

IDENTIFYN RESOLUTION STEPDOWN

PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 488

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

7

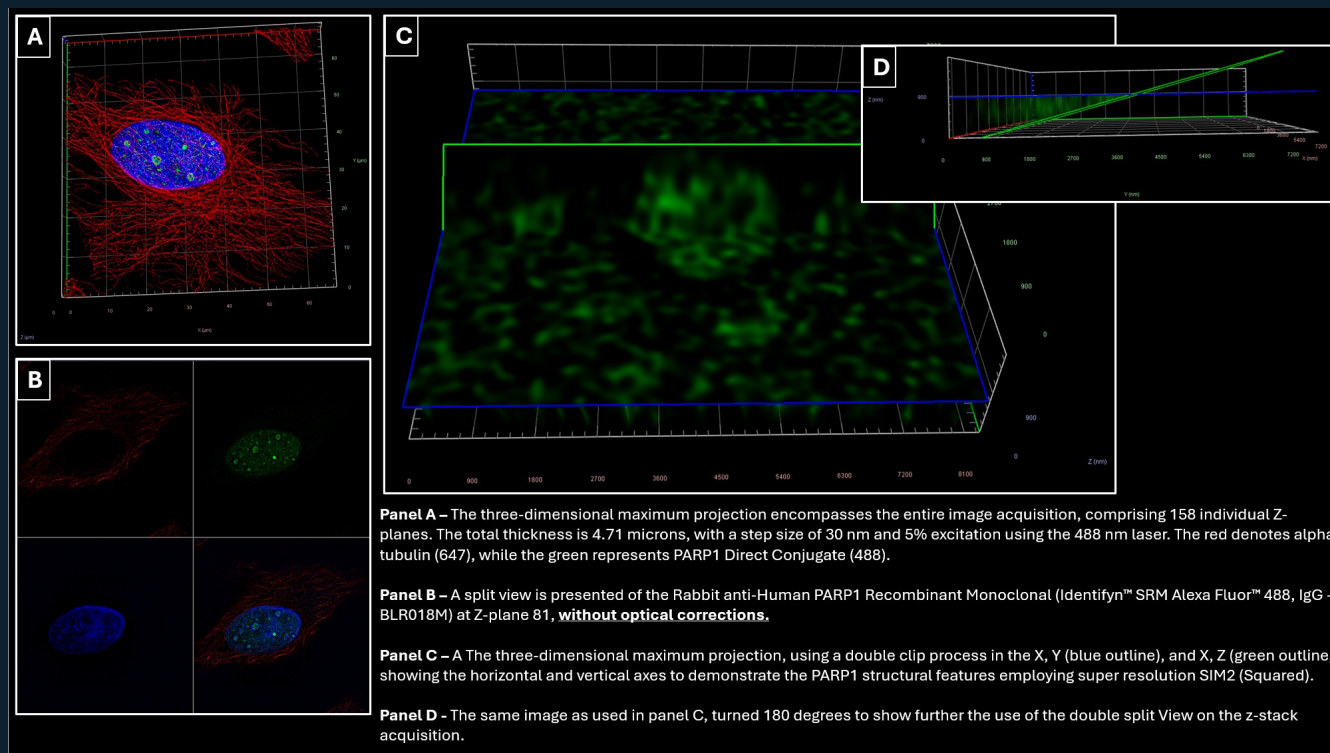
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000009 | 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-000009 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-000009 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	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	2; 38 eGFP; 50 Cy5; 49 DAPI; 450 - 490; 625 - 655; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 38 HE eGFP; 50 Cy5; 49 DAPI; 450 - 490; 625 - 655; 335-383; 500 - 550
Confocal	405/DAPI; 488	2; None; 488nm @0.3%; 405nm @0.2%; 493; 353; 517; 465
Airyscan	405/DAPI; 488	2; None; 488nm @0.3%; 405nm @0.2%; 493; 353; 517; 465
SIM	405/DAPI; 488; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T1-Axiocam 820 -SR; 488; 640
SIM ²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T1-Axiocam 820 -SR; T3-Axiocam 820 #2 -SR; 488

MODALITY ASSESSMENT / PART 1 OF 7

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 488.

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): 0.110µm X 0.110µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 400ms; 40ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @8.15%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 48.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 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: 97 Slices (9.6µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 200.00nm; Image size (Pixels): 512 X 496; Image Size (Pixels): 512 X 496; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 400ms; 50ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20.00%; X-Cite Xylis Lamp Intensity @6%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 49.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 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: 151 Slices (4.50µm); Channels: 2; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.030µm; Image size (Pixels): 1017 X 1017; Image Size (Pixels): 1017 X 1017; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt 3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.33µs; Frame Time: 3.36s. Illumination: Laser Wavelength: 488nm @0.3%; 405nm @0.2%. Optical/scan: Pinhole: 1.00AU / 49µm; 1.0 AU / 42µm; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 6.8 (3D, Auto); 6.1 (3D, Auto). Structured source metadata values: 59.

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.

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: 131 Slices (3.90µm); Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 0.030nm; Image size (Pixels): 646 X 792; Image Size (Pixels): 646 X 792; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.10µs; Frame Time: 18.77s. Illumination: Laser Wavelength: 488nm @0.3%; 405nm @0.2%. Optical/scan: Pinhole: 5.00AU / 250µm; 5.46 AU / 235µm; Scan Zoom: 2.0; LSM Scan Speed: 6; 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; AiryScan Mode: SuperResolution 9.5 (3D, Auto). Structured source metadata values: 55.

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.

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, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 158 Slices (4.71µm); Channels: 2; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; Image Size (Pixels): 5056 X 5056; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820. Timing: Exposure Time: 150ms; 120ms; Frame Time: 1.98s; 1.59s. Illumination: Laser Wavelength: 488nm @5.0%; 640nm @1.5%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; 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: 52.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 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, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 158 Slices (4.71µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 631 X 595; Image Size (Pixels): 5056 X 5056; 631 X 595; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820; AxioCam 820 #2. Timing: Exposure Time: 150ms; 120ms; Frame Time: 1.98s; 1.59s. Illumination: Laser Wavelength: 488nm @5.0%; 640nm @1.5%; Illumination Wavelength: 488; 640. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 68.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000009 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

Widefield SIM — Apotome / Scaling (Per Pixel): $0.143\mu\text{m} \times 0.143\mu\text{m} \times 200.00\text{nm} \rightarrow 0.14285\mu\text{m} \times 0.14286\mu\text{m} \times 0.10000\mu\text{m}$. 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)=512 X 496; Image Size (Scaled)=73.14 μm X 70.86 μm ; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): $13.20\text{nm} \times 13.20\text{nm} \times 30.00\text{nm} \rightarrow 0.01320\mu\text{m} \times 0.01320\mu\text{m} \times 0.03000\mu\text{m}$. 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)=66.72 μm X 66.72 μm ; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): $13.20\text{nm} \times 13.20\text{nm} \times 30.00\text{nm} \rightarrow 0.01320\mu\text{m} \times 0.01320\mu\text{m} \times 0.03000\mu\text{m}$. 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)=66.72 μm X 66.72 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 ; 2 μM $\rightarrow$ Etoposide = 2 μM . The governing scientist identified every 200 μM occurrence as a technician transcription error. The canonical historical concentration is 2 μM . Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

BENNETT 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

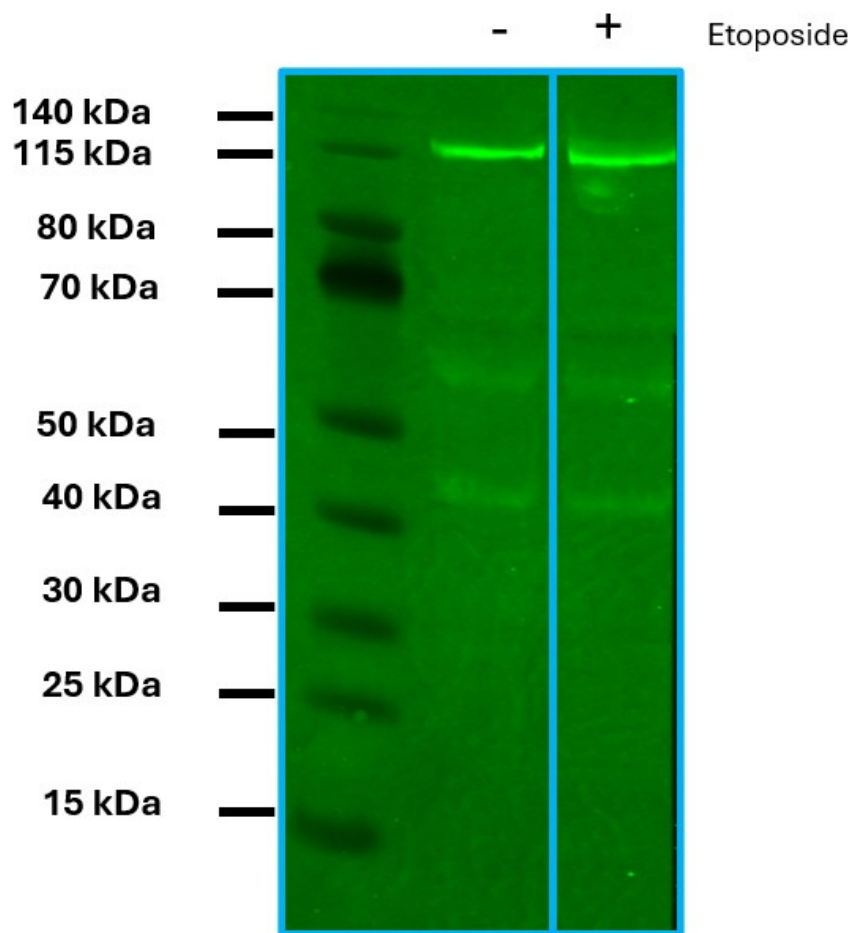
This dossier preserves the complete available evidence progression for DC-000009. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

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	488
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	488nm
Emission Filter	513±17nm
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 PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

Expected Molecular Weight: 116 kDa

HEK293 cells were damaged with 200 µM etoposide for 24 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 (100µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12%, Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, 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 Mouse anti-Human PARP1 Monoclonal, IgG1, for 2 hours, followed by five washes in 1X PBS. Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Control Data - Primary Unconjugated Antibody

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

Scan Settings

Detection mode = Fluorescence

Laser = 488nm

Emission Filter = 513±17nm

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

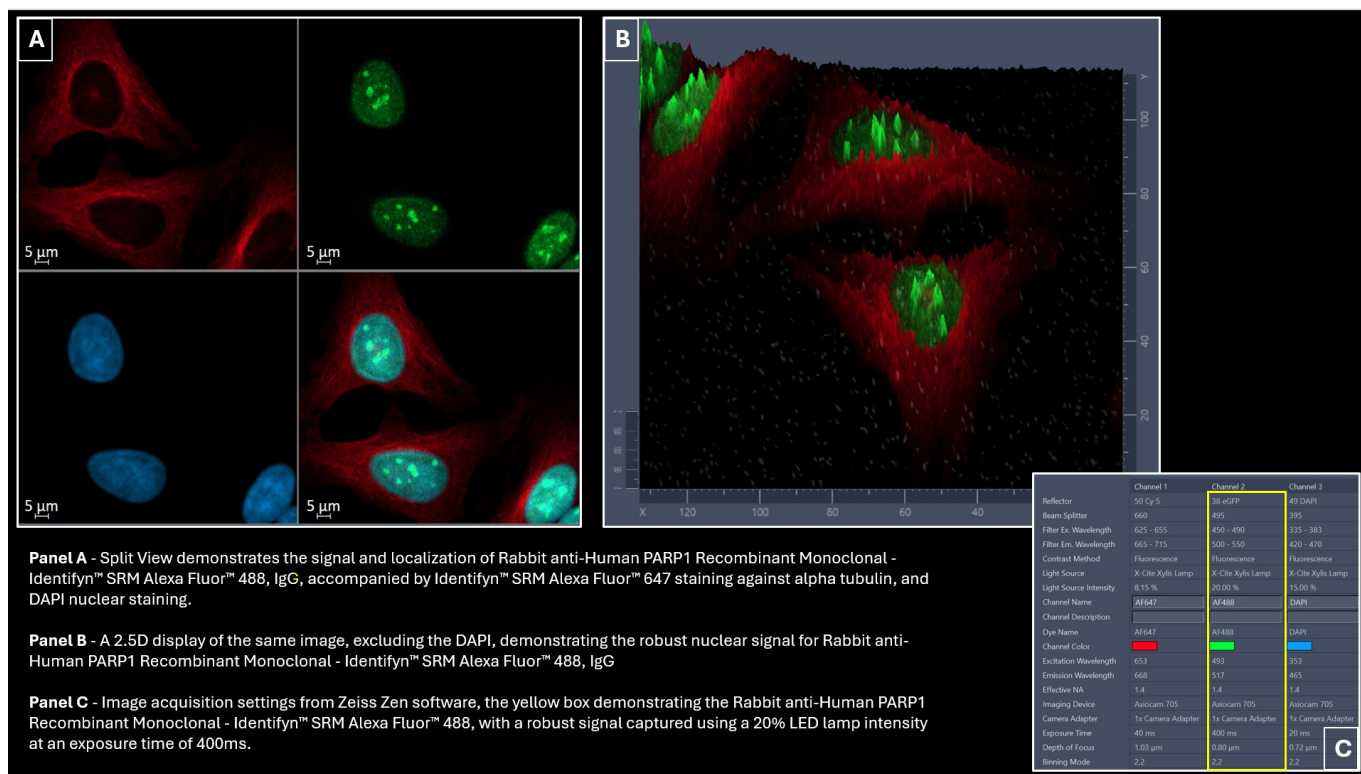
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000009
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	0304850BC4FEDEA9477F7CD2917AE1DD14A7DDD338C856D5E1EB13595129C636
Method PDF SHA-256	0E88ECE417D1D523E9E44CC34541D32B8BF6C74B36EE342AEC69372DDE270108

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View demonstrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 488, IgG, accompanied by Identifyn™ SRM Alexa Fluor™ 647 staining against alpha tubulin, and DAPI nuclear staining.

Panel B - A 2.5D display of the same image, excluding the DAPI, demonstrating the robust nuclear signal for Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 488, IgG

Panel C - Image acquisition settings from Zeiss Zen software, the yellow box demonstrating the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 488, with a robust signal captured using a 20% LED lamp intensity at an exposure time of 400ms.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	48

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)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	38 eGFP 50 Cy5 49 DAPI
Beam Splitter	495 660 395
Filter Excitation Wavelength	450 - 490 625 - 655 335 - 383
Filter Emission Wavelength	500 - 550 665 - 715 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @8.15% X-Cite Xylis Lamp Intensity @15%
Exposure Time	400ms 40ms 20ms
Excitation Wavelength	493 653 353
Emission Wavelength	517 668 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.80µm 1.03µm 0.72µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	17
Angle Y	-180
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

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™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000059

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

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) = 0.110µm X 0.110µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18µm X 112.59µm

Bit Depth = 14 Bit

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

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

488 -

Reflector = 38 eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @20%
 Exposure Time = 400ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80µm
 Binning Mode = 2,2

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 @8.15%
 Exposure Time = 40ms
 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

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15%
 Exposure Time = 20ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 2,2

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface

Grid distance = 5

Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 17

Angle Y = -180

Z Scaling = 1.0

Ambient =50

Specular = 20

Shininess = 50

Light Height = 2.0

Summary

Panel A - Split View demonstrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG, accompanied by Identifyn® SRM Alexa Fluor™ 647 staining against alpha tubulin, and DAPI nuclear staining.

Panel B - A 2.5D display of the same image, excluding the DAPI, demonstrating the robust nuclear signal for Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG

Panel C - Image acquisition settings from Zeiss Zen software, the yellow box demonstrating the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, with a robust signal captured using a 20% LED lamp intensity at an exposure time of 400ms.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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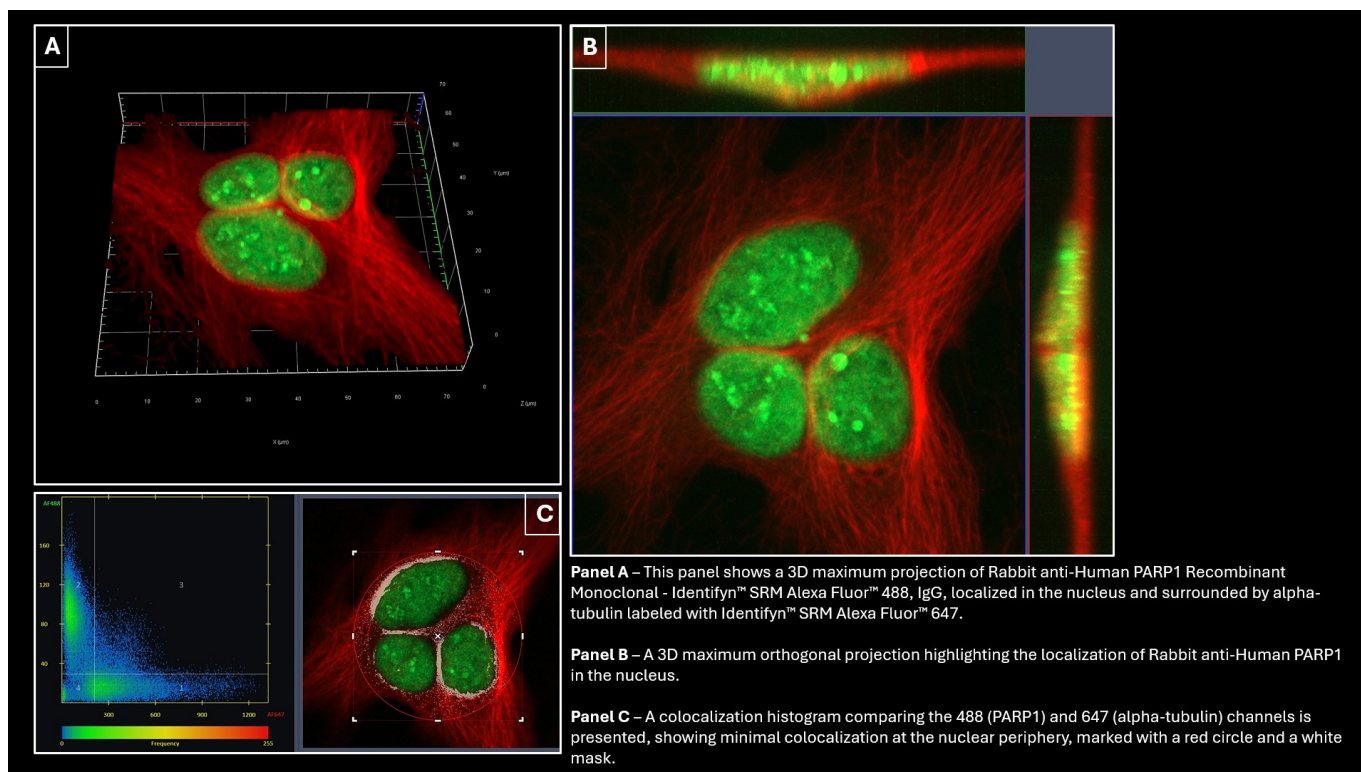
SOURCE PROVENANCE

Catalog	DC-000009
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	48
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	F8892419CEBD4B6E52915A7CD2C843489B9202262C3C83DAF05FBD47BA124DEE
Method PDF SHA-256	39C98C21A7987A21CAFABE334F3C6BE9DB80ED38784A00C13A56691C12D2A02C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



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

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	97 Slices (9.6µm)
Channels	3
Scaling (Per Pixel)	0.14285 µm X 0.14286 µm X 0.10000 µm
Image Size (Pixels)	512 X 496
Image Size (Scaled)	73.14µm X 70.86µm
Bit Depth	14 Bit
Image Center Position	X: 52.02mm, Y: 47.38mm
Stage Position	X: 52.02mm, Y: 47.38mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	38 HE eGFP 50 Cy5 49 DAPI
Beam Splitter	495 660 395
Filter Excitation Wavelength	450 - 490 625 - 655 335-383
Filter Emission Wavelength	500 - 550 665 - 715 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20.00% X-Cite Xylis Lamp Intensity @6%
Exposure Time	400ms 50ms 18ms
Excitation Wavelength	493 653 353
Emission Wavelength	517 668 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.00µm 1.30µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% (488), Threshold 13% (647), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 100%, Tone Mapping was NOT Enabled
Projection	View Angle35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Cut Lines	X 303, Y 287, Z 53

FIELD	SOURCE-DOCUMENTED VALUE
Line Width	1, all
Cut Line Opacity	50

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™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000059

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

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 = 97 Slices (9.6µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm X 200.00nm
Image Size (Pixels) = 512 X 496
Image Size (Scaled) = 73.14µm X 70.86µm
Bit Depth = 14 Bit
Image Center Position = X: 52.02mm, Y: 47.38mm
Stage Position = X: 52.02mm, Y: 47.38mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20.00%
Exposure Time = 400ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

647 -

Reflector = 50 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6%
Exposure Time = 50ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

DAPI - Not Shown in Data Presentation

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335-383
 Filter Emission Wavelength = 420-470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @6%
 Exposure Time = 18ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 0.90µm
 Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 13% (488), Threshold 13% (647), 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)

Orthogonal View - Panel B

Maximum Intensity Opacity (MIP)

Cut Lines = X 303, Y 287, Z 53

Line Width = 1, all

Cut Line Opacity = 50

Colocalization View - Panel C

Z-Plane 43 of 97
 Horizontal Axis - AF647, Threshold 209, Range Auto
 Vertical Axis - AF488, Threshold 29, Range Auto
 Mask - Circular
 Regions - 3 Colocalization, mask white

Summary

Panel A - This panel presents a 3-dimensional maximum projection of the total acquisition, highlighting the localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG (BLR018M). It is depicted within the nuclear compartment and surrounded by alpha-tubulin, which is labeled with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, IgG (H + L) as a secondary stain.

Panel B - This panel displays a 3-dimensional maximum orthogonal projection, further illustrating the localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG (BLR018M) specifically within the nucleus.

Panel C - Here, a colocalization histogram comparing the 488 (PARP1) and 647 (alpha-tubulin) channels (AF488/AF647) is shown, further demonstrating the localization of PARP1. A red circle highlights a region of interest. A mask is overlaid in white to indicate minimal colocalization at the nuclear periphery, which is expected, with no additional colocalization observed elsewhere.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

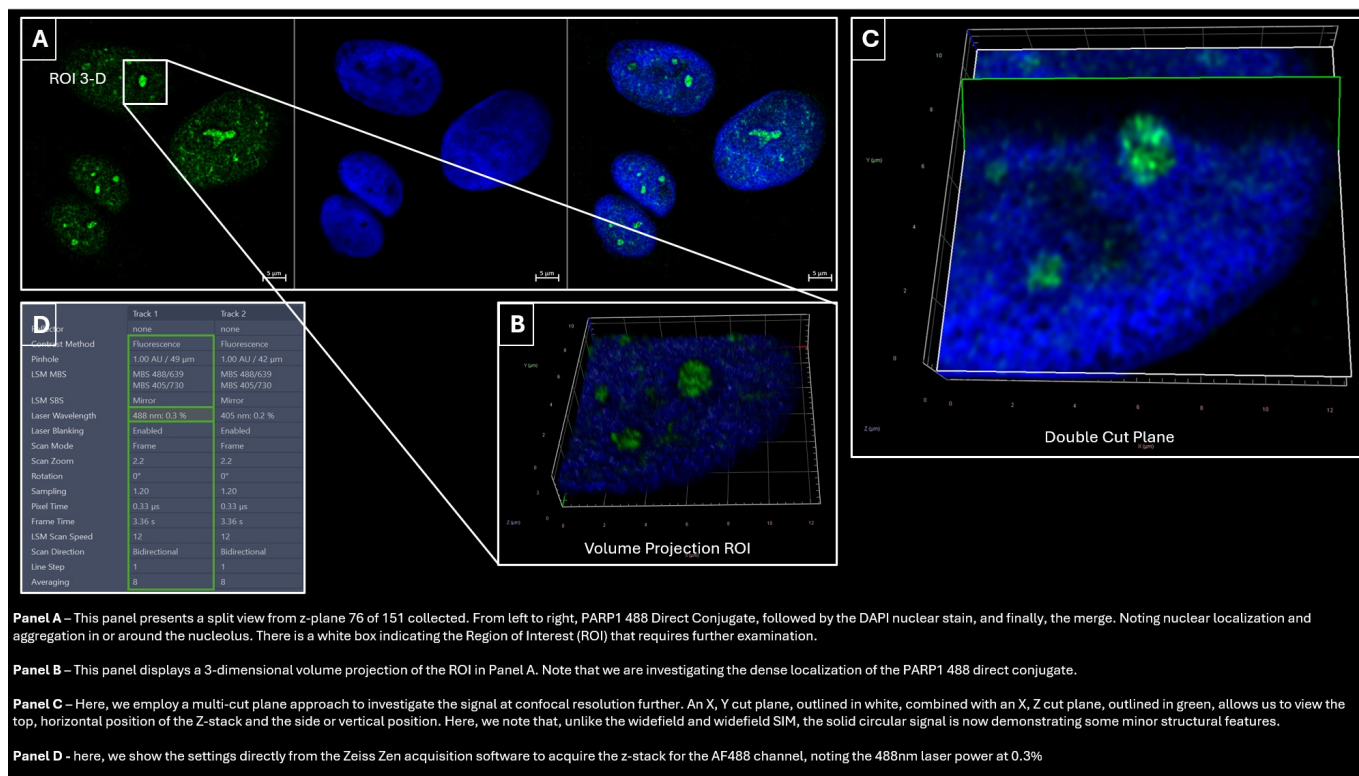
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000009
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7CD343695B8F8938437BE15F3699899753BD80805E3C9BD909F747925F6E69D6
Method PDF SHA-256	20CA6C02A9943A3649188E20AE0421A16721312849E327B22A3DE634C8C17EC6

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A – This panel presents a split view from z-plane 76 of 151 collected. From left to right, PARP1 488 Direct Conjugate, followed by the DAPI nuclear stain, and finally, the merge. Noting nuclear localization and aggregation in or around the nucleolus. There is a white box indicating the Region of Interest (ROI) that requires further examination.

Panel B – This panel displays a 3-dimensional volume projection of the ROI in Panel A. Note that we are investigating the dense localization of the PARP1 488 direct conjugate.

Panel C – Here, we employ a multi-cut plane approach to investigate the signal at confocal resolution further. An X, Y cut plane, outlined in white, combined with an X, Z cut plane, outlined in green, allows us to view the top, horizontal position of the Z-stack and the side or vertical position. Here, we note that, unlike the widefield and widefield SIM, the solid circular signal is now demonstrating some minor structural features.

Panel D – here, we show the settings directly from the Zeiss Zen acquisition software to acquire the z-stack for the AF488 channel, noting the 488nm laser power at 0.3%

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	59

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	151 Slices (4.50µm)
Channels	2
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.030µm
Image Size (Pixels)	1017 X 1017
Image Size (Scaled)	59.81µm X 59.81µm
Bit Depth	16 Bit
Image Center Position	X: 100.93mm, Y: 53.45mm
Stage Position	X: 100.93mm, Y: 53.45mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 49µm 1.0 AU / 42µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	Mirror
Laser Wavelength	488nm @0.3% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.20
Pixel Time	0.33µs
Frame Time	3.36s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 353
Emission Wavelength	517 465
Effective NA	1.4
Detection Wavelength	480 - 545 410 - 470
Imaging Device	GaAsP-Pmt 3

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	6.8 (3D, Auto) 6.1 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% (488), Threshold 2% (DAPI), Ramp 98% (488), Ramp 98% (DAPI), Maximum 100% both channels 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 Brightness100%, 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 Maximum Projection	Precise
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Y	Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	0% -22%
Clip X	-0° -60°
Clip Y	0°
X-Z	Active was selected, textured opaque was chosen to remove a vertical portion of the Z-Stack of the cell from the image, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. 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)

Z-Stack = 151 Slices (4.50µm)

Channels = 2

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

Image Size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81µm X 59.81µm

Bit Depth = 16 Bit

Image Center Position = X: 100.93mm, Y: 53.45mm

Stage Position = X: 100.93mm, Y: 53.45mm

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

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 49µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 488nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.33µs
 Frame Time = 3.36s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 480 - 545
 Imaging Device = GaAsP-Pmt 3
 Detector Type = GaAsP-PMT
 Detector Gain = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.8 (3D, Auto)

DAPI -

Reflector - None
 Contrast Method - Fluorescence
 Pinhole = 1.0 AU / 42µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.33µs
 Frame Time = 3.36s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsP-Pmt 3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.1 (3D, Auto)

3D Image Processing - Panel B

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

3D Image Processing - Panel C

3D Maximum Projection = Precise
 Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, Tone Mapping Enabled
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
 Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Y = Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%

Clip X = -0°

Clip Y = 0°

X-Z = Active was selected, textured opaque was chosen to remove a vertical portion of the Z-Stack of the cell from the image, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected

Position of Clip = -22%

Clip X = -60°

Clip Y = 0°

Summary

Panel A - This panel presents a split view from z-plane 76 of 151 collected. From left to right, PARP1 488 Direct Conjugate, followed by the DAPI nuclear stain, and finally, the merge. Noting nuclear localization and aggregation in or around the nucleolus. There is a white box indicating the Region of Interest (ROI) that requires further examination.

Panel B - This panel displays a 3-dimensional volume projection of the ROI in Panel A. Note that we are investigating the dense localization of the PARP1 488 direct conjugate.

Panel C - Here, we employ a multi-cut plane approach to investigate the signal at confocal resolution further. An X, Y cut plane, outlined in white, combined with an X, Z cut plane, outlined in green, allows us to view the top, horizontal position of the Z-stack and the side or vertical position. Here, we note that, unlike the widefield and widefield SIM, the solid circular signal is now demonstrating some minor structural features.

Panel D - here, we show the settings directly from the Zeiss Zen acquisition software to acquire the z-stack for Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR018M), noting the 488nm laser power at 0.3%

Image Corrections

None

Image by J.K. Bennett

Analysis and Presentation by B.T. Bennett

Data Presentation by P.D. Amistoso

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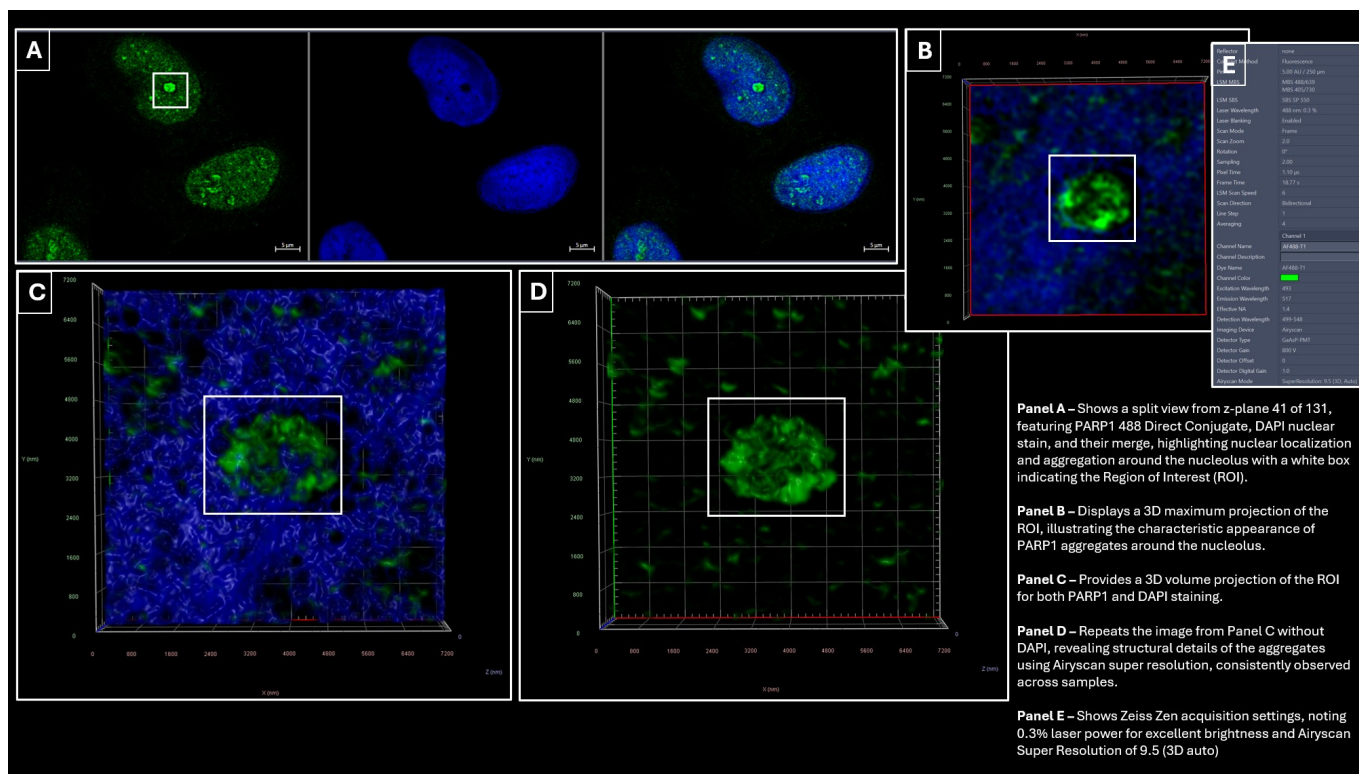
SOURCE PROVENANCE

Catalog	DC-000009
Stage	Confocal
Instrument	ZEISS LSM 980

SOURCE PROVENANCE

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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3229666CD6EEB4B5028FBBFEF5C00A2BDED0B8E44A6E521FD98AA0F64C3CE079
Method PDF SHA-256	91625F4CD5A8122BDD064BF889AB237B3498760D29486A58D4128CB2DAFB07D1

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	55

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	131 Slices (3.90µm)
Channels	2
Scaling (Per Pixel)	35.30nm X 35.30nm X 0.030nm
Image Size (Pixels)	646 X 792
Image Size (Scaled)	22.80µm X 17.37µm
Bit Depth	16 Bit
Image Center Position	X: 99.65mm, Y: 52.36mm
Stage Position	X: 99.65mm, Y: 52.36mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 250µm 5.46 AU / 235µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 550 SBS SP 505
Laser Wavelength	488nm @0.3% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	2.00
Pixel Time	1.10µs
Frame Time	18.77s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	493 353
Emission Wavelength	517 465
Effective NA	1.4
Detection Wavelength	499 - 548 350 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 9.5 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels. Threshold 2% (488), Threshold 2% (DAPI), Ramp 98% (488), Ramp 98% (DAPI), Maximum 100% both channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest - Panel B
X-Y	Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-20%
Clip X	-0°
Clip Y	0°

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™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

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:

Control Data - Primary Unconjugated Antibody

Z Stack - (3D)

Z-Stack = 131 Slices (3.90µm)

Channels = 2

Scaling (Per Pixel) = 35.30nm X 35.30nm X 0.030nm

Image Size (Pixels) = 646 X 792

Image Size (Scaled) = 22.80µm X 17.37µm

Bit Depth = 16 Bit

Image Center Position = X: 99.65mm, Y: 52.36mm

Stage Position = X: 99.65mm, Y: 52.36mm

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

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 250µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = SBS SP 550
 Laser Wavelength = 488nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.10µs
 Frame Time = 18.77s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 499 - 548
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 9.5 (3D, Auto)

DAPI -

Reflector - None
 Contrast Method - Fluorescence
 Pinhole = 5.46 AU / 235µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = SBS SP 505
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.10µs
 Frame Time = 18.77s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 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
 Airyscan Mode = SuperResolution 9.5 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing - Panel B

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

3D Image Processing - Panels C, D

3D Volume Projection = Precise

Appearance = Threshold 2% (488), Threshold 2% (DAPI), Ramp 98% (488), Ramp 98% (DAPI), Maximum 100% both 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 = Clipping planes are introduced to pronounce regions of special interest - Panel B

X-Y = Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -20%

Clip X = -0°

Clip Y = 0°

Summary

Panel A - Shows a split view from z-plane 41 of 131, featuring PARP1 488 Direct Conjugate, DAPI nuclear stain, and their merge, highlighting nuclear localization and aggregation around the nucleolus with a white box indicating the Region of Interest (ROI).

Panel B - Displays a 3D maximum projection of the ROI, illustrating the characteristic appearance of PARP1 aggregates around the nucleolus.

Panel C - Provides a 3D volume projection of the ROI for both PARP1 and DAPI staining.

Panel D - Repeats the image from Panel C without DAPI, revealing structural details of the aggregates using Airyscan super resolution, consistently observed across samples.

Panel E - Shows Zeiss Zen acquisition settings, noting 0.3% laser power for excellent brightness and Airyscan Super Resolution of 9.5 (3D auto)

Image Corrections

None

Image by J.K. Bennett

Analysis and Presentation by B.T. Bennett

Data Presentation by P.D. Amistoso

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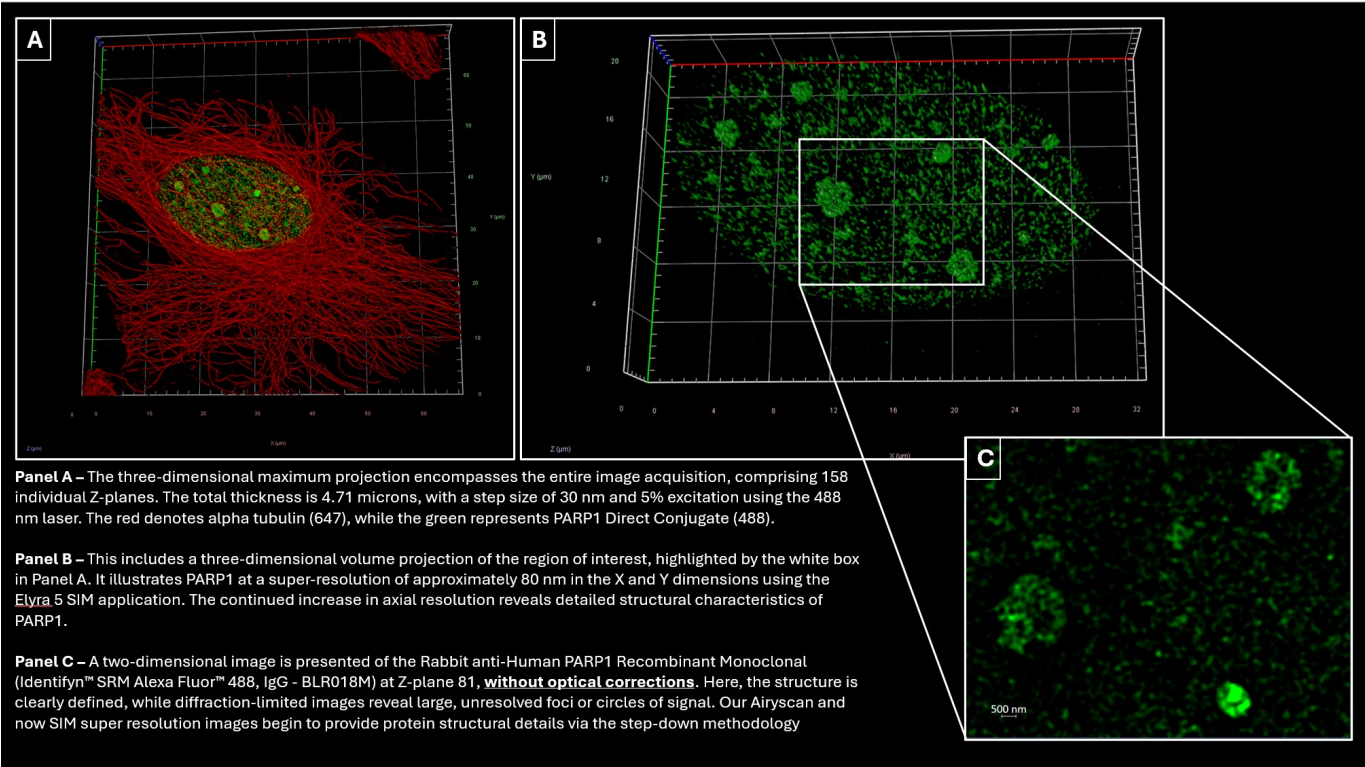
SOURCE PROVENANCE

Catalog	DC-000009
Stage	Airyscan
Instrument	ZEISS LSM 980

SOURCE PROVENANCE

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	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	02909BCF3626BD02947443100C20DC52A0A0028509541D817637D406F602E5C7
Method PDF SHA-256	5FBC60F7E80A6AE2302061DE519142D4BFF1B9AE690AC33ABA24FFFE5EB6AC0A

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	52

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	158 Slices (4.71µm)
Channels	2
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056
Image Size (Scaled)	66.72µm X 66.72µm
Bit Depth	16 Bit
Image Center Position	X: 60.31mm, Y: 38.57mm
Stage Position	X: 60.31mm, Y: 38.57mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 640
Channel Name	T1-Axiocam 820-SR T1-Axiocam 820 -SR
Excitation Wavelength	488 640
Emission Wavelength	525 729
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820
Camera Adapter	1X
Exposure Time	150ms 120ms
Depth of Focus	0.81µm 1.13µm
Binning Mode	2,2
Laser Wavelength	488nm @5.0% 640nm @1.5%
Frame Time	1.98s 1.59s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.1219
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (488), Ramp 0% all channels, Maximum 100% all channels Threshold 2% (647), Threshold 2% (488), Ramp 98% (488), Ramp 98% (488), Maximum 100% both channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
Z-Plane	81/158
Channel	Green (488) PARP1 Direct Conjugate

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™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000064

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

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

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D)

Z-Stack = 158 Slices (4.71µm)

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 66.72µm X 66.72µm

Bit Depth = 16 Bit

Image Center Position = X: 60.31mm, Y: 38.57mm

Stage Position = X: 60.31mm, Y: 38.57mm

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

488 -

Reflectors = SR HE LBF 405/488/561/642

Beam Splitter = 405, 488, 561, 642

Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642

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

Contrast Method = Fluorescence

Illumination Wavelength = 488

Channel Name = T1-Axiocam 820-SR

Excitation Wavelength = 488

Emission Wavelength = 525

Effective NA = 1.4

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

Imaging Device = Axiocam 820

Camera Adapter = 1X

Exposure Time = 150ms

Depth of Focus = 0.81µm

Binning Mode = 2,2

Laser Wavelength = 488nm @5.0%

Frame Time = 1.98s

Scan Direction = Unidirectional

Grating 36.5µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 640
 Channel Name = T1-Axiocam 820 -SR
 Excitation Wavelength = 640
 Emission Wavelength = 729
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 1.13µm
 Binning Mode = 2,2
 Laser Wavelength = 640nm @1.5%
 Frame Time = 1.59s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 2
 Channels = 2
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 10.1219

3D Image Processing - Panel A

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

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 2% (647), Threshold 2% (488), Ramp 98% (488), Ramp 98% (488), Maximum 100% both 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)

2-Dimensional Image - Panel C

Maximum Projection

Z-Plane = 81/158

Channel = Green (488) PARP1 Direct Conjugate

Summary

Panel A - The three-dimensional maximum projection encompasses the entire image acquisition, comprising 158 individual Z-planes. The total thickness is 4.71 microns, with a step size of 30 nm and 5% excitation using the 488 nm laser. The red denotes alpha tubulin (647), while the green represents PARP1 Direct Conjugate (488).

Panel B - This includes a three-dimensional volume projection of the region of interest, highlighted by the white box in Panel A. It illustrates PARP1 at a super-resolution of approximately 80 nm in the X and Y dimensions using the Elyra 5 SIM application. The continued increase in axial resolution reveals detailed structural characteristics of PARP1.

Panel C - A two-dimensional image is presented of the Rabbit anti-Human PARP1 Recombinant Monoclonal (Identifyn® SRM Alexa Fluor™ 488, IgG - BLR018M) at Z-plane 81, without optical corrections. Here, the structure is clearly defined, while diffraction-limited images reveal large, unresolved foci or circles of signal. Our Airyscan and now SIM super resolution images begin to provide protein structural details via the step-down methodology

Image Corrections

None

Image by P. Raut

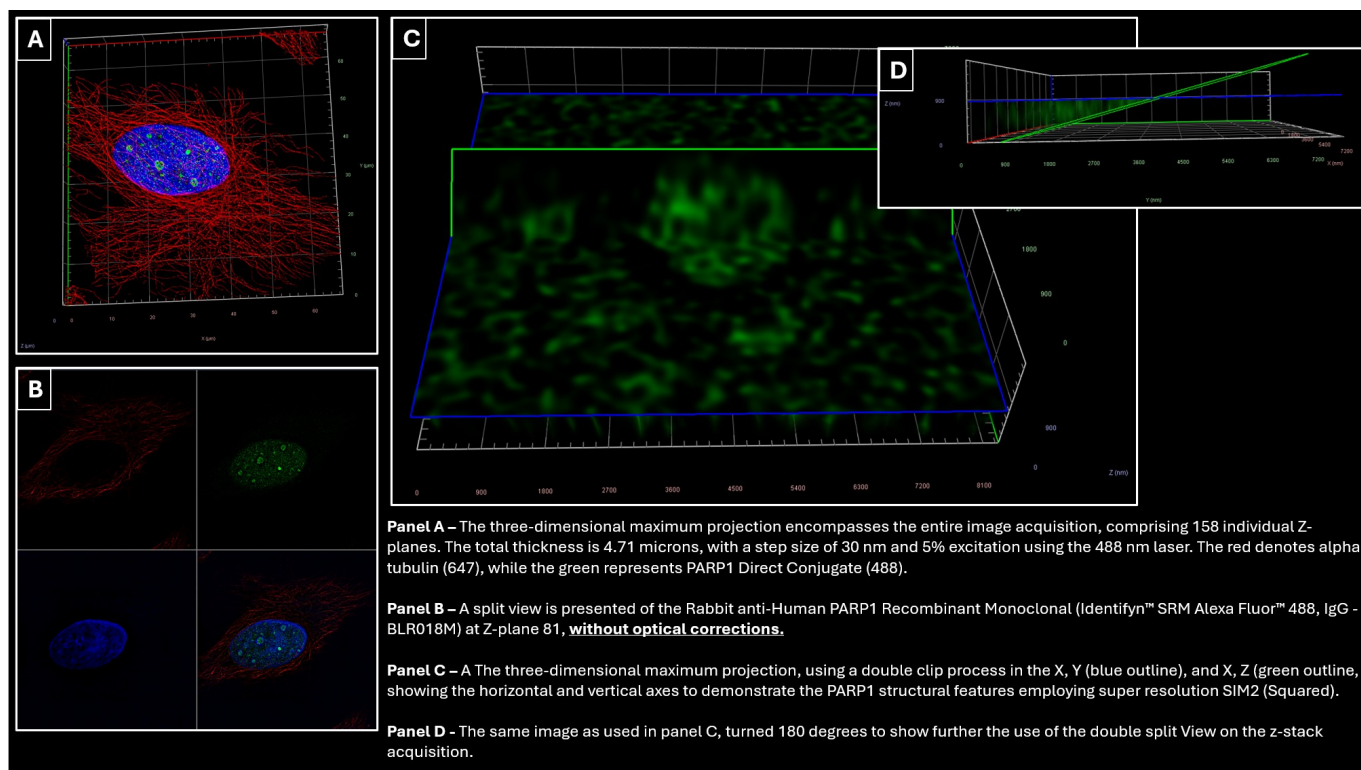
Analysis and Presentation by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000009
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E04362E7ECD33613469A952D5F3FC2C06DE873710F25FE7A9E28ADA8CD905D0E
Method PDF SHA-256	19A0782108539D423CD8852173513F91850B3BF46ADB29CA143DC57CCE9DDFD6

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	68

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	158 Slices (4.71µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 631 X 595
Image Size (Scaled)	66.72µm X 66.72µm 8.33µm X 7.85µm
Bit Depth	16 Bit
Image Center Position	X: 60.31mm, Y: 38.57mm
Stage Position	X: 60.31mm, Y: 38.57mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 640 405
Channel Name	T1-Axiocam 820-SR T1-Axiocam 820 -SR T3-Axiocam 820 #2 -SR
Excitation Wavelength	488 640 405
Emission Wavelength	525 729 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150ms 120ms 50ms
Depth of Focus	0.81µm 1.13µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @5.0% 640nm @1.5% 640nm @7.0%
Frame Time	1.98s 1.59s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.5026
Order Combination	Weiner filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (488), 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)
Z-Plane	81/158
Channel	Green (488) PARP1 Direct Conjugate, Red (647) alpha tubulin, and blue (DAPI)

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™ 488, IgG - (BLR018M)
Cat ID# - DC 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000064

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™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. 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, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 158 Slices (4.71µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 60.31mm, Y: 38.57mm
Stage Position = X: 60.31mm, Y: 38.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel C, D, and E

Z-Stack = 158 Slices (4.71µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 631 X 595
Image Size (Scaled) = 8.33µm X 7.85µm
Bit Depth = 16 Bit
Image Center Position = X: 60.31mm, Y: 38.57mm
Stage Position = X: 60.31mm, Y: 38.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @5.0%
Frame Time = 1.98s
Scan Direction = Unidirectional
Grating 36.5µm grating

647 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 640
 Channel Name = T1-Axiocam 820 -SR
 Excitation Wavelength = 640
 Emission Wavelength = 729
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 1.13µm
 Binning Mode = 2,2
 Laser Wavelength = 640nm @1.5%
 Frame Time = 1.59s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2 -SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503-546, 576-617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 640nm @7.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

 Post Processing - SIM2 Processing
 Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 3

Algorithm = SIM2
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.5026
 Order Combination = Weiner filter
 Regularization = None
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Histogram = Scale to Min/Max

3D Image Processing - Panels A, C, and D

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

2-Dimensional Split View - Panel B

Maximum Projection

Z-Plane = 81/158

Channel = Green (488) PARP1 Direct Conjugate, Red (647) alpha tubulin, and blue (DAPI)

Summary

Panel A - The three-dimensional maximum projection encompasses the entire image acquisition, comprising 158 individual Z-planes. The total thickness is 4.71 microns, with a step size of 30 nm and 5% excitation using the 488 nm laser. The red denotes alpha tubulin (647), while the green represents PARP1 Direct Conjugate (488).

Panel B - A split view is presented of the Rabbit anti-Human PARP1 Recombinant Monoclonal (Identifyn® SRM Alexa Fluor™ 488, IgG - BLR018M) at Z-plane 81, without optical corrections.

Panel C - A The three-dimensional maximum projection, using a double clip process in the X, Y (blue outline), and X, Z (green outline, showing the horizontal and vertical axes to demonstrate the PARP1 structural features employing super resolution SIM2 (Squared)

Panel D - The same image as used in panel C, but turned 180 degrees to further show the use of the double split View on the z-stack acquisition.

Image Corrections

None

Image by P. Raut

Analysis and Presentation by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000009
Stage	SIM ²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	06D23B9C071CD5889F0A0079BA039F73237D93475420B70CCFE88F68C9CA5D97
Method PDF SHA-256	49F4A3576928B673DEF1499B3C0913F1821F7E355D053776982B941DAD30300C