, at the same z-plane, 53. We note, as we have demonstrated for all PARP1 data across different sample preparations and microscopes, the nucleolar localization, and that super resolution provides some level of structural detail.

Panels C - A 3-dimensional volume projection was rendered of the same image from panel B again. Airyscan super-resolution, achieved at ~100-120 nm in the X and Y dimensions, demonstrates that the previously observed significant circular non-informational signal, evident in diffraction-limited techniques such as widefield, widefield SIM, and even confocal microscopy, now provides structural information.

Based on this distribution pattern and given PARP1's known biochemical activity, we hypothesize that the structures we have captured in these super resolution Airyscan branched poly(ADP-ribose) (PAR) assemblies represent the product of PARylation. In this process, ADP-ribose units are covalently attached to target proteins and extended into long, branched chains, often referred to informally as "PARylation".

Image Corrections

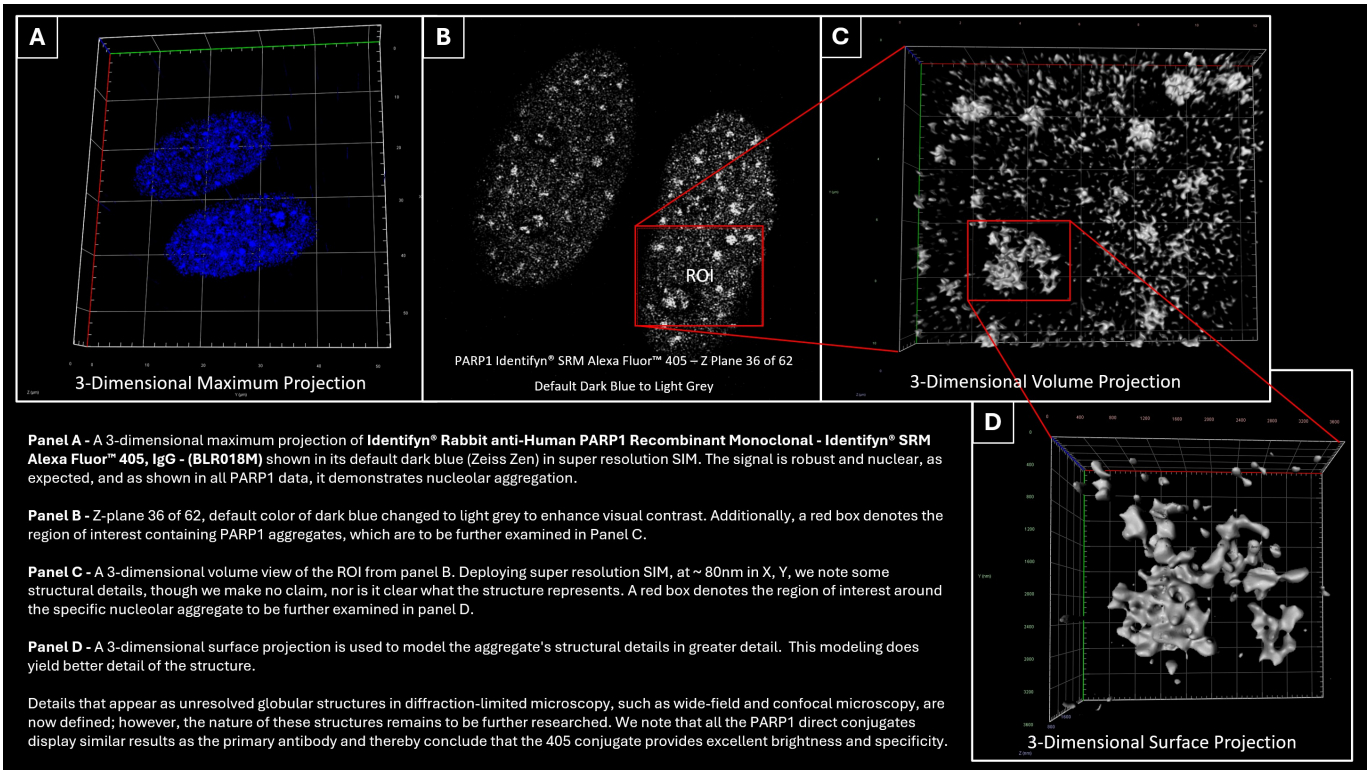
Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from dark blue, the default color, to white to provide visual contrast between the 405/594 channels.

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

SOURCE PROVENANCE

Catalog	DC-000054
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BD9DE0BD01AE5DDECC6199FFE755D9F108FD9A157447E63EBA5DA7D98B280B24
Method PDF SHA-256	801385D289771F0FD60543554C3A2D9B99F388F07590A8AC2374296E679E9F94

ORIGINAL IMAGE EVIDENCE / SIM



Panel A - A 3-dimensional maximum projection of **Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)** shown in its default dark blue (Zeiss Zen) in super resolution SIM. The signal is robust and nuclear, as expected, and as shown in all PARP1 data, it demonstrates nucleolar aggregation.

Panel B - Z-plane 36 of 62, default color of dark blue changed to light grey to enhance visual contrast. Additionally, a red box denotes the region of interest containing PARP1 aggregates, which are to be further examined in Panel C.

Panel C - A 3-dimensional volume view of the ROI from panel B. Deploying super resolution SIM, at ~ 80nm in X, Y, we note some structural details, though we make no claim, nor is it clear what the structure represents. A red box denotes the region of interest around the specific nucleolar aggregate to be further examined in panel D.

Panel D - A 3-dimensional surface projection is used to model the aggregate's structural details in greater detail. This modeling does yield better detail of the structure.

Details that appear as unresolved globular structures in diffraction-limited microscopy, such as wide-field and confocal microscopy, are now defined; however, the nature of these structures remains to be further researched. We note that all the PARP1 direct conjugates display similar results as the primary antibody and thereby conclude that the 405 conjugate provides excellent brightness and specificity.

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

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	62 Slices (1.83 µm)
Channels	1 | 3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	2656 X 2469 | 595 X 492 | 179 X 171
Image Size (Scaled)	56.08 µm X 52.13 µm | 12.56 µm X 10.39 µm | 3.78 µm X 3.61 µm
Bit Depth	16 Bit
Image Center Position	X: 61.13 mm, Y: 38.42 mm
Stage Position	X: 61.13 mm, Y: 38.42 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
Channel Name	T1-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 @5.00%
Frame Time	676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.3057 (405)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 2%, Ramp 0% , Maximum 100% | Threshold 4%, Ramp 96% , Maximum 100% | Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping | Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
3D Surface 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 PARP1 Recombinan tMonoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 36 of 62 total - Panel B

Z Stack - (3D) - Panel A (Maximum Projection), and Panel B (2-dimensional)

Z-Stack = 62 Slices (1.83 μm)

Channels = 1
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 2656 X 2469
Image Size (Scaled) = 56.08 μm X 52.13 μm
Bit Depth = 16 Bit
Image Center Position = X: 61.13 mm, Y: 38.42 mm
Stage Position = X: 61.13 mm, Y: 38.42 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - Panel C

Z-Stack = 62 Slices (1.83 µm)

Channels = 1
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 595 X 492
Image Size (Scaled) = 12.56 µm X 10.39 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.13 mm, Y: 38.42 mm
Stage Position = X: 61.13 mm, Y: 38.42 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D (Surface Projection)

Z-Stack = 62 Slices (1.83 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 179 X 171
Image Size (Scaled) = 3.78 µm X 3.61 µm
Bit Depth = 16 Bit
Image Center Position = X: 61.13 mm, Y: 38.42 mm
Stage Position = X: 61.13 mm, Y: 38.42 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 = T1-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 @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 1
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 10.3057 (405)
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 2%, Ramp 0% , Maximum 100%
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 4%, Ramp 96% , Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel D

3D Surface Projection = Precise
Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%
Background = Black
Light = Azimuth 80°, Elongation -130°, 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)

Summary

Panel A - A 3-dimensional maximum projection of Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M) shown in its default dark blue (Zeiss Zen) in super resolution SIM. The signal is robust and nuclear, as expected, and as shown in all PARP1 data, it demonstrates nucleolar aggregation.

Panel B - Z-plane 36 of 62, default color of dark blue changed to light grey to enhance visual contrast. Additionally, a red box denotes the region of interest containing PARP1 aggregates, which are to be further examined in Panel C.

Panel C - A 3-dimensional volume view of the ROI from panel B. Deploying super resolution SIM, at ~ 80nm in X, Y, we note some structural details, though we make no claim, nor is it clear what the structure represents. A red box denotes the region of interest around the specific nucleolar aggregate to be further examined in panel D.

Panel D - A 3-dimensional surface projection is used to model the aggregate's structural details in greater detail. This modeling does yield better detail of the structure.

Details that appear as unresolved globular structures in diffraction-limited microscopy, such as wide-field and confocal microscopy, are now defined; however, the nature of these structures remains to be further researched. We note that all the PARP1 direct conjugates display similar results as the primary antibody and thereby conclude that the 405 conjugate provides excellent brightness and specificity.

Image Corrections

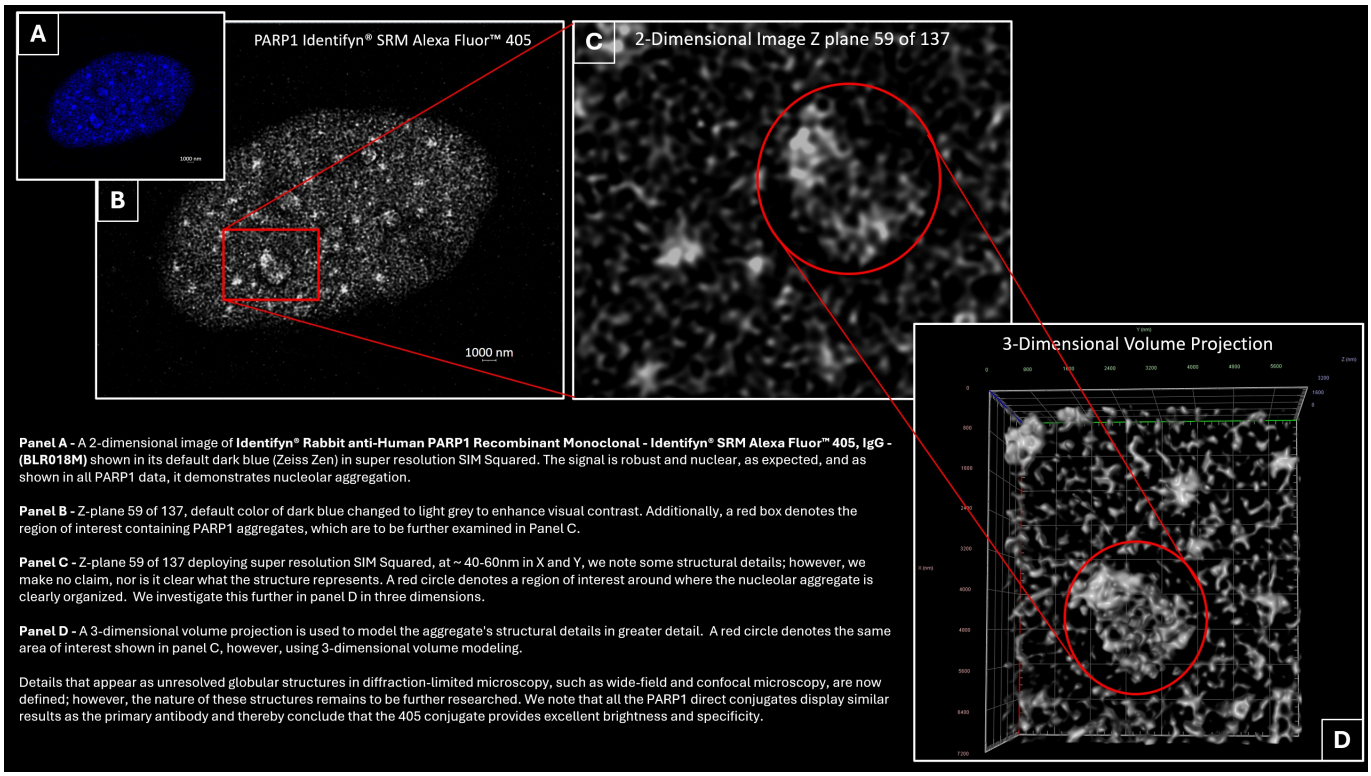
Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M) was psuedo colored from the default dark blue to light grey to provide enhanced visualization in panels B, C, and D.

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

SOURCE PROVENANCE

Catalog	DC-000054
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	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4C723D963E53F6BC95F0ADF25993E0572FEEC2CFC13E7CAF3960FA2EF638D9AD
Method PDF SHA-256	E08DCB4779BF94C0A27C98EFC2CC3477CA67D41ABB347EB2BB9BEA64379D64E2

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - A 2-dimensional image of **Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)** shown in its default dark blue (Zeiss Zen) in super resolution SIM Squared. The signal is robust and nuclear, as expected, and as shown in all PARP1 data, it demonstrates nucleolar aggregation.

Panel B - Z-plane 59 of 137, default color of dark blue changed to light grey to enhance visual contrast. Additionally, a red box denotes the region of interest containing PARP1 aggregates, which are to be further examined in Panel C.

Panel C - Z-plane 59 of 137 deploying super resolution SIM Squared, at ~ 40-60nm in X and Y, we note some structural details; however, we make no claim, nor is it clear what the structure represents. A red circle denotes a region of interest around where the nucleolar aggregate is clearly organized. We investigate this further in panel D in three dimensions.

Panel D - A 3-dimensional volume projection is used to model the aggregate's structural details in greater detail. A red circle denotes the same area of interest shown in panel C, however, using 3-dimensional volume modeling.

Details that appear as unresolved globular structures in diffraction-limited microscopy, such as wide-field and confocal microscopy, are now defined; however, the nature of these structures remains to be further researched. We note that all the PARP1 direct conjugates display similar results as the primary antibody and thereby conclude that the 405 conjugate provides excellent brightness and specificity.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	45

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
Channels	1
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	1658 X 1335 | 342 X 295
Image Size (Scaled)	35.00 µm X 28.19 µm | 7.22 µm X 6.23 µm
Bit Depth	16 Bit
Image Center Position	X: 62.41 mm, Y: 38.97 mm
Stage Position	X: 62.41 mm, Y: 38.97 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Z-Stack	137 Slices (4.08 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
Channel Name	T1-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 @5.00%
Frame Time	676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2%, Ramp 98% , Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 68%, 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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M)
Cat ID# - DC 000054

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD+; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed five times in PBS and then mounted using Prolong Diamond Antifade Mountant (cat# 36961).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D) - Z-plane 59 of 137 total - Panel A, B, and C

Channels = 1
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 1658 X 1335
Image Size (Scaled) = 35.00 μm X 28.19 μm
Bit Depth = 16 Bit
Image Center Position = X: 62.41 mm, Y: 38.97 mm
Stage Position = X: 62.41 mm, Y: 38.97 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - Panel D

Z-Stack = 137 Slices (4.08 µm)

Channels = 1
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 342 X 295
Image Size (Scaled) = 7.22 µm X 6.23 µm
Bit Depth = 16 Bit
Image Center Position = X: 62.41 mm, Y: 38.97 mm
Stage Position = X: 62.41 mm, Y: 38.97 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 = T1-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 @5.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = 4
Channels = 1
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel D

3D Volume Projection = Precise
Appearance = Threshold 2%, Ramp 98% , Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 68%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - A 2-dimensional image of Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M) shown in its default dark blue (Zeiss Zen) in super resolution SIM Squared. The signal is robust and nuclear, as expected, and as shown in all PARP1 data, it demonstrates nucleolar aggregation.

Panel B - Z-plane 59 of 137, default color of dark blue changed to light grey to enhance visual contrast. Additionally, a red box denotes the region of interest containing PARP1 aggregates, which are to be further examined in Panel C.

Panel C - Z-plane 59 of 137 deploying super resolution SIM Squared, at ~ 40-60nm in X and Y, we note some structural details; however, we make no claim, nor is it clear what the structure represents. A red circle denotes a region of interest around where the nucleolar aggregate is clearly organized. We investigate this further in panel D, examining it in three dimensions.

Panel D - A 3-dimensional volume projection is used to model the aggregate's structural details in greater detail. A red circle denotes the same area of interest shown in panel C, however, using 3-dimensional volume modeling.

Details that appear as unresolved globular structures in diffraction-limited microscopy, such as wide-field and confocal microscopy, are now defined; however, the nature of these structures remains to be further researched. We note that all the PARP1 direct conjugates display similar results as the primary antibody and thereby conclude that the 405 conjugate provides excellent brightness and specificity.

Image Corrections

Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 405, IgG - (BLR018M) was psuedo colored from the default dark blue to light grey to provide enhanced visualization in panels B, C, and D.

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

SOURCE PROVENANCE

Catalog	DC-000054
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	45

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	305E90C7FD7CA9A472DF3B9CFD5800666646BF244C6D7027336F7EDDA1ABA879
Method PDF SHA-256	C9E8F1427E4CB5902EBBC2C86BB11A9E6544CD0D023F0E6D5679D17EE8581A9D