

IDENTIFYN RESOLUTION STEPDOWN

PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 594

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

7

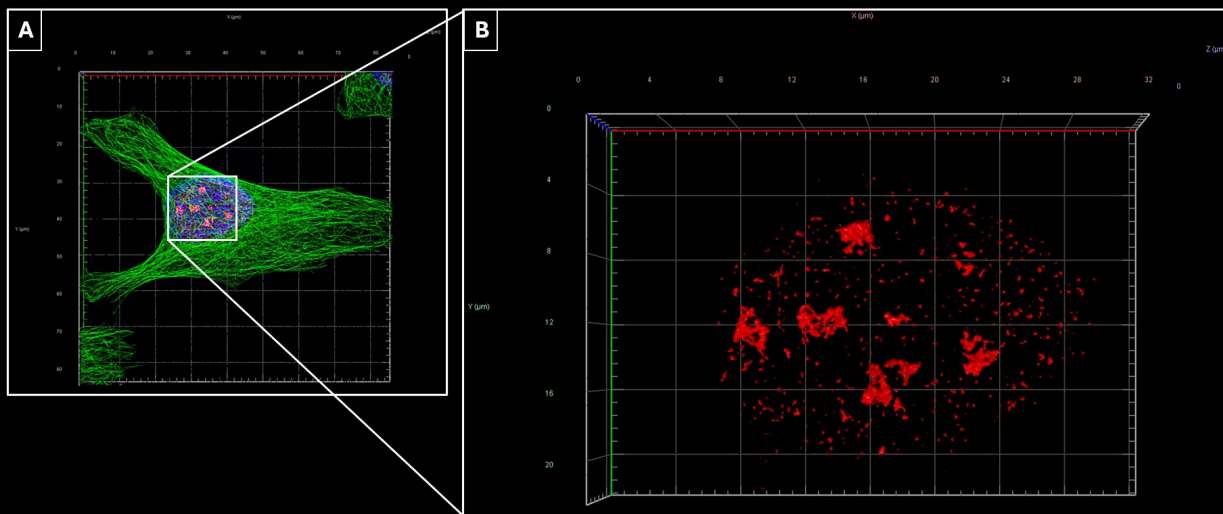
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



Panel A – This panel presents a 3-dimensional maximum projection of the z-stack acquired. Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 direct conjugate (shown in red). Alpha tubulin primary antibody counterstained with Identifyn™ SRM Alexa Fluor™ 488 secondary (in green). DAPI nuclear staining (in blue).

Panel B – This panel presents a 3-dimensional Surface projection of the Region of Interest (ROI) shown in Panel A; this is the same area examined in the SIM resolution image, however, in two dimensions (same product). Here, we see enhanced structural detail of the PARP1 594 direct conjugate at the aggregate level.

We note that the aggregates appear over all direct conjugate and primary/secondary images to have similar structures across all resolutions, while we do not claim as to PARP1 biological meaning, we do find it interesting that the structural details across many samples, sample preps and images are consistent, and we have confidence this is representative of PARP1 localization and further investigation in examining PARP1 known protein interactions could yield new findings.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000012 | 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-000012 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-000012 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

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

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594; 647	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561 nm @0.3%; 488 nm @0.01%; 405 nm @0.2%; 590; 493; 353
Airyscan	405/DAPI; 488; 594; 750	3; None; 561 nm @0.2%; 488 nm @0.02%; 405 nm @0.2%; 590; 493; 353
SIM	405/DAPI; 488; 594; 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 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561
SIM ²	405/DAPI; 488; 594	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 561

MODALITY ASSESSMENT / PART 1 OF 7

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 7

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 μm X 0.110 μm ; Image size (Pixels): 653 X 808; Image Size (Pixels): 653 X 808; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 705. Timing: Exposure Time: 200 ms; 50 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 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; 594; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 93 Slices (9.2 μm); Channels: 3; Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm ; Image size (Pixels): 1600 X 1104; 403 X 478; Image Size (Pixels): 1600 X 1104; 403 X 478; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 100 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @12.00%; 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: 52.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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: 181 Slices (5.4 μm); Channels: 3; Scaling (Per Pixel): 0.060 μm X 0.060 μm X 0.030 μm ; Image size (Pixels): 1101 X 1101; Image Size (Pixels): 1101 X 1101; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.31 μs ; Frame Time: 3.63 s. Illumination: Laser Wavelength: 561 nm @0.3%; 488 nm @0.01%. Optical/scan: Pinhole: 1.00 AU / 61 μm ; 1.00 AU / 49 μm ; Scan Zoom: 2.0; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.4 (3D, Auto); 7.1 (3D, Auto). Structured source metadata values: 65.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 7

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 291 Slices (8.7 μm); Channels: 3; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 1711 X 1711; 407 X 236; Image Size (Pixels): 1711 X 1711; 407 X 236; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.21 μs ; Frame Time: 17.06 s. Illumination: Laser Wavelength: 561 nm @0.2%; 488 nm @0.02%. Optical/scan: Pinhole: 5.00 AU / 309 μm ; 5.00 AU / 247 μm ; Scan Zoom: 2.2; 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); 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; AiryScan Mode: SuperResolution 9.8 (3D, Auto); SuperResolution 8.9 (3D, Auto). Structured source metadata values: 67.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 7

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 167 Slices (4.98 μ m); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5033 X 3837; 1659 X 1225; Image Size (Pixels): 5033 X 3837; 1659 X 1225; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @2.00%; 488 nm @1.50%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 62.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 594; 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, 1.25X Tubelens, was used with the following parameters for each dye: Acquisition: Z-Stack: 167 Slices (4.98 μ m); Channels: 3; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1918 X 1334; Image Size (Pixels): 5056 X 5056; 1918 X 1334; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.59 s; 676.00 ms. Illumination: Laser Wavelength: 561 nm @2.00%; 488 nm @1.50%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 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: 69.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000012 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): 35.29 nm X 35.29 nm X 30.00 nm -> 0.03529 μ m X 0.03529 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1711 X 1711; Image Size (Scaled)=60.37 μ m X 60.37 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 μ m X 0.01689 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5033 X 3837; Image Size (Scaled)=85.01 μ m X 64.81 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm -> 0.01689 μ m X 0.01689 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=85.40 μ m X 85.40 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

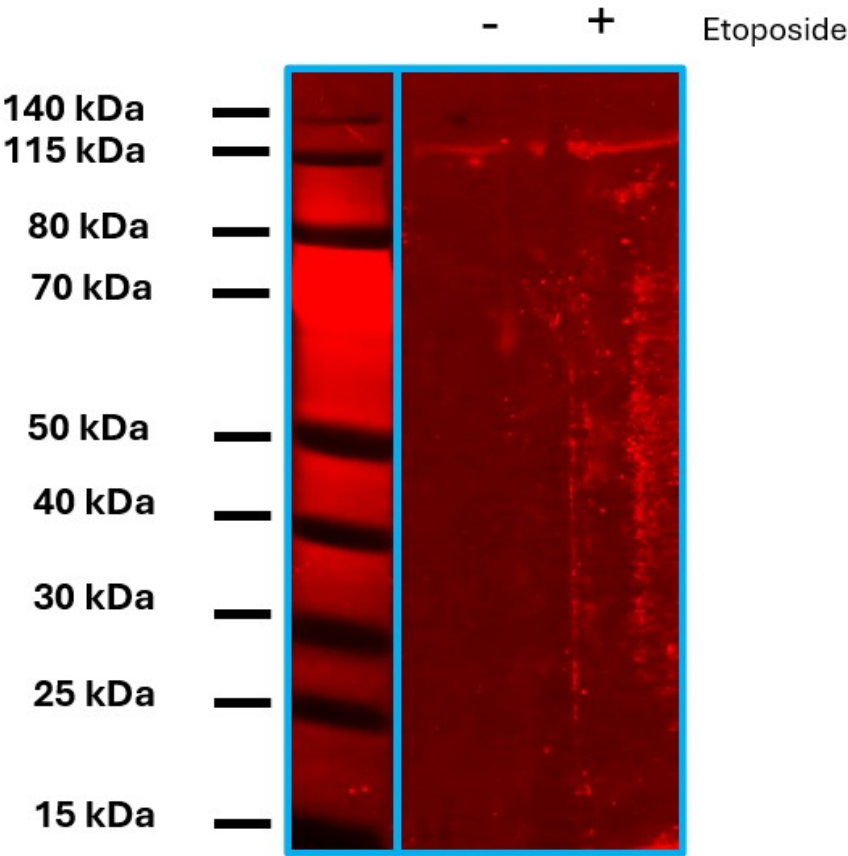
This dossier preserves the complete available evidence progression for DC-000012. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	594
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

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

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR014M)
Cat ID# - DC 000012

Expected Molecular Weight: 116 kDa

HEK293 cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down the cell debris. The lysate supernatant (100ug) 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 washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR014M) for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence

Laser = 532nm

Emission Filter = 572±28nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

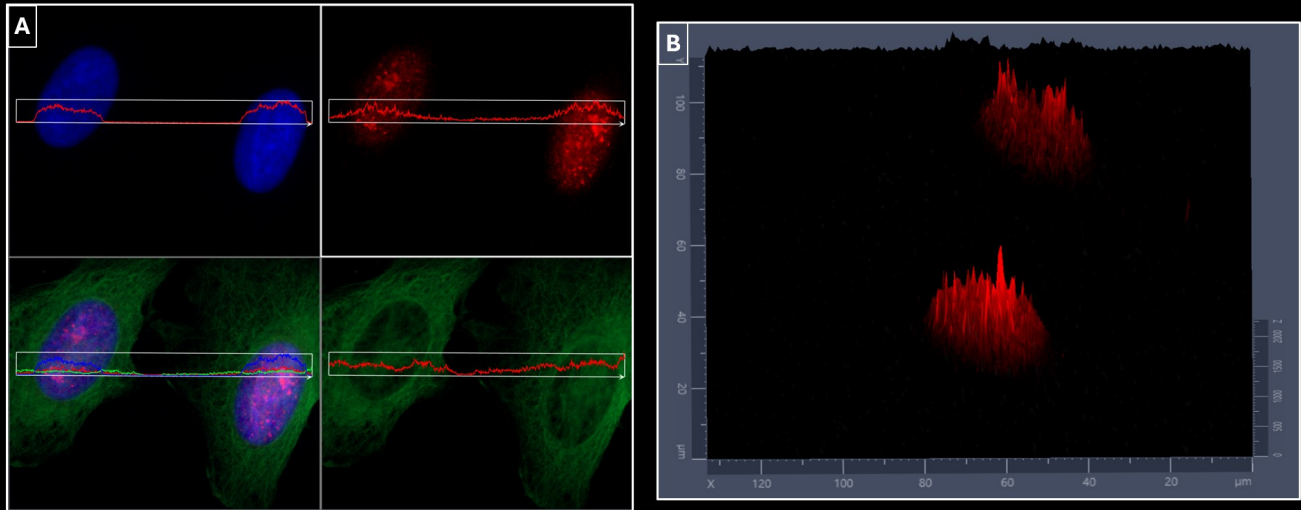
Image: K. Small

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SOURCE PROVENANCE

Catalog	DC-000012
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	A18AEFDF61265257764FF3C4EFBAE30F0772F85452CF1BEDDD98093D6C3B3927
Method PDF SHA-256	97A0AE0A5A3DFB1323E68C2B190DED7048F4645B043409771F809F70EB136223

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 647, IgG, Direct Conjugate. The Upper left displays DAPI (blue), PARP1 647 direct conjugate (red), upper right, PARP1 647 direct conjugate (red), alpha tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 is in the lower right, and the lower left presents a merged image of the three channels.

Panel B – Displays a 2.5D view of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 647, IgG, Direct Conjugate signal being captured was captured using 20% LED for 200ms. The image settings indicate that the 594 direct conjugate is bright and easily captured.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 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	3
Scaling (Per Pixel)	0.110 μ m X 0.110 μ m
Image size (Pixels)	653 X 808
Image Size (Scaled)	71.52 μ m X 88.50 μ m
Bit Depth	14 Bit
Image Center Position	X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflector	45 Texas Red 38 HE eGFP 49 DAPI
Beam Splitter	585 495 395
Filter Excitation Wavelength	540 - 580 450 - 490 335 - 383
Filter Emission Wavelength	593 - 668 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	200 ms 50 ms 15 ms
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96 μ m 0.80 μ m 0.72 μ m
Binning Mode	2,2
Render Mode	Surface
Grid distance	1
Angle X	54
Angle Y	-179
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™ 594, IgG - (BLR018M)
Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000017

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™ 594, 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, 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® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) 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 = 3

Scaling (Per Pixel) = 0.110 µm X 0.110 µm

Image size (Pixels) = 653 X 808

Image Size (Scaled) = 71.52 µm X 88.50 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

594 -

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

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10%
 Exposure Time = 50 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80 μ m
 Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10.00%
 Exposure Time = 15 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72 μ m
 Binning Mode = 2,2

Split View - Panel A

The top-left panel features DAPI nuclear staining, the top-right panel shows Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR018M) after DNA damage, the bottom-left panel features the merged image of all channels, and the bottom-right panel features Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L).

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface

Grid distance = 1

Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 54

Angle Y = -179

Z Scaling = 1.0

Ambient = 50

Specular = 20

Shininess = 50

Light Height = 2.0

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, Direct Conjugate. The Upper left displays DAPI (blue), PARP1 647 direct conjugate (red), the upper right presents PARP1 647 direct conjugate (red), alpha tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 in the lower right, and the lower left presents a merged image of the three channels.

Panel B - Displays a 2.5D view of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, Direct Conjugate signal being captured was captured using 20% LED for 200ms. The image settings indicate that the 594 direct conjugate is bright and easily captured.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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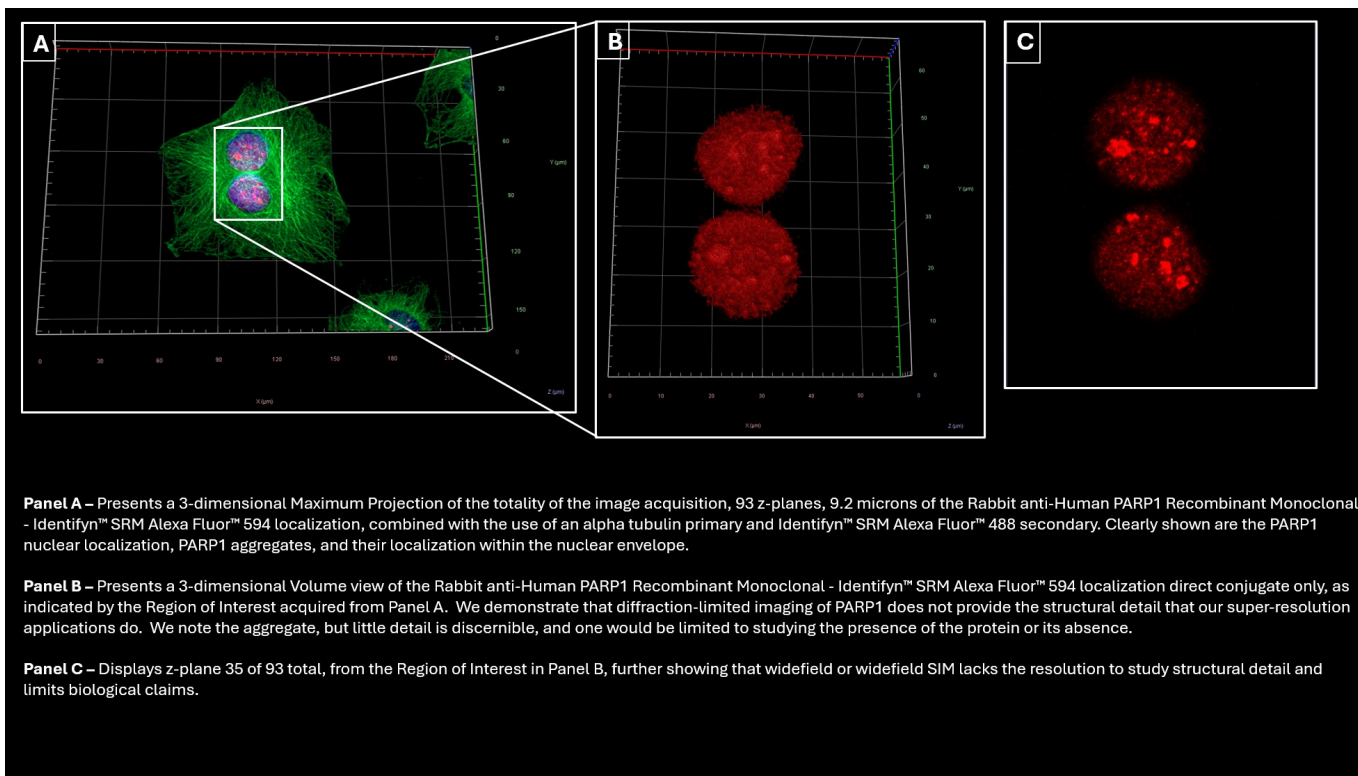
SOURCE PROVENANCE

Catalog	DC-000012
Stage	Widefield

SOURCE PROVENANCE

Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylys Lamp, was used with the following parameters for each dye:
Structured source metadata values	48
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	616364481DA439049A0C4A44F57BD2B75C6613363C4D5CCEFC331A662F2AC5D3
Method PDF SHA-256	681367CFC548BA539EF125F2DC1965837F64A7969FDBD559C4B828EE5666CB75

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	52

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	93 Slices (9.2 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	1600 X 1104 403 X 478
Image Size (Scaled)	228.57 µm X 157.71 µm 57.57 µm X 68.29 µm
Bit Depth	14 Bit
Image Center Position	X: 49.78 mm, Y: 51.54 mm
Stage Position	= X: 49.78 mm, Y: 51.54 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	45 Texas Red 38 HE eGFP 49 DAPI
Beam Splitter	585 495 395
Filter Excitation Wavelength	540 - 580 450 - 490 335 - 383
Filter Emission Wavelength	593 - 668 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @12.00% X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @8.00%
Exposure Time	100 ms 150 ms 20 ms
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.20 µm 1.00 µm 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels. Threshold 10%, Ramp 50% all, Maximum 100%
Background	Black
Light	Brightness 100%, Tone Mapping Enabled Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

FIELD	SOURCE-DOCUMENTED VALUE
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR018M)
Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000017

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™ 594, 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, 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® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) 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) - Panel A

Z-Stack = 93 Slices (9.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 1600 X 1104
Image Size (Scaled) = 228.57 µm X 157.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 49.78 mm, Y: 51.54 mm
Stage Position = = X: 49.78 mm, Y: 51.54 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 93 Slices (9.2 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 403 X 478
Image Size (Scaled) = 57.57 µm X 68.29 µm
Bit Depth = 14 Bit
Image Center Position = X: 49.78 mm, Y: 51.54 mm
Stage Position = = X: 49.78 mm, Y: 51.54 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @12.00%
Exposure Time = 100 ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @20%
 Exposure Time = 150 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.00 μm
 Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @8.00%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.90 μm
 Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, 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 B

3D Volume Projection = Precise

Appearance = Threshold 10%, Ramp 50% all, 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)

Summary

Panel A - Presents a 3-dimensional Maximum Projection of the totality of the image acquisition, 93 z-planes, 9.2 microns of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM AlexaFluor™ 594 localization, combined with the use of an alpha tubulin primary and Identifyn® SRM AlexaFluor™ 488 secondary. Clearly shown are the PARP1 nuclear localization, PARP1 aggregates, and their localization within the nuclear envelope.

Panel B - Presents a 3-dimensional Volume view of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594 localization direct conjugate only, as indicated by the Region of Interest acquired from Panel A. We demonstrate that diffraction-limited imaging of PARP1 does not provide the structural detail that our super-resolution applications do. We note the aggregate, but little detail is discernible, and one would be limited to studying the presence of the protein or its absence.

Panel C - Displays z-plane 35 of 93 total, from the Region of Interest in Panel B, further showing that widefield or widefield SIM lacks the resolution to study structural detail and limits biological claims.

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

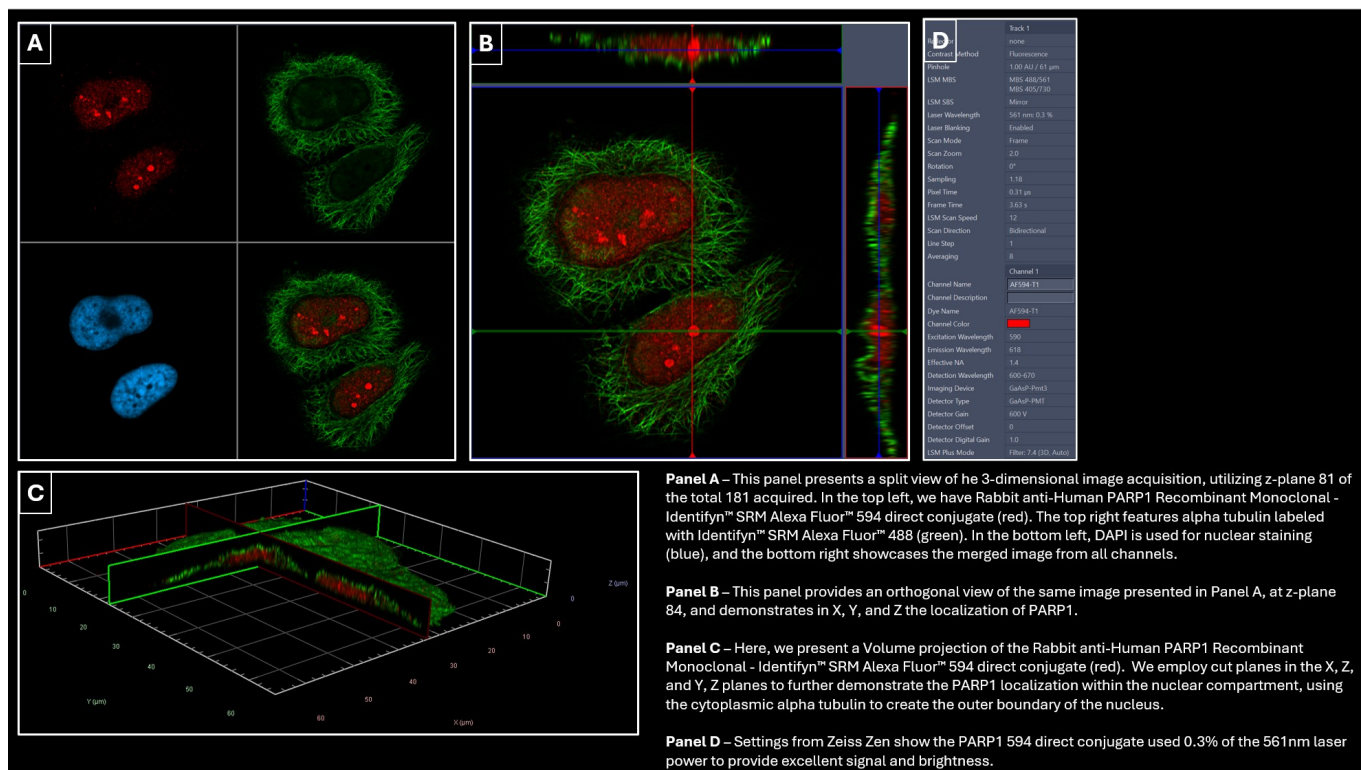
Catalog	DC-000012
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	08FB3306BE5CB420751805BC903803032F67D29A06CBDE72D4C505DDF1952644

SOURCE PROVENANCE

Method PDF SHA-256

E42887BC96CE69A140DA5B23997712DF4A7EB529C7DF1402BA63F74171E74580

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	65

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	181 Slices (5.4 μm)
Channels	3
Scaling (Per Pixel)	0.060 μm X 0.060 μm X 0.030 μm
Image Size (Pixels)	1101 X 1101
Image Size (Scaled)	65.91 μm X 65.91 μm
Bit Depth	16 Bit
Image Center Position	X: 95.56 mm, Y: 51.50 mm
Stage Position	X: 95.56 mm, Y: 51.50 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 61 μm 1.00 AU / 49 μm 0.50 AU / 21 μm
LSM MBS	MBS 488/561, 405/730
LSM SBS	Mirror
Laser Wavelength	561 nm @0.3% 488 nm @0.01% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	1.18
Pixel Time	0.31 μs
Frame Time	3.63 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.4
Detection Wavelength	600-670 480-549 410-470
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V 550 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.4 (3D, Auto) 7.1 (3D, Auto) 6.4 (3D, Auto)
Cut Lines	X 657, Y 726, Z 84
Line Width	X 5, Y 6, Z 5
Cut Line Opacity	60
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 127%, 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 C
X-Z	Active was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-30% 6%
Clip X	0°
Clip Y	0°
Y-Z	Active was selected, textured opaque was chosen, and a dark red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

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Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000017

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Method

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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® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) 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 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 = 181 Slices (5.4 µm)

Channels = 3

Scaling (Per Pixel) = 0.060 µm X 0.060 µm X 0.030 µm

Image Size (Pixels) = 1101 X 1101

Image Size (Scaled) = 65.91 µm X 65.91 µm

Bit Depth = 16 Bit

Image Center Position = X: 95.56 mm, Y: 51.50 mm

Stage Position = X: 95.56 mm, Y: 51.50 mm

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

594 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00 AU / 61 µm

LSM MBS = MBS 488/561, 405/730

LSM SBS = Mirror

Laser Wavelength = 561 nm @0.3%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.0

Rotation = 0°

Sampling = 1.18

Pixel Time = 0.31 µs

Frame Time = 3.63 s

LSM Scan Speed = 12

Scan Direction = Bidirectional

Line Step = 1

Averaging = 8

Excitation Wavelength = 590

Emission Wavelength = 618

Effective NA = 1.4

Detection Wavelength = 600-670

Imaging Device = GaAsP-Pmt3

Detector Type = GaAsP-PMT

Detector Gain = 600 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = 7.4 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 49 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 488 nm @0.01%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.18
 Pixel Time = 0.31 μ s
 Frame Time = 3.63 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 480-549
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 7.1 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 0.50 AU / 21 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.18
 Pixel Time = 0.31 μ s
 Frame Time = 3.63 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410-470
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.4 (3D, Auto)

Split View - Panel A

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR018M) after DNA damage, the top-right panel features Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(a'') $\square$ (H + L), the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

Orthogonal View - Panel B

Cut Lines = X 657, Y 726, Z 84
 Line Width = X 5, Y 6, Z 5
 Cut Line Opacity = 60
 Maximum Intensity Projection (MIP) NOT selected
 Measure 3D Distance NOT selected

3D Image Processing - Panel C

3D Volume Projection = Precise

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

Background = Black

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

Projection = View Angle 35°, Scale Z 127%, 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 C

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

Position of Clip = -30%

Clip X = 0°

Clip Y = 0°

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

Position of Clip = 6%

Clip X = 0°

Clip Y = 0°

Summary

Panel A - This panel presents a split view of the 3-dimensional image acquisition, utilizing z-plane 81 of the total 181 acquired. In the top left, we have Rabbit anti-Human PARP1 Recombinant Monoclonal -Identifyn® SRM Alexa Fluor™ 594 direct conjugate (red). The top right features alpha tubulin labeled with Identifyn® SRM Alexa Fluor™ 488 (green). In the bottom left, DAPI is used for nuclear staining (blue), and the bottom right showcases the merged image from all channels.

Panel B - This panel provides an orthogonal view of the same image presented in Panel A, at z-plane 84, and demonstrates in X, Y, and Z the localization of PARP1.

Panel C - Here, we present a Volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal -Identifyn® SRM Alexa Fluor™ 594 direct conjugate (red). We employ cut planes in the X, Z, and Y, Z planes to further demonstrate the PARP1 localization within the nuclear compartment, using the cytoplasmic alpha tubulin to create the outer boundary of the nucleus.

Panel D - Settings from Zeiss Zen show the PARP1 594 direct conjugate used 0.3% of the 561nm laser power to provide an excellent signal and brightness.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE	
Catalog	DC-000012
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	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0C947C958617C223EB1E5F47E785BE9D11B453A47AA53E107712C11D6127EC20
Method PDF SHA-256	72C99FC99C44515E659C8CBD2D4549FE2310B63F980D30272113449F4FAAC1A9

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	291 Slices (8.7 μm)
Channels	3
Scaling (Per Pixel)	0.03529 μm X 0.03529 μm X 0.03000 μm
Image Size (Pixels)	1711 X 1711 407 X 236
Image Size (Scaled)	60.37 μm X 60.37 μm 14.36 μm X 8.33 μm
Bit Depth	16 Bit
Image Center Position	X: 90.29 mm, Y: 54.26 mm
Stage Position	X: 90.29 mm, Y: 54.26 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 309 μm 5.00 AU / 247 μm 5.46 AU / 235 μm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 525 SBS SP 550 SBS SP 505
Laser Wavelength	561 nm @0.2% 488 nm @0.02% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	2.00
Pixel Time	1.21 μs
Frame Time	17.06 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.4
Detection Wavelength	527-735 380-548 350-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V 750 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 9.8 (3D, Auto) SuperResolution 8.9 (3D, Auto) SuperResolution 7.9 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels. Threshold 11%, Ramp 80%, Maximum 100%
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 68%, 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-Z	Active was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-42%
Clip X	0°
Clip Y	0°
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR018M)
Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000017

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™ 594, 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, 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® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) 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 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 = 291 Slices (8.7 µm)

Channels = 3

Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm

Image Size (Pixels) = 1711 X 1711

Image Size (Scaled) = 60.37 µm X 60.37 µm

Bit Depth = 16 Bit

Image Center Position = X: 90.29 mm, Y: 54.26 mm

Stage Position = X: 90.29 mm, Y: 54.26 mm

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

Z Stack - (3D) - Panel B

Z-Stack = 291 Slices (8.7 µm)

Channels = 3

Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm

Image Size (Pixels) = 407 X 236

Image Size (Scaled) = 14.36 µm X 8.33 µm

Bit Depth = 16 Bit

Image Center Position = X: 90.29 mm, Y: 54.26 mm

Stage Position = X: 90.29 mm, Y: 54.26 mm

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

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 309 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = SBS LP 525
 Laser Wavelength = 561 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.21 μ s
 Frame Time = 17.06 s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 527-735
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 9.8 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 247 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = SBS SP 550
 Laser Wavelength = 488 nm @0.02%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.21 μ s
 Frame Time = 17.06 s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 380-548
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 8.9 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.46 AU / 235 µm
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = SBS SP 505
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.2
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.21 µs
 Frame Time = 17.06 s
 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 = 750 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 7.9 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
 Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.
 X-Z = Active was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -42%

Clip X = 0°

Clip Y = 0°

3D Image Processing - Panel B

3D Volume Projection = Precise

Appearance = Threshold 11%, Ramp 80%, 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 - This panel features a 3-dimensional maximum projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594 direct conjugate (red). This is combined with an alpha tubulin primary antibody and Identifyn® SRM Alexa Fluor™ 488 secondary (green), along with DAPI nuclear staining (blue). Additionally, we employ a cut plane in the X and Y axes, outlined in blue, to allow for clear visualization of the PARP1 594 direct conjugate within the nuclear compartment.

Panel B - This panel features a 3-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594 direct conjugate from the Region of Interest shown in panel A. We note that the use of Airyscan super resolution is where we begin to identify discernible structure, which in our super resolution SIM and SIM squared, we further resolve.

Panel C - A clip from the Zeiss Zen image acquisition software shows that the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594 direct conjugate required 0.2% of 561nm laser in providing optimal image brightness over the 291 z-planes collected.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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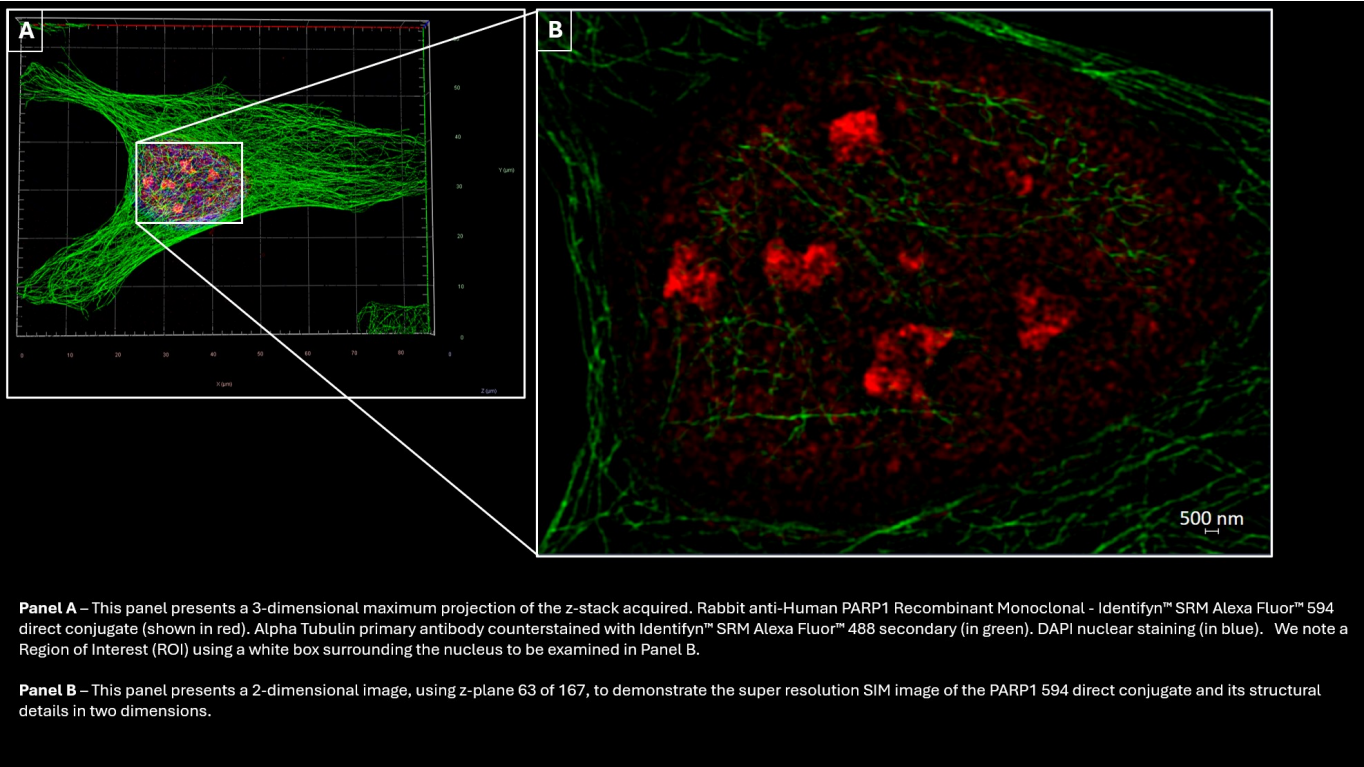
SOURCE PROVENANCE

Catalog	DC-000012
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	67

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	55A76A4EAB59BE0BBCCC61E3ACB1959B73D422C85A31D9F223560B1131762C80
Method PDF SHA-256	A2FEF7EBB0234FBABC83E17075862C0A301ABA462A8F98264C8CB2D187BF6815

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647
METADATA VALUES	62

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	167 Slices (4.98 μm)
Channels	3
Scaling (Per Pixel)	0.01689 μm X 0.01689 μm X 0.03000 μm
Image Size (Pixels)	5033 X 3837 1659 X 1225
Image Size (Scaled)	85.01 μm X 64.81 μm 28.02 μm X 20.69 μm
Bit Depth	16 Bit
Image Center Position	X: 63.63 mm, Y: 32.89 mm
Stage Position	X: 63.63 mm, Y: 32.89 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 488 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	561 488 405
Emission Wavelength	597 525 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 Axiocam 820
Camera Adapter	1X
Exposure Time	120 ms 50 ms
Depth of Focus	0.92 μm 0.81 μm 0.69 μm
Binning Mode	2,2
Laser Wavelength	561 nm @2.00% 488 nm @1.50% 405 nm @5.00%
Frame Time	1.59 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 μm grating 32 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	9.6812 (647), 10.7314 (488), 8.5340 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 594, IgG - (BLR018M)
Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

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™ 594, 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, 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® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) 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, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 167 Slices (4.98 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5033 X 3837
Image Size (Scaled) = 85.01 µm X 64.81 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.63 mm, Y: 32.89 mm
Stage Position = X: 63.63 mm, Y: 32.89 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 167 Slices (4.98 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 1659 X 1225
Image Size (Scaled) = 28.02 µm X 20.69 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.63 mm, Y: 32.89 mm
Stage Position = X: 63.63 mm, Y: 32.89 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @2.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 503 - 546, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μ m
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 32 μ m 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 = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @5.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D

Result Sampling = 4

Process Sampling = N/A

Channels = 3

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.6812 (647), 10.7314 (488), 8.5340 (DAPI)

Fitting = Fast Fit

Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise

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

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

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

Summary

Panel A - This panel presents a 3-dimensional maximum projection of the z-stack acquired. Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM AlexaFluora™ 594 direct conjugate (shown in red). Alpha Tubulin primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 secondary (in green), DAPI nuclear staining (in blue). We note a Region of Interest (ROI) using a white box surrounding the nucleus to be examined in Panel B.

Panel B - This panel presents a 2-dimensional image, using z-plane 63 of 167, to demonstrate the super resolution SIM image of the PARP1 594 direct conjugate and its structural details in two dimensions.

Image Corrections

None

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

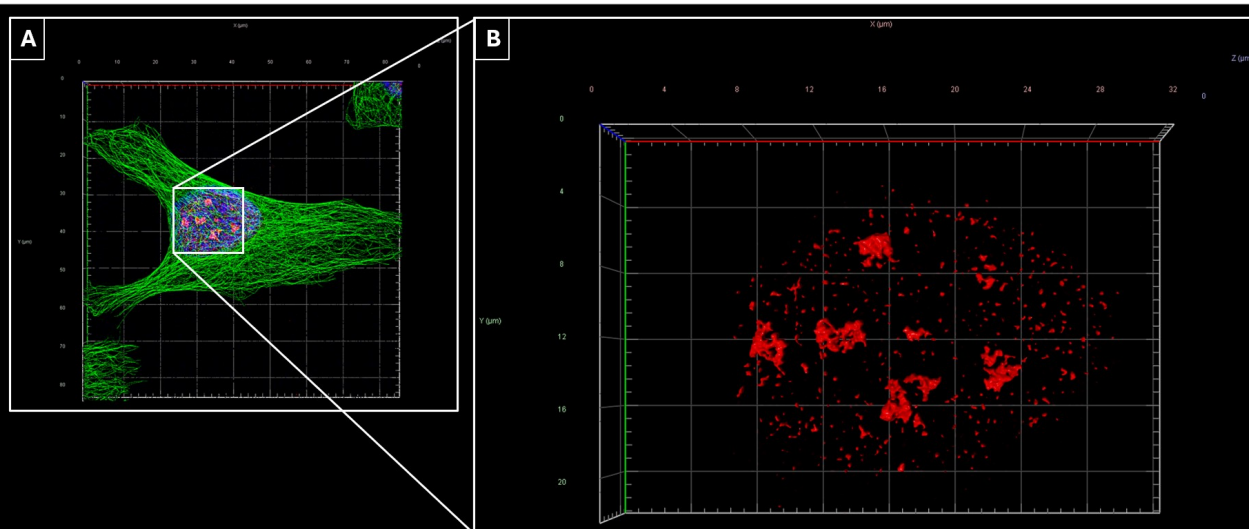
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SOURCE PROVENANCE

Catalog	DC-000012
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5CD0904C29664D07213C9F64B62977D6BAF271A8F8BD2A1E3E3581CECCB6B6B7
Method PDF SHA-256	3DB50A77BEC0C016F57DA9BA92BAEE9BAC3EB8DC17E032B7A36391187C6ACE28

ORIGINAL IMAGE EVIDENCE / SIM²

Panel A – This panel presents a 3-dimensional maximum projection of the z-stack acquired. Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 594 direct conjugate (shown in red). Alpha tubulin primary antibody counterstained with Identifyn™ SRM Alexa Fluor™ 488 secondary (in green). DAPI nuclear staining (in blue).

Panel B – This panel presents a 3-dimensional Surface projection of the Region of Interest (ROI) shown in Panel A; this is the same area examined in the SIM resolution image, however, in two dimensions (same product). Here, we see enhanced structural detail of the PARP1 594 direct conjugate at the aggregate level.

We note that the aggregates appear over all direct conjugate and primary/secondary images to have similar structures across all resolutions, while we do not claim as to PARP1 biological meaning, we do find it interesting that the structural details across many samples, sample preps and images are consistent, and we have confidence this is representative of PARP1 localization and further investigation in examining PARP1 known protein interactions could yield new findings.

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	69

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	167 Slices (4.98 µm)
Channels	3
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 1918 X 1334
Image Size (Scaled)	85.40 µm X 85.40 µm 32.40 µm X 22.53 µm
Bit Depth	16 Bit
Image Center Position	X: 63.63 mm, Y: 32.89 mm
Stage Position	X: 63.63 mm, Y: 32.89 mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 488 405
Channel Name	T1-Axiocam 820 #2-SR T2-Axiocam 820-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	561 488 405
Emission Wavelength	597 525 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 503 - 546, 657 - 800
Imaging Device	Axiocam 820 #2 Axiocam 820
Camera Adapter	1X
Exposure Time	120 ms 50 ms
Depth of Focus	0.92 µm 0.81 µm 0.69 µm
Binning Mode	2,2
Laser Wavelength	561 nm @2.00% 488 nm @1.50% 405 nm @5.00%
Frame Time	1.59 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 µm grating 32 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Algorithm	SIM ²
SIM ² Settings	Standard
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light, 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 Mixed Projection	Precise
Transparency	Threshold 2%, Ramp 20%, Maximum 100%
Surface	Threshold 25%, Ambient Light 10%, Specular Light 100%, Shininess 20%

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™ 594, IgG - (BLR018M)
Cat ID# - DC 000012

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

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™ 594, 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, 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® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) 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, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 167 Slices (4.98 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 85.40 µm X 85.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.63 mm, Y: 32.89 mm
Stage Position = X: 63.63 mm, Y: 32.89 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 167 Slices (4.98 µm)

Channels = 3
Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm
Image Size (Pixels) = 1918 X 1334
Image Size (Scaled) = 32.40 µm X 22.53 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.63 mm, Y: 32.89 mm
Stage Position = X: 63.63 mm, Y: 32.89 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

594 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 561
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @2.00%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 503 - 546, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μ m
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 32 μ m 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 = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @5.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM² Processing

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

3D Image Processing - Panel A

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

3D Image Processing - Panel B

3D Mixed Projection = Precise
 Transparency = Threshold 2%, Ramp 20%, Maximum 100%
 Surface = Threshold 25%, Ambient Light 10%, Specular Light 100%, Shininess 20%
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light, 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 presents a 3-dimensional maximum projection of the z-stack acquired Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM AlexaFluor™ 594 direct conjugate (shown in red), Alpha tubulin primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 secondary (in green). DAPI nuclear staining (in blue).

Panel B - This panel presents a 3-dimensional Surface projection of the Region of Interest (ROI) shown in Panel A; this is the same area examined in the SIM resolution image, however, in two dimensions (same product). Here, we see enhanced structural detail of the PARP1 594 direct conjugate at the aggregate level.

We note that the aggregates appear over all direct conjugate and primary/secondary images to have similar structures across all resolutions, while we do not claim as to PARP1 biological meaning, we do find it interesting that the structural details across many samples, sample preps and images are consistent, and we have confidence this is representative of PARP1 localization and further investigation in examining PARP1 known protein interactions could yield new findings.

Image Corrections

None

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000012
Stage	SIM ²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7C62A38BD797D22DC1F1A620BE9AD4B86D714A43569D487CF06B40397BC2A0EE
Method PDF SHA-256	CAA3769BAAB78553F6BE7FCDED5CFC3677243AF6BF6D51968C51DAC92D0F042E