

IDENTIFYN RESOLUTION STEPDOWN

PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 555

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

7

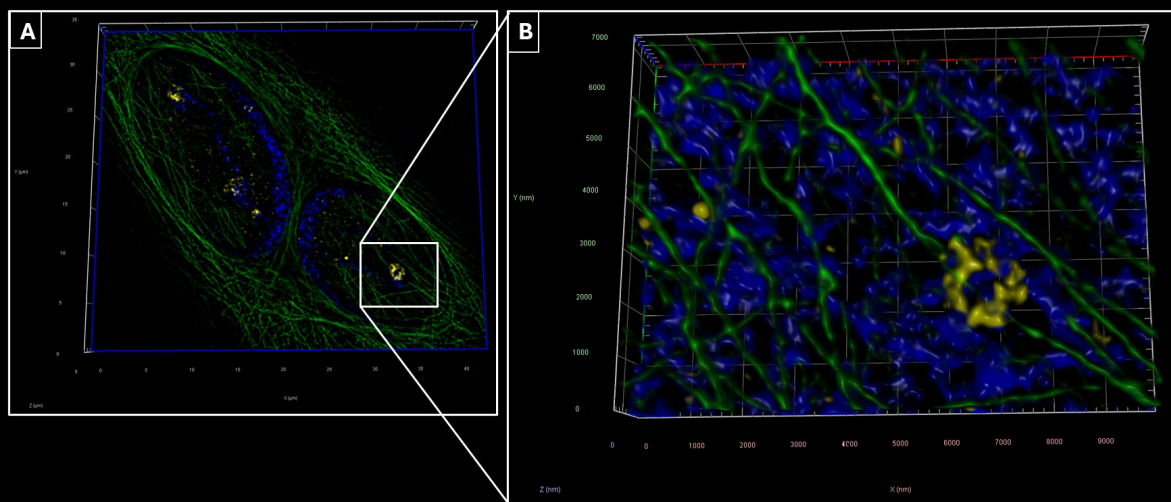
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



Panel A – This panel presents a 3-dimensional maximum projection of the entire image acquisition, comprising 135 z-planes at 4.02 microns. It displays the localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 555, combined with an alpha tubulin primary and Identifyn™ SRM Alexa Fluor™ 488 secondary, along with a DAPI nuclear stain. The nuclear localization of PARP1 and the presence of PARP1 aggregates are visible. A white box marks the Region of Interest (ROI) for further investigation.

Panel B – This panel shows a 3-dimensional volume projection of the ROI from Panel A, including all three channels. Upon closer examination of SIM squared super resolution, it is observed that the DAPI signal is not pan-nuclear, as this high-resolution technique allows for the observation of chromatin structures (~40-60 nm). Consequently, one would conclude that the staining is providing structural detail. Furthermore, there is an observable "interweaving" of the DAPI and PARP1 signals. While this does not imply colocalization that could allow for the interpretation of any direct interactions, it does homogeneously demonstrate the limitations of diffraction-limited images in providing such detail. We do not make any claims regarding biological relevance, but the findings suggest a need for further study and peer-reviewed literature regarding PARP1 mechanisms in DNA repair, which certainly supports this image.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000011 | 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-000011 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-000011 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	555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 555	3; AF532-49014; 38 eGFP; 49 DAPI; 515 - 545; 450 - 490; 335 - 383; 555 - 595
Widefield SIM — Apotome	405/DAPI; 488; 555	3; AF532-49014; 38 HE eGFP; 49 DAPI; 515 - 545; 450 - 490; 335 - 383; 555 - 595
Confocal	405/DAPI; 488; 555	3; None; 561 nm @0.3%; 488 nm @0.01%; 405 nm @0.2%; 553; 493; 353
Airyscan	405/DAPI; 488; 555; 750	2; None; 561 nm @0.5%; 488nm @0.02%; 405 nm @0.2%; 553; 493; 353
SIM	405/DAPI; 488; 555	3; 2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820-SR
SIM ²	405/DAPI; 488; 555	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 555.

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): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 200 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 41.

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; 555.

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: 111 Slices (11 μm); Channels: 3; Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm ; Image size (Pixels): 922 X 1069; Image Size (Pixels): 922 X 1069; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 100 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @12.00%. 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: 46.

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; 555.

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): 514 X 518; 380 X 255; Image Size (Pixels): 514 X 518; 380 X 255; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.31 μs ; Frame Time: 3.60 s. Illumination: Laser Wavelength: 561 nm @0.3%; 488 nm @0.01%. Optical/scan: Pinhole: 1.00 AU / 57 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 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.3 (3D, Auto); 6.9 (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; 555.

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: 161 Slices (4.80 μm); Channels: 2; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 1711 X 1711; 645 X 360; Image Size (Pixels): 1711 X 1711; 645 X 360; 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 @0.5%; 488nm @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); Background: Black; AiryScan Mode: SuperResolution 9.2 (3D, Auto); SuperResolution 8.5 (3D, Auto). Structured source metadata values: 61.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 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: 203 Slices (6.06 μm); 73 Slices (2.16 μm); Channels: 3; 2; Scaling (Per Pixel): 16.89 nm X 16.89 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 1096 X 1102; Image Size (Pixels): 5056 X 5056; 1096 X 1102; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.98 s; 1.59 s. Illumination: Laser Wavelength: 561 nm @4.00%; 488 nm @1.00%; Illumination Wavelength: 561; 488. 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); View Angle 35°, Scale Z 125%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 72.

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; 555.

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: 135 Slices (4.02 μm); 50 Slices (1.47 μm); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm; 0.013 μm X 0.013 μm X 0.030 μm ; Image size (Pixels): 3146 X 2681; 752 X 549; Image Size (Pixels): 3146 X 2681; 752 X 549; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820. Timing: Exposure Time: 120 ms; 50 ms; Frame Time: 1.98 s; 1.59 s. Illumination: Laser Wavelength: 561 nm @4.00%; 488 nm @1.00%; Illumination Wavelength: 561; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 30°, 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: 73.

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; 555.

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-000011 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)=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 - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm -> 0.01320 μ m X 0.01320 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)=3146 X 2681; Image Size (Scaled)=41.51 μ m X 35.38 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 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

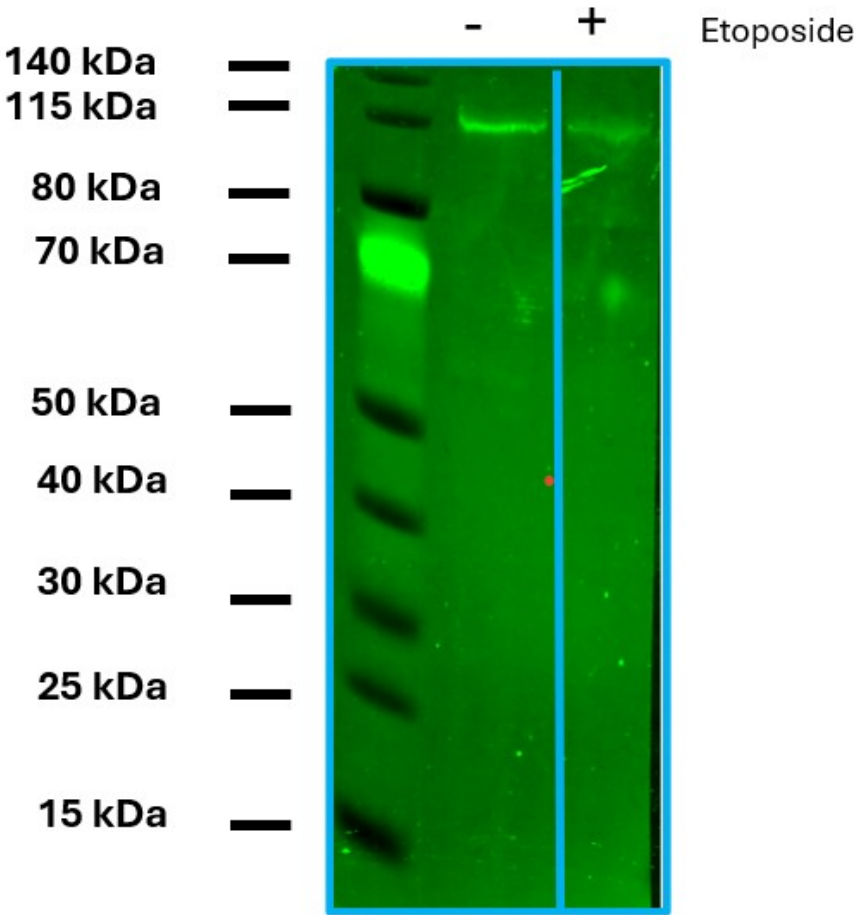
This dossier preserves the complete available evidence progression for DC-000011. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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	555
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™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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 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 ThermFisher's'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 then incubated with Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG (BLR018M) for 2 hours, followed by 5X PBS wash. 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

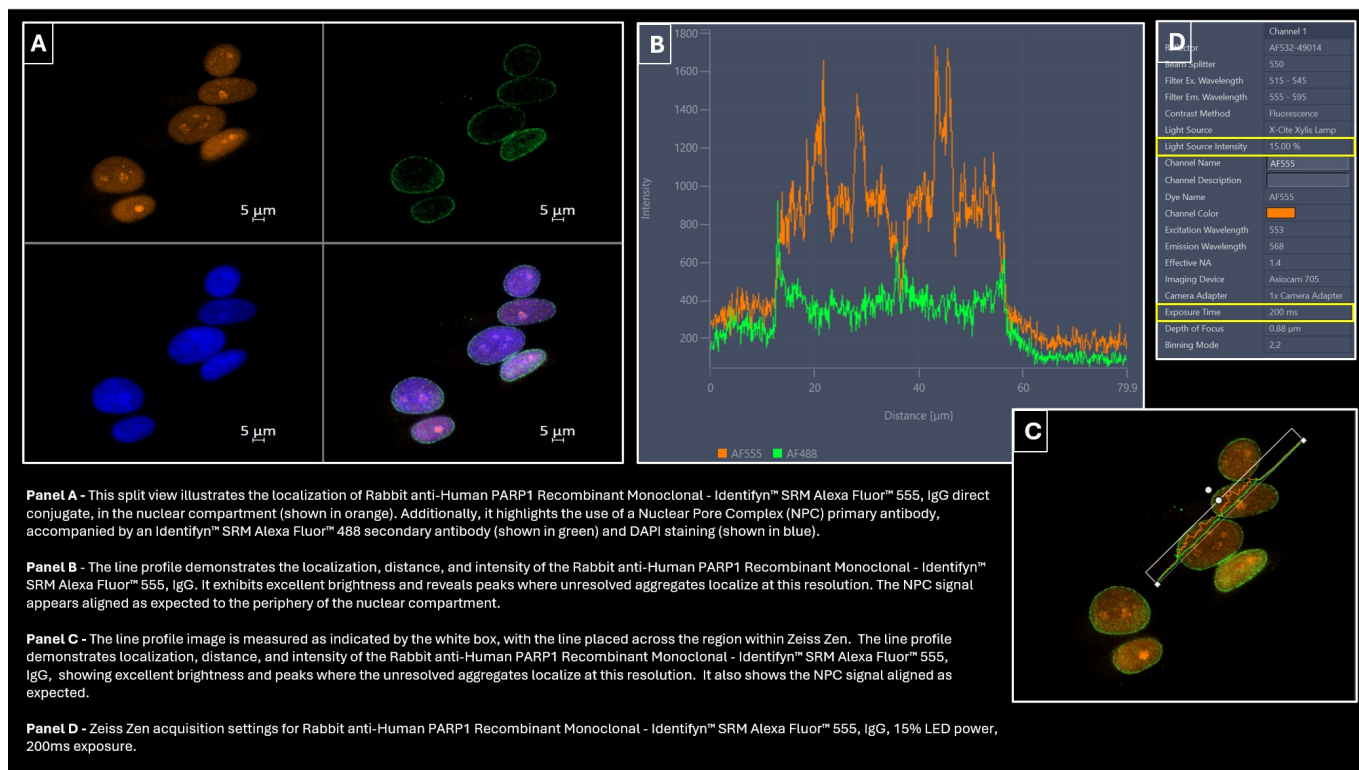
Image: K. Small

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000011
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	9CF2DA79ED26F1724925500AE8B5512B9234AE34D7D13E101F873F8F9B937328
Method PDF SHA-256	224ED800AC4EA269A2195C7D55D85CFB9FFE355CD438E97181DD19C95E0F04B3

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	41

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 μm X 0.110 μm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 μm X 112.59 μm
Bit Depth	14 Bit
Image Center Position	X: 0.00 μm , Y: 0.00 μm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	AF532-49014 38 eGFP 49 DAPI
Beam Splitter	550 495 395
Filter Excitation Wavelength	515 - 545 450 - 490 335 - 383
Filter Emission Wavelength	555 - 595 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @12%
Exposure Time	200 ms 100 ms 20 ms
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.88 μm 0.80 μm 0.72 μm
Binning Mode	2,2
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 79.9), Y (Min 45 and Max 1820)

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™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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™ 555, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, 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) = 1216 X 1028

Image Size (Scaled) = 133.18 µm X 112.59 µm

Bit Depth = 14 Bit

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

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

555 -

Reflector = AF532-49014
 Beam Splitter = 550
 Filter Excitation Wavelength = 515 - 545
 Filter Emission Wavelength = 555 - 595
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15.00%
 Exposure Time = 200 ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.88 μm
 Binning Mode = 2,2

488 -

Reflector = 38 eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10%
 Exposure Time = 100 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80 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 @12%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72 μm
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M) after DNA damage, the top-right panel features Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L), the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

Profile View Display - Panel B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 79.9), Y (Min 45 and Max 1820)

Summary

Panel A - This split view illustrates the localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG direct conjugate, in the nuclear compartment (shown in orange). Additionally, it highlights the use of a Nuclear Pore Complex (NPC) primary antibody, accompanied by an Identifyn® SRM Alexa Fluor™ 488 secondary antibody (shown in green) and DAPI staining (shown in blue).

Panel B - The line profile demonstrates the localization, distance, and intensity of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG. It exhibits excellent brightness and reveals peaks where unresolved aggregates localize at this resolution. The NPC signal appears aligned as expected to the periphery of the nuclear compartment.

Panel C - The line profile image is measured as indicated by the white box, with the line placed across the region within Zeiss Zen. The line profile demonstrates localization, distance, and intensity of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG, showing excellent brightness and peaks where the unresolved aggregates localize at this resolution. It also shows the NPC signal aligned as expected.

Panel D - Zeiss Zen acquisition settings for Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG, 15% LED power, 200ms exposure.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

© Copyright 2026 GMD12 LLC.

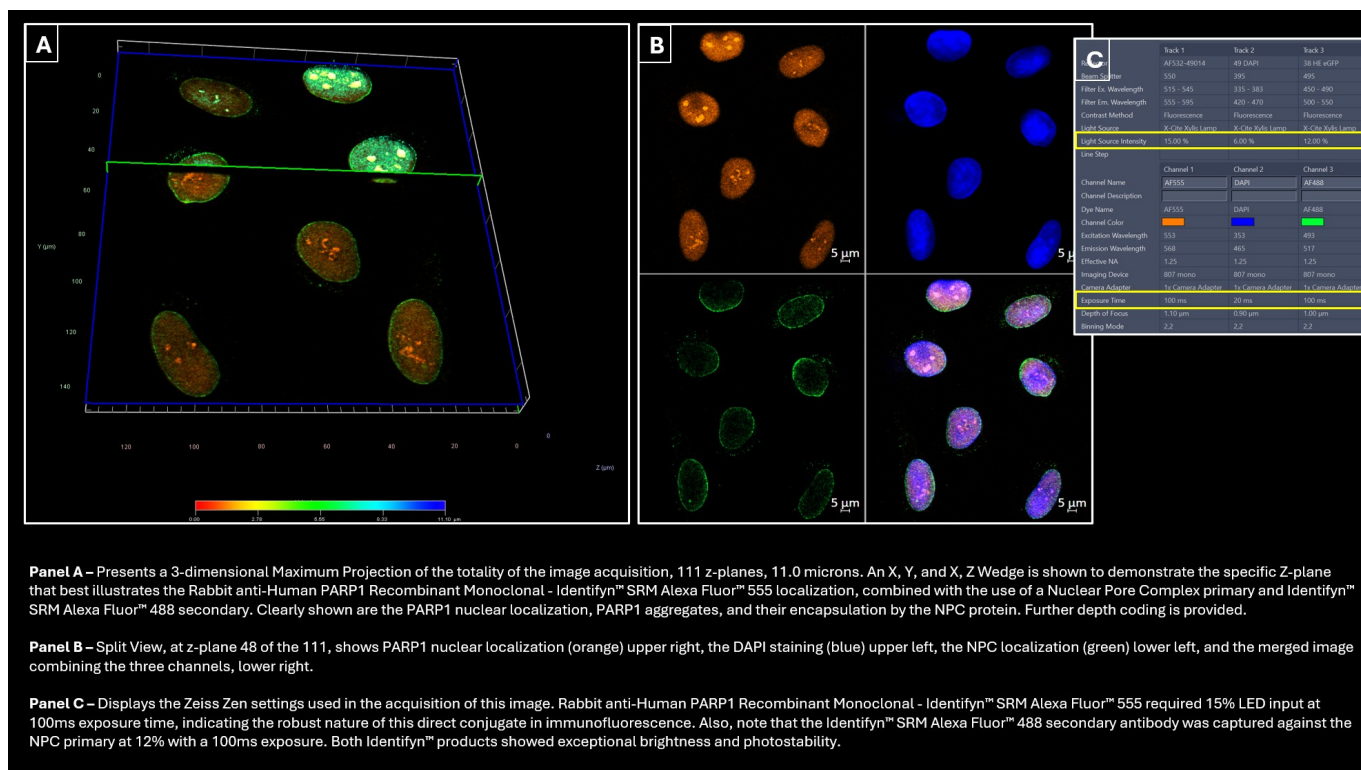
SOURCE PROVENANCE

Catalog	DC-000011
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:

SOURCE PROVENANCE

Structured source metadata values	41
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A3A3C9BEA437089FA333E6C26C26376335E53E1D02E635AE0B6B5532E7541958
Method PDF SHA-256	680CD52DB32083FC3653834CC3ABE827411A6BFC590F22FAD4464261C8806C73

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; 555
METADATA VALUES	46

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	111 Slices (11 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	922 X 1069
Image Size (Scaled)	131.71 µm X 152.71 µm
Bit Depth	14 Bit
Image Center Position	X: 50.24 mm, Y: 52.84 mm
Stage Position	X: 50.24 mm, Y: 52.84 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	AF532-49014 38 HE eGFP 49 DAPI
Beam Splitter	550 495 395
Filter Excitation Wavelength	515 - 545 450 - 490 335 - 383
Filter Emission Wavelength	555 - 595 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% X-Cite Xylis Lamp Intensity @12.00% X-Cite Xylis Lamp Intensity @6.00%
Exposure Time	100 ms 20 ms
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.10 µm 1.00 µm 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 100%, 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)

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™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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#375273. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, 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, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified 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 Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylys Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 111 Slices (11 µm)

Channels = 3
 Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
 Image Size (Pixels) = 922 X 1069
 Image Size (Scaled) = 131.71 µm X 152.71 µm
 Bit Depth = 14 Bit
 Image Center Position = X: 50.24 mm, Y: 52.84 mm
 Stage Position = X: 50.24 mm, Y: 52.84 mm
 ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

555 -

Reflector = AF532-49014
 Beam Splitter = 550
 Filter Excitation Wavelength = 515 - 545
 Filter Emission Wavelength = 555 - 595
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15.00%
 Exposure Time = 100 ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.10 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 @12.00%
 Exposure Time = 100 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 @6.00%
 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 μ m
 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)

X-Y and X-Z Wedge selected

Depth Coding selected

Split View - Panel B

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M) after DNA damage, the top-right panel features DAPI nuclear staining, the bottom-left panel features Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L), and the bottom-right panel is the merged image of all channels.

Summary

Panel A - Presents a 3-dimensional Maximum Projection of the totality of the image acquisition, 111 z-planes, 11.0 microns. An X, Y, and X, Z Wedge is shown to demonstrate the specific Z-plane that best illustrates the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 localization, combined with the use of a Nuclear Pore Complex primary and Identifyn® SRM Alexa Fluor™ 488 secondary. Clearly shown are the PARP1 nuclear localization, PARP1 aggregates, and their encapsulation by the NPC protein. Further depth coding is provided.

Panel B - Split View, at z-plane 48 of the 111, shows PARP1 nuclear localization (orange) upper right, the DAPI staining (blue) upper left, the NPC localization (green) lower left, and the merged image combining the three channels,

lower right.

Panel C - Displays the Zeiss Zen settings used in the acquisition of this image. Rabbit anti-HumanPARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 required 15% LED input at 100msec exposure time, indicating the robust nature of this direct conjugate in immunofluorescence. Also, note that the Identifyn® SRM Alexa Fluor™ 488 secondary antibody was captured against the NPC primary at 12% with a 100 ms exposure. Both Identifyn products showed exceptional brightness and photostability.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

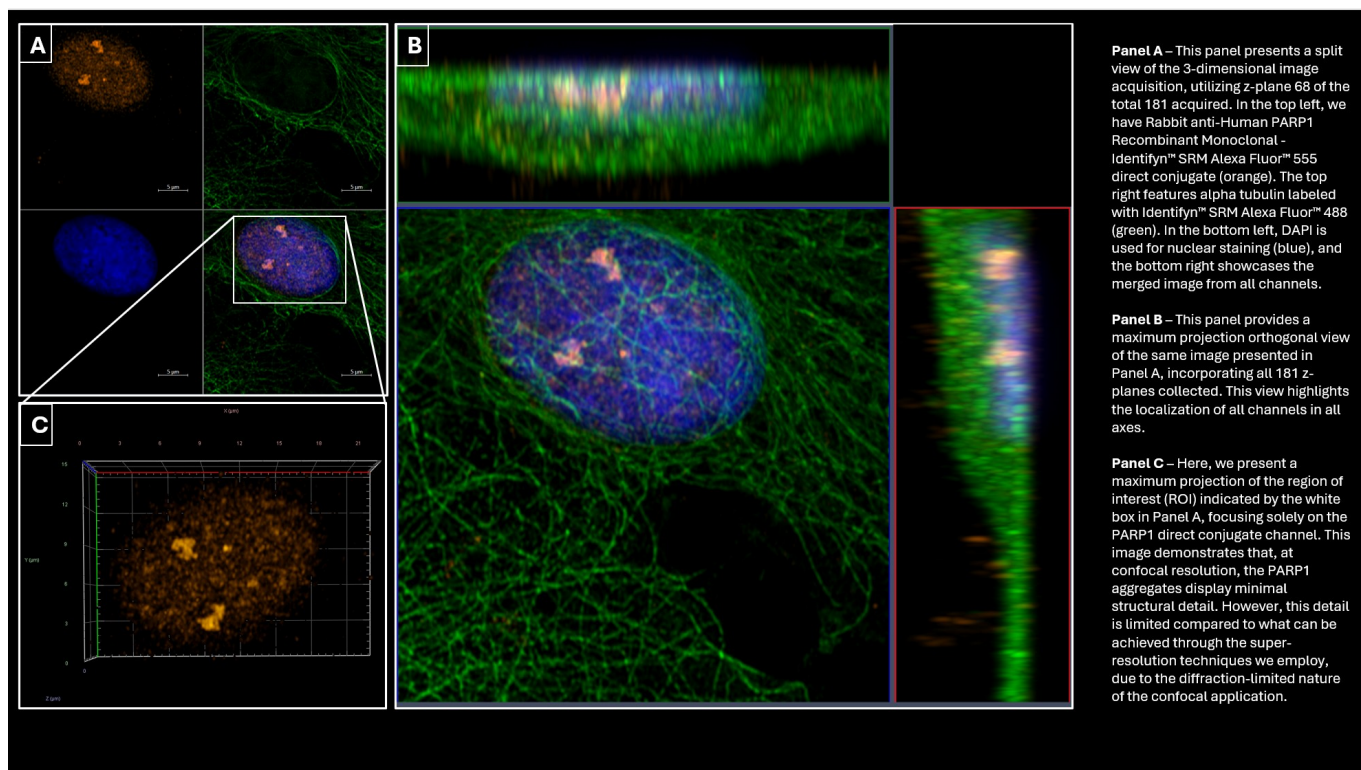
Data Presentation by P.D. Amistoso

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000011
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, Xiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2315CF1EE7232B408D7F70EA25A31E56E862B243C0C6D2C23FA5B646A4DB0735
Method PDF SHA-256	3DAA42E52741F736F640EC0CEBC4159E9477E59FB3872AECB2CFB9C30DDBC7C1

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
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	181 Slices (5.4 μm)
Channels	3
Scaling (Per Pixel)	0.060 μm X 0.060 μm X 0.030 μm
Image Size (Pixels)	514 X 518 380 X 255
Image Size (Scaled)	31.04 μm X 31.29 μm 22.95 μm X 15.40 μm
Bit Depth	16 Bit
Image Center Position	X: 93.57 mm, Y: 52.08 mm
Stage Position	X: 93.57 mm, Y: 52.08 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 57 μm 1.00 AU / 49 μm 1.0 AU / 30 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	1.17
Pixel Time	0.31 μs
Frame Time	3.60 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	550-630 480-549 410-470
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.3 (3D, Auto) 6.9 (3D, Auto) 6.3 (3D, Auto)
Cut Lines	X 178, Y 155, Z 71
Line Width	1 for all axes
Cut Line Opacity	50
3D Maximum Projection	Precise
Appearance	Threshold 1%, Ramp 0%, Maximum 100%
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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™ 555, 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 = 181 Slices (5.4 µm)

Channels = 3

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

Image Size (Pixels) = 514 X 518

Image Size (Scaled) = 31.04 µm X 31.29 µm

Bit Depth = 16 Bit

Image Center Position = X: 93.57 mm, Y: 52.08 mm

Stage Position = X: 93.57 mm, Y: 52.08 mm

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

Z Stack - (3D) - Panel C

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) = 380 X 255

Image Size (Scaled) = 22.95 µm X 15.40 µm

Bit Depth = 16 Bit

Image Center Position = X: 93.57 mm, Y: 52.08 mm

Stage Position = X: 93.57 mm, Y: 52.08 mm

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

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 57 μ m
 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 = 1.17
 Pixel Time = 0.31 μ s
 Frame Time = 3.60 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 550-630
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 7.3 (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 = 1.17
 Pixel Time = 0.31 μ s
 Frame Time = 3.60 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 = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.9 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.0 AU / 30 µm
 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 = 1.17
 Pixel Time = 0.31 µs
 Frame Time = 3.60 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 = 6.3 (3D, Auto)

Split View - Panel A

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, 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(ab')₂ (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

Maximum Intensity Projection (MIP)

Cut Lines = X 178, Y 155, Z 71
 Line Width = 1 for all axes
 Cut Line Opacity = 50

3D Image Processing - Panel C

3D Maximum Projection = Precise

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

Background = Black

Light = Brightness 100%, Tone Mapping Enabled

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

Summary

Panel A - This panel presents a split view of the 3-dimensional image acquisition, utilizing z-plane 68 of the total 181 acquired. In the top left, we have Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555 direct conjugate (orange). 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 a maximum projection orthogonal view of the same image presented in Panel A, incorporating all 181 z-planes collected. This View highlights the localization of all channels in all axes.

Panel C - Here, we present a maximum projection of the region of interest (ROI) indicated by the white box in Panel A, focusing solely on the PARP1 direct conjugate channel. This image demonstrates that, at confocal resolution, the PARP1 aggregates display minimal structural detail. However, this detail is limited compared to what can be achieved through the super-resolution techniques we employ due to the diffraction-limited nature of the confocal application.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

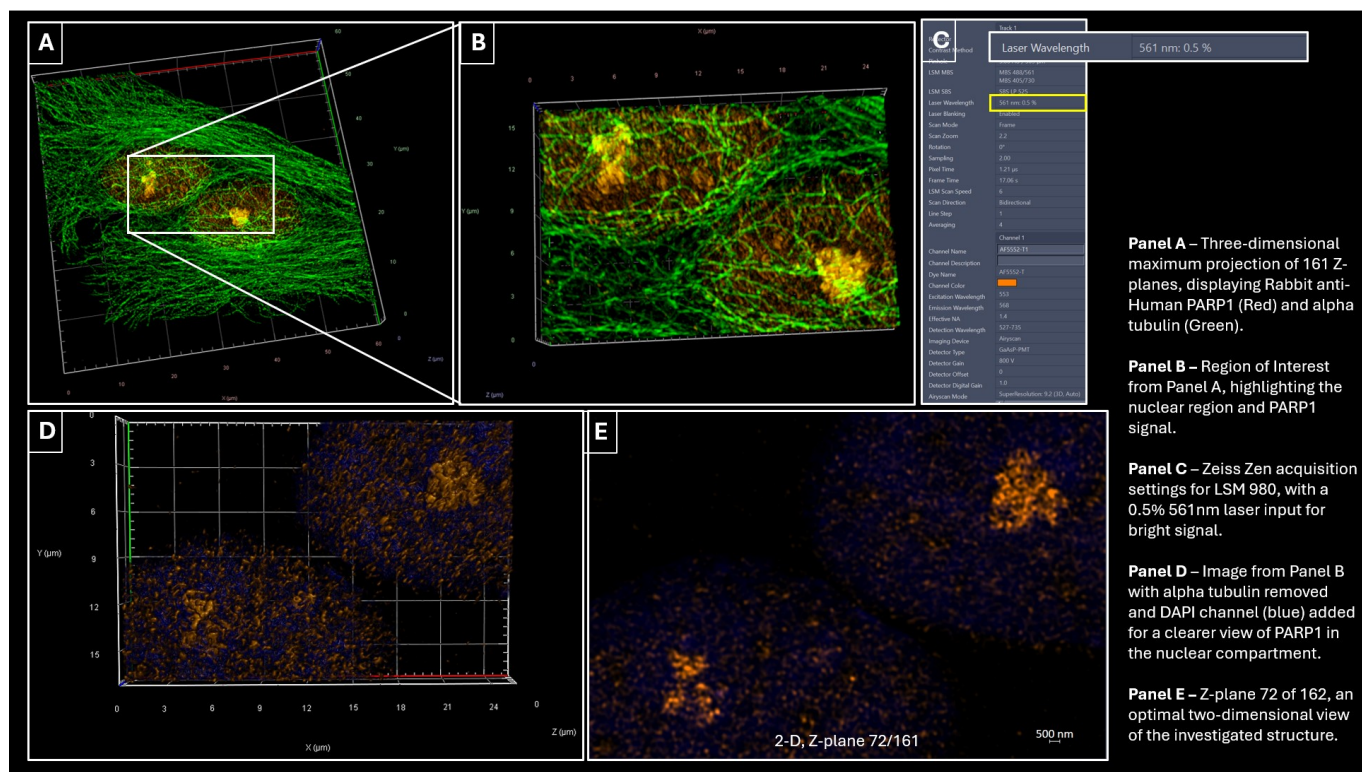
Catalog	DC-000011
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	189AF052DC975C0E5A081976DE9D45DFB807420567F89E5ADC89330B9ECA0F07

SOURCE PROVENANCE

Method PDF SHA-256

135BDEDDBD38C6630CC976FDBA2E1559012A57D63B1DDA7550C39996E2E533559

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 750
METADATA VALUES	61

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	161 Slices (4.80 µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.03000 µm
Image Size (Pixels)	1711 X 1711 645 X 360
Image Size (Scaled)	60.37 µm X 60.37 µm 22.76 µm X 12.70 µm
Bit Depth	16 Bit
Image Center Position	X: 94.43 mm, Y: 50.07 mm
Stage Position	X: 94.43 mm, Y: 50.07 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 Plate 10° SBS SP 505
Laser Wavelength	561 nm @0.5% 488nm @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	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	527-735 420-480, 495-550 350-499
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 9.2 (3D, Auto) SuperResolution 8.5 (3D, Auto) SuperResolution 7.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (555), Threshold 1% (488), Ramp 0% all channels, Maximum 100% all channels. Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% both channels, 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

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™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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#37527X. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250.

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 = 161 Slices (4.80 µm)

Channels = 2

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: 94.43 mm, Y: 50.07 mm

Stage Position = X: 94.43 mm, Y: 50.07 mm

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

Z Stack - (3D) - Panels B, D, & E

Z-Stack = 161 Slices (4.80 µm)

Channels = 2

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

Image Size (Pixels) = 645 X 360

Image Size (Scaled) = 22.76 µm X 12.70 µm

Bit Depth = 16 Bit

Image Center Position = X: 94.43 mm, Y: 50.07 mm

Stage Position = X: 94.43 mm, Y: 50.07 mm

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

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 309 μm
LSM MBS = MBS 488/561, 405/730
LSM SBS = SBS LP 525
Laser Wavelength = 561 nm @0.5%
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 = 553
Emission Wavelength = 568
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 9.2 (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 Plate 10°
 Laser Wavelength = 488nm @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 = 420-480, 495-550
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 8.5 (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 7.6 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing - Panels A & B

3D Maximum Projection = Precise
 Appearance = Threshold 1% (555), 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 D

3D Volume Projection = Precise

Appearance = Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% both channels, 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)

Summary

Panel A - Three-dimensional maximum projection of 161 Z-planes, displaying Rabbit anti-Human PARP1 (Red) and alpha tubulin (Green).

Panel B - Region of Interest from Panel A, highlighting the nuclear region and PARP1 signal.

Panel C - Zeiss Zen acquisition settings for LSM 980, with a 0.5% 561nm laser input for bright signal.

Panel D - Image from Panel B with alpha tubulin removed and DAPI channel (blue) added for a clearer view of PARP1 in the nuclear compartment.

Panel E - Z-plane 72 of 162, an optimal two-dimensional view of the investigated structure.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

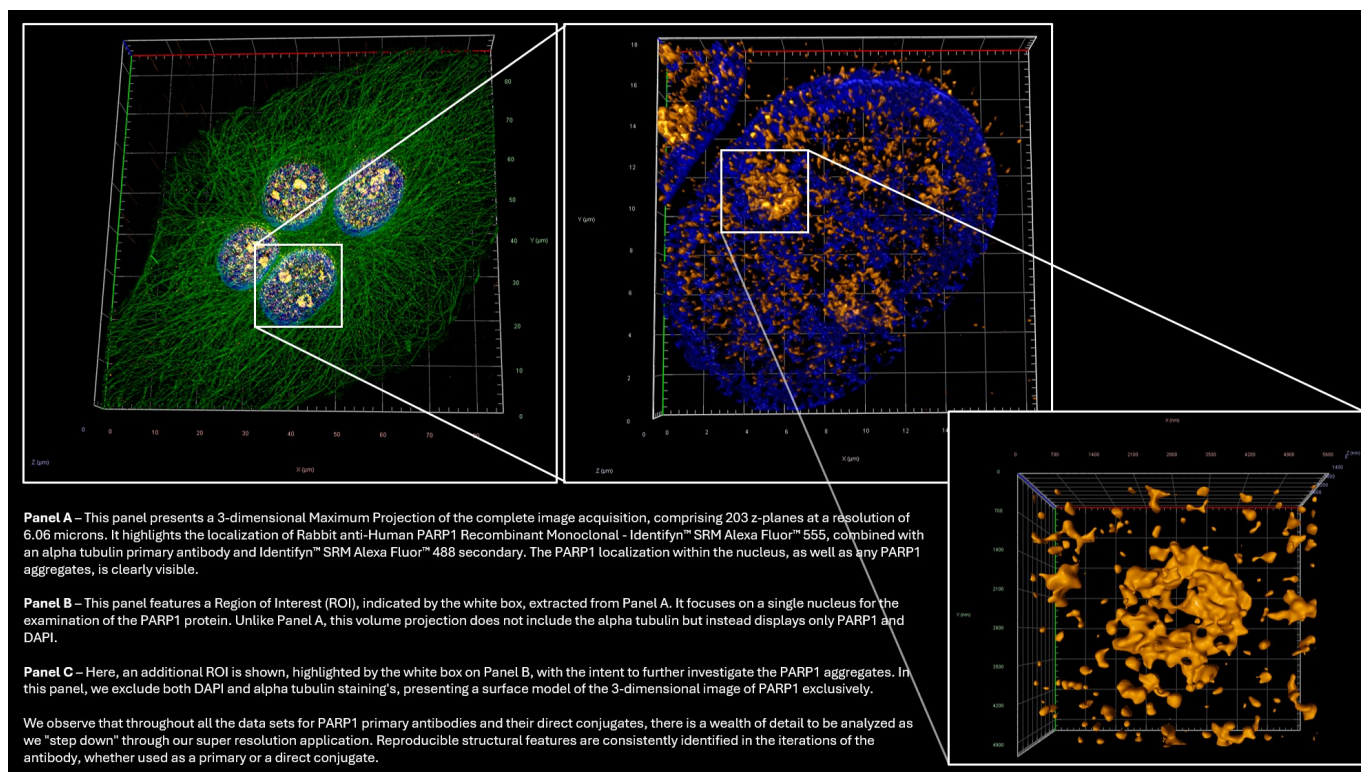
Data Presentation by P.D. Amistoso

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000011
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	61
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A43E6BEB9F5E54E2C647CF4F9D9B2AF3AFBFCCD7D5EA17C1F73FC8A96E9E9024
Method PDF SHA-256	A1EE27396F394D618B5B7434096B4B4314F068A618C1CF88386312477F21D8B4

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	72

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	203 Slices (6.06 µm) 73 Slices (2.16 µm)
Channels	3 2 1
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 1096 X 1102 266 X 268
Image Size (Scaled)	85.40 µm X 85.40 µm 18.51 µm X 18.61 µm 4.49 µm X 4.53 µm
Bit Depth	16 Bit
Image Center Position	X: 57.86 mm, Y: 34.37 mm
Stage Position	X: 57.86 mm, Y: 34.37 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
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 @4.00% 488 nm @1.00% 405 nm @5.00%
Frame Time	1.98 s 1.59 s 676.00 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	2
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.6556
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels Threshold 20%, Ambient Light 5%, Specular Light 100%, Shininess 35%
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 125%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise
3D Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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™ 555, 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 + ') 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 = 203 Slices (6.06 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: 57.86 mm, Y: 34.37 mm

Stage Position = X: 57.86 mm, Y: 34.37 mm

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

Z Stack - (3D) - Panel B

Z-Stack = 203 Slices (6.06 µm)

Channels = 2

Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm

Image Size (Pixels) = 1096 X 1102

Image Size (Scaled) = 18.51 µm X 18.61 µm

Bit Depth = 16 Bit

Image Center Position = X: 57.86 mm, Y: 34.37 mm

Stage Position = X: 57.86 mm, Y: 34.37 mm

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

Z Stack - (3D) - Panel C

Z-Stack = 73 Slices (2.16 µm)

Channels = 1

Scaling (Per Pixel) = 16.89 nm X 16.89 nm X 30.00 nm

Image Size (Pixels) = 266 X 268

Image Size (Scaled) = 4.49 µm X 4.53 µm

Bit Depth = 16 Bit

Image Center Position = X: 57.86 mm, Y: 34.37 mm

Stage Position = X: 57.86 mm, Y: 34.37 mm

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

555 -

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 μ m
 Binning Mode = 2,2
 Laser Wavelength = 561 nm @4.00%
 Frame Time = 1.98 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m 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 = 419 - 470, 503 - 546, 576 - 617, 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 @1.00%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 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 s
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = 2
 Channels = 3
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.6556
 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 Volume Projection = Precise
 Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise

Appearance = Threshold 20%, Ambient Light 5%, Specular Light 100%, Shininess 35%

Background = Black

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

Projection = View Angle 35°, Scale Z 125%, 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 complete image acquisition, comprising 203 z-planes at a resolution of 6.06 microns. It highlights the localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, combined with an alpha tubulin primary antibody and Identifyn® SRM Alexa Fluor™ 488 secondary. The PARP1 localization within the nucleus, as well as any PARP1 aggregates, is visible.

Panel B - This panel features a Region of Interest (ROI), indicated by the white box extracted from Panel A. It focuses on a single nucleus for the examination of the PARP1 protein. Unlike Panel A, this volume projection does not include the alpha tubulin but instead displays only PARP1 and DAPI.

Panel C - Here, an additional ROI is shown, highlighted by the white box on Panel B, with the intent to further investigate the PARP1 aggregates. In this panel, we exclude both DAPI and alpha tubulin stainings, presenting a surface model of the 3-dimensional image of PARP1 exclusively.

We observe that throughout all the data sets for PARP1 primary antibodies and their direct conjugates, there is a wealth of detail to be analyzed as we "step down" through our super resolution application. Reproducible structural features are consistently identified in the iterations of the antibody, whether used as a primary or a direct conjugate.

Image Corrections

None

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

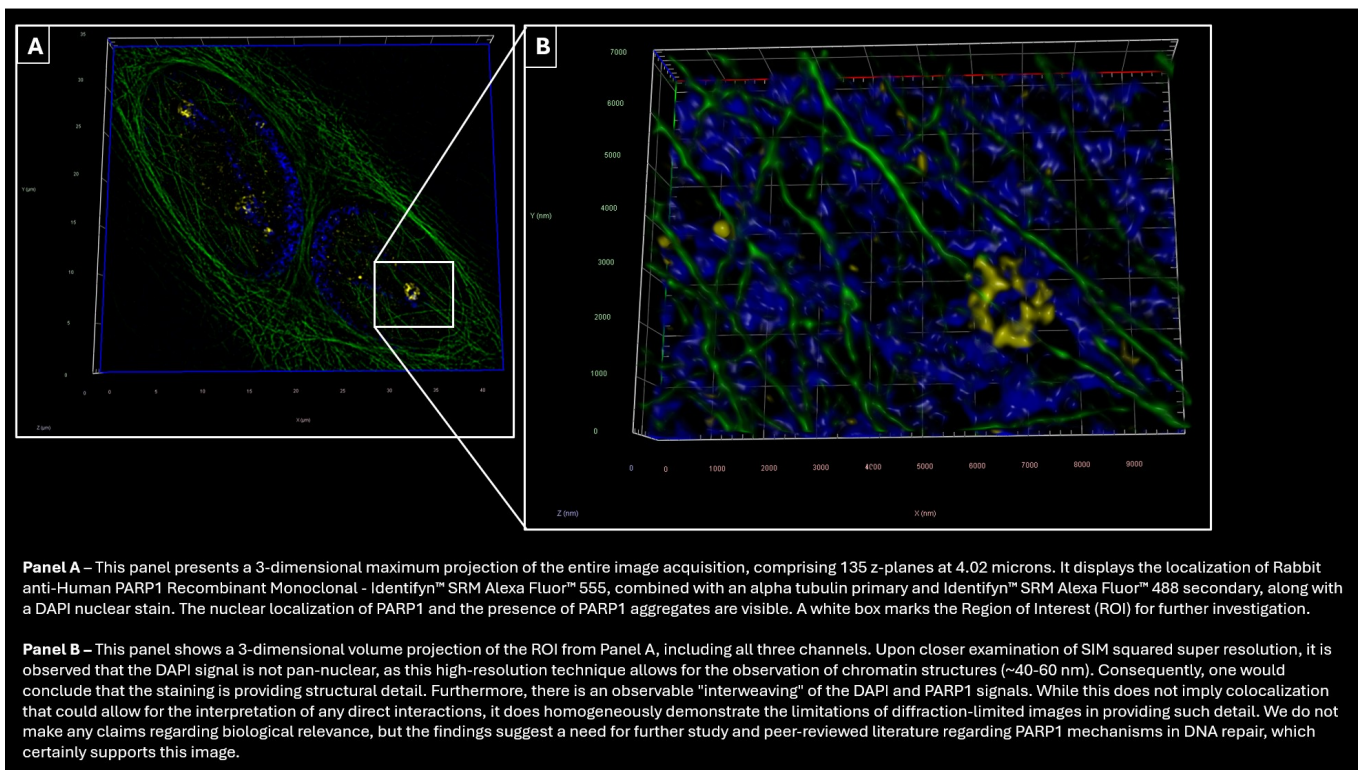
© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000011
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	72
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	868A4FDA09B321FB450DFB95565AD5FACE6EA6077184CD516EE5439FC56B9B97
Method PDF SHA-256	DE2D0A03B9B7D02D4EF98E881E2686D8601E9620ADA84ADB9F8407E9E6AC6EC2

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	73

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	135 Slices (4.02 µm) 50 Slices (1.47 µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm 0.013 µm X 0.013 µm X 0.030 µm
Image Size (Pixels)	3146 X 2681 752 X 549
Image Size (Scaled)	41.51 µm X 35.38 µm 9.92 µm X 7.24 µm
Bit Depth	16 Bit
Image Center Position	X: 58.67 mm, Y: 33.83 mm
Stage Position	X: X: 58.67 mm, Y: 33.83 mm X: 58.67 mm, Y: 33.83 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
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 @4.00% 488 nm @1.00% 405 nm @5.00%
Frame Time	1.98 s 1.59 s 676.00 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM ²
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.8884
Order Combination	Weiner filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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 30°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M)
Cat ID# - DC 000011

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™ 555, 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 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 = 135 Slices (4.02 µm)

Channels = 3

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

Image Size (Pixels) = 3146 X 2681

Image Size (Scaled) = 41.51 µm X 35.38 µm

Bit Depth = 16 Bit

Image Center Position = X: 58.67 mm, Y: 33.83 mm

Stage Position = X: 58.67 mm, Y: 33.83 mm

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

Z Stack - (3D) - Panel B

Z-Stack = 50 Slices (1.47 µm)

Channels = 3

Scaling (Per Pixel) = 0.013 µm X 0.013 µm X 0.030 µm

Image Size (Pixels) = 752 X 549

Image Size (Scaled) = 9.92 µm X 7.24 µm

Bit Depth = 16 Bit

Image Center Position = X: 58.67 mm, Y: 33.83 mm

Stage Position = X: 58.67 mm, Y: 33.83 mm

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

555 -

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 @4.00%

Frame Time = 1.98 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 = 419 - 470, 503 - 546, 576 - 617, 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 @1.00%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 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 s
 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
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 8.8884
 Order Combination = Weiner filter
 Regularization = None
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Histogram = Scale to Min/Max
 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)

X-Y and X-Z Wedge Selected

Depth Coding Selected

3D Image Processing - Panel B

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 30°, 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 entire image acquisition, comprising 135 z-planes at 4.02 microns. It displays the localization of Rabbit anti-Human PARP1Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, combined with an alpha tubulin primary and Identifyn® SRM Alexa Fluor™ 488 secondary, along with a DAPI nuclear stain. The nuclear localization of PARP1 and the presence of PARP1 aggregates are visible. A white box marks the Region of interest (ROI) for further investigation.

Panel B - This panel shows a 3-dimensional volume projection of the ROI from Panel A, including all three channels. Upon closer examination of SIM squared super resolution, it is observed that the DAPI signal is not pan-nuclear, as this high-resolution technique allows for the observation of chromatin structures (~40-60 nm). Consequently, one

would conclude that the staining is providing structural detail. Furthermore, there is an observable "interweaving" of the DAPI and PARP1 signals. While this does not imply colocalization that could allow for the interpretation of any direct interactions, it does homogeneously demonstrate the limitations of diffraction-limited images in providing such detail. We do not make any claims regarding biological relevance. Still, the findings suggest a need for further study and peer-reviewed literature regarding PARP1 mechanisms in DNA repair, which certainly supports this image.

Image Corrections

None

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

© Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	DC-000011
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	73
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	46E67AA59326C8D7D98CDEEF12076E59958702AA16BDDDB018CCBD9E7D90CB637
Method PDF SHA-256	D4BF9BD0EF8CC89C7E9D4C6B103BBCCFE0AF2BC8375C0AC79EF39B52B8A70344