BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

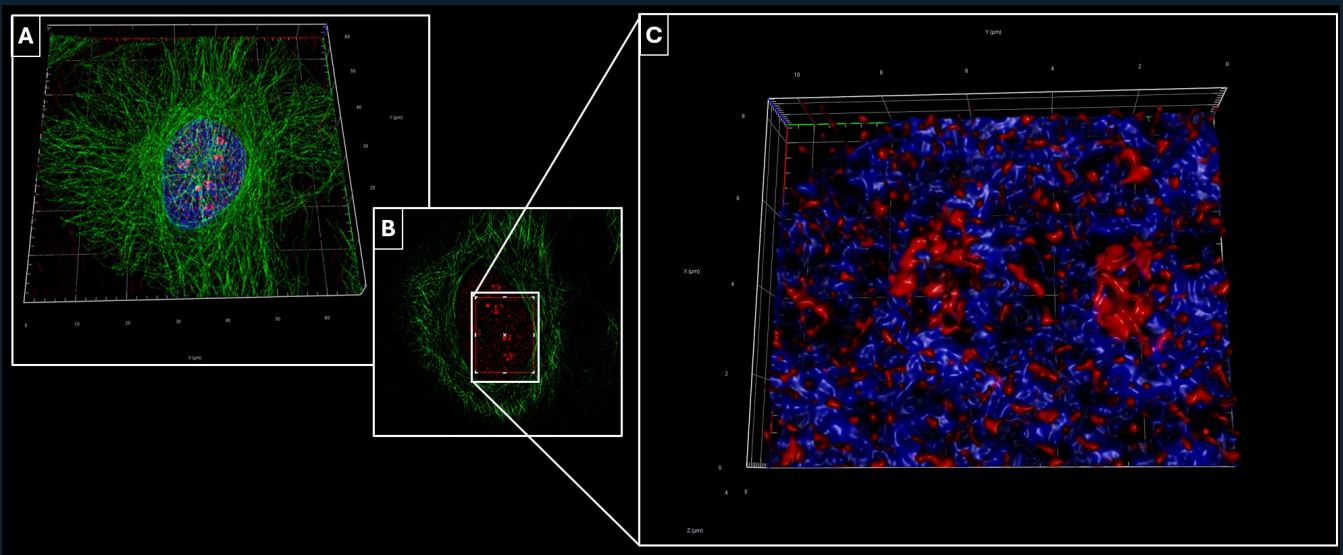
PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680

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

7	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	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™ 680 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 illustrates a single z-plane at position 40 of the 164 planes collected, including a white box denoting the Region of Interest (ROI) we selected to investigate further the PARP1 680 direct conjugate structural details available using super resolution SIM2, which provides ~ 40 – 60nm in the X, and Y planes.

Panel C – This panel presents a 3-dimensional volume projection of the z-stack acquired using only the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate channel. We note, as has been repeated throughout our image acquisition and analysis, that as we "step down" in resolution, we gain more detailed structural information. However, this structure is relatively large at scale, and the difference between super resolution SIM and SIM2 is left to the investigator.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

DC-000024 | 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-000024 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-000024 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	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 680	3; 50 Cy 5; AF532-49014; 49 DAPI; 625 - 655; 515 - 545; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 532; 680	3; 50 Cy 5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 532; 680; 750	3; None; 639 nm @0.3%; 488 nm @0.10%; 405 nm @0.2%; 679; 528; 353
Airyscan	405/DAPI; 488; 532; 680; 750	3; None; 639 nm @4.00%; 488 nm @0.2%; 405 nm @0.2%; 679; 528; 353
SIM	405/DAPI; 488; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 680	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640

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 680.

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): 0.110µm X 0.110µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 400 ms; 50 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 38.

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; 532; 555; 680.

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: 83 Slices (8.2 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 (Pixels): 1600 X 1104; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%; X-Cite Xylis Lamp Intensity @10.00%. 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: 47.

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; 532; 680.

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 (5.40 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1066 X 1066; 409 X 410; Image Size (Pixels): 1066 X 1066; 409 X 410; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.32 µs; Frame Time: 3.52 s. Illumination: Laser Wavelength: 639 nm @0.3%; 488 nm @0.10%. Optical/scan: Pinhole: 1.00AU / 68 µm; 1.00AU / 54 µm; Scan Zoom: 2.1; 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: 8.0 (3D, Auto); 9.2 (3D, Auto). Structured source metadata values: 67.

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; 532; 680; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 141 Slices (4.2 µm); Channels: 3; Scaling (Per Pixel): 35.29 nm X 35.29 nm X 30.00 nm; Image size (Pixels): 2221 X 2221; 476 X 486; Image Size (Pixels): 2221 X 2221; 476 X 486; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.93 µs; 93 µs; Frame Time: 22.09 s. Illumination: Laser Wavelength: 639 nm @4.00%; 488 nm @0.2%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 261 µm; Scan Zoom: 1.7; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution 9.4 (3D, Auto); SuperResolution 9.9 (3D, Auto). Structured source metadata values: 59.

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; 532; 680; 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, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 178 Slices (5.31 µm); 53 Slices (1.56 µm); Channels: 3; Scaling (Per Pixel): 21.11 nm X 21.11 nm X 30.00 nm; 0.021 µm X 0.021 µm X 0.030 µm; Image size (Pixels): 5056 X 5056; 478 X 478; Image Size (Pixels): 5056 X 5056; 478 X 478; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 150 ms; 100 ms; Frame Time: 1.98 s; 1.33 s. Illumination: Laser Wavelength: 640 nm @5.0%; 488 nm @1.50%; Illumination Wavelength: 640; 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); Background: Black. Structured source metadata values: 65.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 7

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 164 Slices (4.89 µm); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm; Image size (Pixels): 5056 X 5056; 643 X 810; Image Size (Pixels): 5056 X 5056; 643 X 810; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.98 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @5.00%; 488 nm @1.50%; Illumination Wavelength: 640; 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: 64.

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; 680.

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-000024 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 µm X 0.03529 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2221 X 2221; Image Size (Scaled)=78.38 µm X 78.38 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm -> 0.01320 µm X 0.01320 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=66.72 µm X 66.72 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

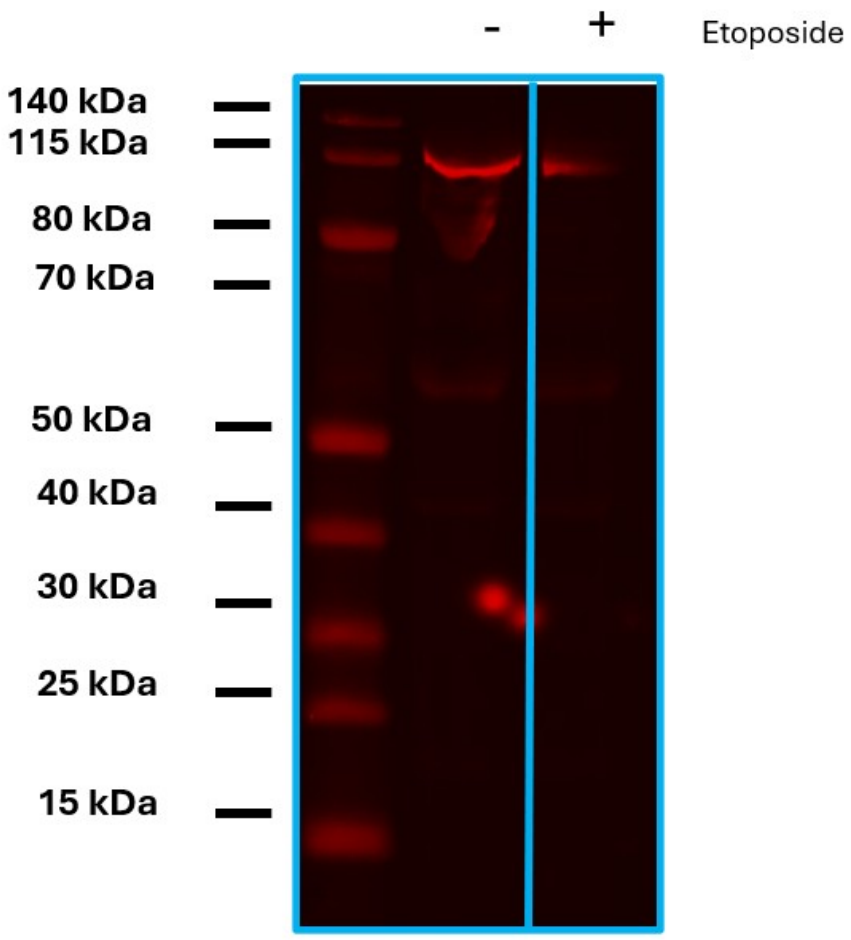
This dossier preserves the complete available evidence progression for DC-000024. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HEK293
DETECTED DYES	680
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	685nm
Emission Filter	720±24 nm
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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

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 (100 µg) 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™ 680, IgG - (BLR018M) 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 = 685nm
Emission Filter = 720±24 nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

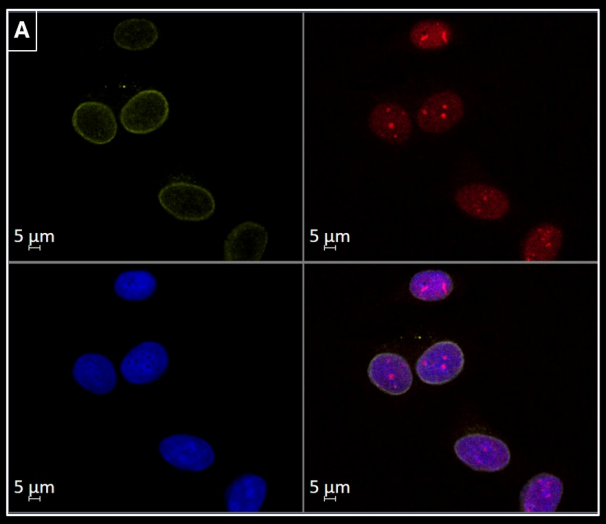
None

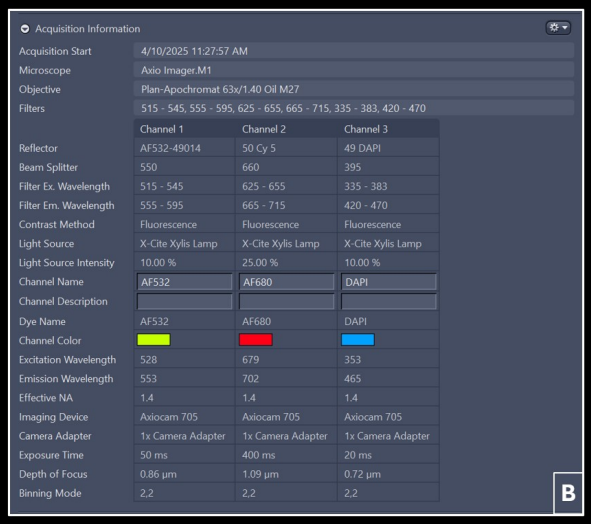
Image: K. Small
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
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	4B1EE788EF47D0D4C283D5B667B804CD9BBB81614557AF81EEB256D66F4FE6E8
Method PDF SHA-256	BAF75589C85BB83836B98AD563F98D63EAE936F7A4CD9888DD0239CC50B9C60A

ORIGINAL IMAGE EVIDENCE / WIDEFIELD





Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG, Direct Conjugate. In the upper left, we have the Nuclear Pore Complex (NPC) primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488. The upper right shows PARP1 680 direct conjugate (red), while the lower left displays DAPI (blue). The lower right presents a merged image of the three channels.

Panel B - Displays the settings used for image acquisition within Zeiss Zen software. The Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680, IgG, Direct Conjugate was captured using 25% LED for 400 ms, while the Identifyn™ SRM Alexa Fluor™ 488 was captured with 10% LED for 50 ms. The image settings indicate that the 680 direct conjugate is bright and easily captured with LED, as is the Identifyn™ 488.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 680
METADATA VALUES	38

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)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	50 Cy 5 | AF532-49014 | 49 DAPI
Beam Splitter	660 | 550 | 395
Filter Excitation Wavelength	625 - 655 | 515 - 545 | 335 - 383
Filter Emission Wavelength	665 - 715 | 555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00% | X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	400 ms | 50 ms | 20 ms
Excitation Wavelength	679 | 532 | 353
Emission Wavelength	702 | 553 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.09 µm | 0.86 µm | 0.72 µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000029

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™ 680, 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™ 532 Affinity Purified Goat Anti-Mouse, F(ab')□ (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) = 0.110μm X 0.110μm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18μm X 112.59μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00μm, Y: 0.00μm
ROI Center Offset = X: 0.00μm, Y: 0.00μm

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25.00%
Exposure Time = 400 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.09 µm
Binning Mode = 2,2

532 -

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 @10.00%
Exposure Time = 50 ms
Excitation Wavelength = 532
Emission Wavelength = 553
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.86 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 @10.00%
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 features Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L), the top-right panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR018M) after DNA damage, and the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR018M). In the upper left, we have the Nuclear Pore Complex (NPC) primary antibody labeled with Identifyn® SRM Alexa Fluor™ 532. The upper right shows PARP1 680 direct conjugate (red), while the lower left displays DAPI (blue). The lower right presents a merged image of the three channels.

Panel B - Displays the settings used for image acquisition within Zeiss Zen software. The Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG, Direct Conjugate was captured using 25% LED for 400 ms. In contrast, the Identifyn® SRM Alexa Fluor™ 532 was captured with 10% LED for 50 ms. The image settings indicate that the 680 direct conjugate is bright and easily captured with LED, as is the Identifyn® 532.

Image Correction

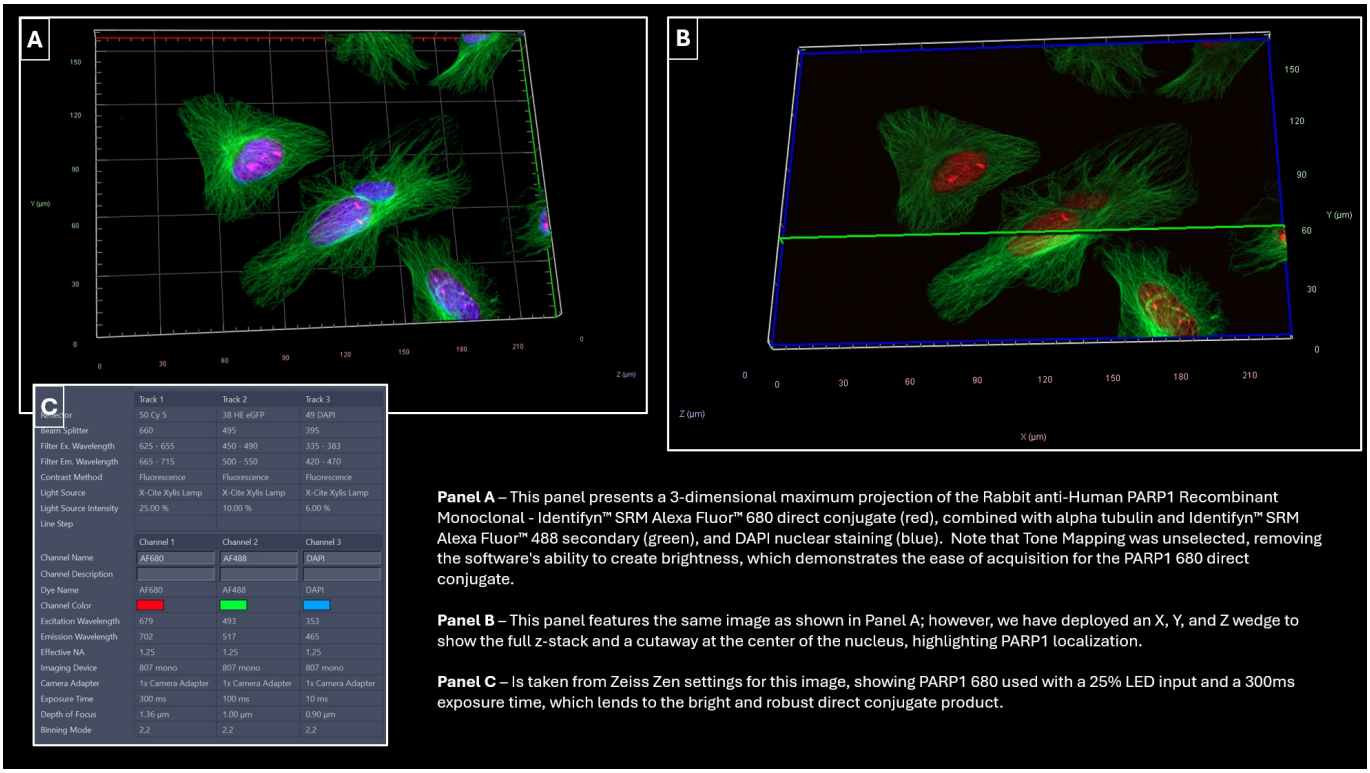
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B834572D88B271ABE2F2CD04E98F27A01C76CE822596144C608D6C10B166A3E4
Method PDF SHA-256	A5041989D7915B1C317F68F1CC4DD8CE5E6C85F37C0D3AF2400764B4221E9310

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A – This panel presents a 3-dimensional maximum projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate (red), combined with alpha tubulin and Identifyn™ SRM Alexa Fluor™ 488 secondary (green), and DAPI nuclear staining (blue). Note that Tone Mapping was unselected, removing the software's ability to create brightness, which demonstrates the ease of acquisition for the PARP1 680 direct conjugate.

Panel B – This panel features the same image as shown in Panel A; however, we have deployed an X, Y, and Z wedge to show the full z-stack and a cutaway at the center of the nucleus, highlighting PARP1 localization.

Panel C – Is taken from Zeiss Zen settings for this image, showing PARP1 680 used with a 25% LED input and a 300ms exposure time, which lends to the bright and robust direct conjugate product.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 680
METADATA VALUES	47

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	83 Slices (8.2 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: 51.03 mm, Y: 48.94 mm
Stage Position	X: 51.03 mm, Y: 48.94 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 HE eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00% | X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @6.00%
Exposure Time	300 ms | 100 ms | 10 ms
Excitation Wavelength	679 | 493 | 353
Emission Wavelength	702 | 517 | 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.36 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%, Directional Light Enabled, 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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000031

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™ 680, 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™ 532 Affinity Purified Donkey Anti-Mouse, F(ab')□ (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 = 83 Slices (8.2 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: 51.03 mm, Y: 48.94 mm
Stage Position = X: 51.03 mm, Y: 48.94 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

680 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25.00%
Exposure Time = 300 ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.36 µm
Binning Mode = 2,2

532 -

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 @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807
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 = 10 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
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%, Directional Light Enabled, 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 Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background = Black
Light = Brightness 100%, Directional Light Enabled, 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

Summary

Panel A - This panel presents a 3-dimensional maximum projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate (red), combined with alpha tubulin and Identifyn® SRM Alexa Fluor™ 488 secondary (green), and DAPI nuclear staining (blue). Note that Tone Mapping was unselected, removing the software's ability to create brightness, which demonstrates the ease of acquisition for the PARP1 680 direct conjugate.

Panel B - This panel features the same image as shown in Panel A; however, we have deployed an X, Y, and Z wedge to show the full z-stack and a cutaway at the center of the nucleus, highlighting PARP1 localization.

Panel C - This is taken from Zeiss Zen settings for this image, showing PARP1 680 used with a 25% LED input and a 300ms exposure time, which lends to the bright and robust direct conjugate product.

Image Correction

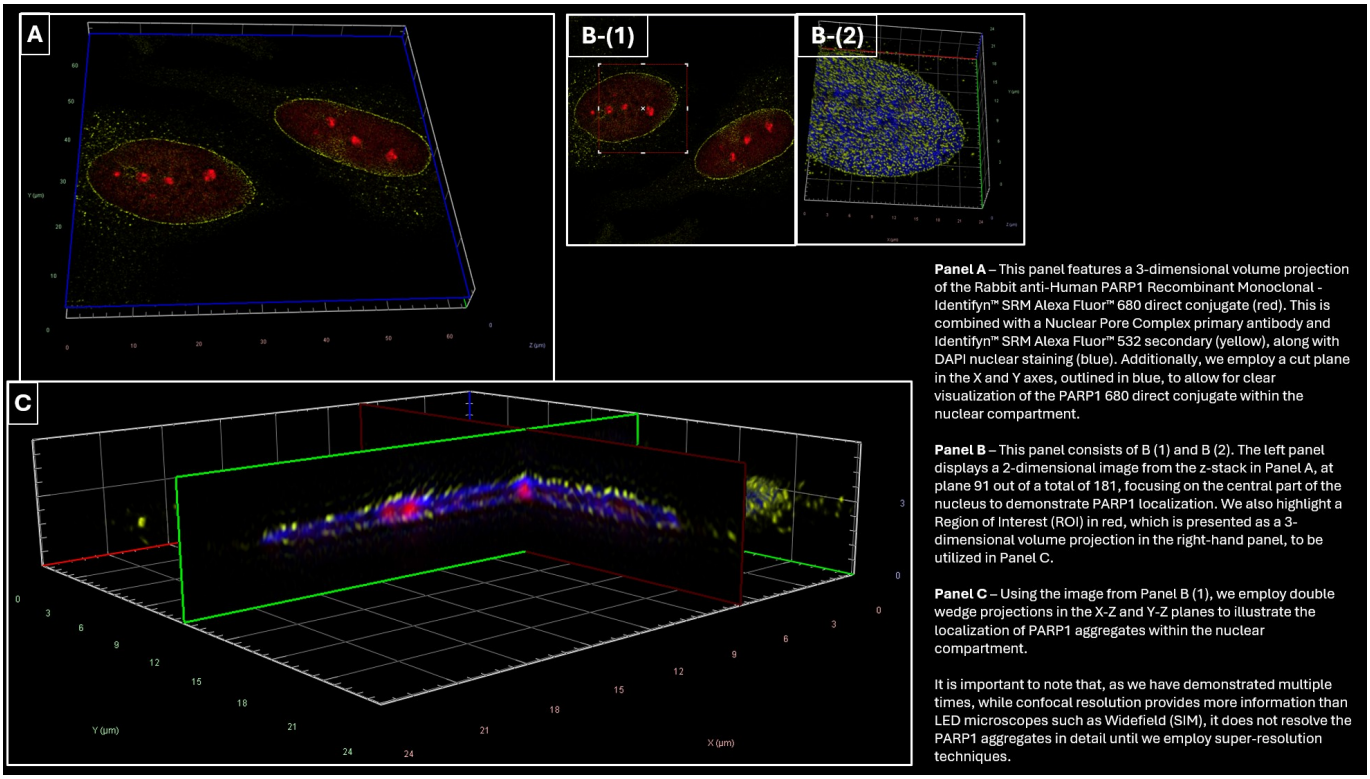
None

Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	EECCAD64420D9F2562B593705E94A4240733203FF1C4528D9AA5A88A6CA986B5
Method PDF SHA-256	F17DA30F9A90FD54A243697C7135D45AF0BCFF11CB3F43A0CAF43F761EC128AF

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A – This panel features a 3-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate (red). This is combined with a Nuclear Pore Complex primary antibody and Identifyn™ SRM Alexa Fluor™ 532 secondary (yellow), 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 680 direct conjugate within the nuclear compartment.

Panel B – This panel consists of B (1) and B (2). The left panel displays a 2-dimensional image from the z-stack in Panel A, at plane 91 out of a total of 181, focusing on the central part of the nucleus to demonstrate PARP1 localization. We also highlight a Region of Interest (ROI) in red, which is presented as a 3-dimensional volume projection in the right-hand panel, to be utilized in Panel C.

Panel C – Using the image from Panel B (1), we employ double wedge projections in the X-Z and Y-Z planes to illustrate the localization of PARP1 aggregates within the nuclear compartment.

It is important to note that, as we have demonstrated multiple times, while confocal resolution provides more information than LED microscopes such as Widefield (SIM), it does not resolve the PARP1 aggregates in detail until we employ super-resolution techniques.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 680; 750
METADATA VALUES	67

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 (5.40 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1066 X 1066 | 409 X 410
Image Size (Scaled)	62.72 µm X 62.72 µm | 24.07 µm X 24.12 µm
Bit Depth	16 Bit
Image Center Position	X: 89.20 mm, Y: 52.71 mm
Stage Position	X: 89.20 mm, Y: 52.71 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 68 µm | 1.00AU / 54 µm | 1.00 AU / 45 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 615 | SBS SP 505 | Mirror
Laser Wavelength	639 nm @0.3% | 488 nm @0.10% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	1.20
Pixel Time	0.32 µs
Frame Time	3.52 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	679 | 528 | 353
Emission Wavelength	702 | 553 | 465
Effective NA	1.4
Detection Wavelength	675-750 | 526-600 | 439-499
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	8.0 (3D, Auto) | 9.2 (3D, Auto) | 7.2 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Y	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	3% | 0% | -41%
Clip X	0°
Clip Y	0°
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.
Y-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

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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000029

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™ 680, 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™ 532 Affinity Purified Goat Anti-Mouse, F(a'')□ (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 (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1066 X 1066
Image Size (Scaled) = 62.72 µm X 62.72 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.20 mm, Y: 52.71 mm
Stage Position = X: 89.20 mm, Y: 52.71 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B & C

Z-Stack = 181 Slices (5.40 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 409 X 410
Image Size (Scaled) = 24.07 µm X 24.12 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.20 mm, Y: 52.71 mm
Stage Position = X: 89.20 mm, Y: 52.71 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 68 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 675-750
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.0 (3D, Auto)

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 54 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 488 nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 526-600
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 9.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 45 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 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 = 439-499
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.2 (3D, Auto)

3D Image Processing - Panel A

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

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

X-Y = 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 = 3%
Clip X = 0°
Clip Y = 0°

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)

X-Z and Y-Z Wedge selected

Clipping Image Process = Clipping planes are introduced to pronounce regions of special interest.

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 = 0%
Clip X = 0°
Clip Y = 0°

Y-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 = -41%
Clip X = 0°
Clip Y = 0°

Summary

Panel A - This panel features a 3-dimensional volume projection of the Rabbit anti-Human PARP1Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate (red). This is combined with a Nuclear Pore Complex primary antibody and Identifyn® SRM Alexa Fluor™ 532 secondary (yellow), 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 680 direct conjugate within the nuclear compartment.

Panel B - This panel consists of B (1) and B (2). The left panel displays a 2-dimensional image from the z-stack in Panel A, at plane 91 out of a total of 181, focusing on the central part of the nucleus to demonstrate PARP1 localization. We also highlight a Region of Interest (ROI) in red, which is presented as a 3-dimensional volume projection in the right-hand panel, to be utilized in Panel C.

Panel C - Using the image from Panel B (1), we employ double wedge projections in the X-Z and Y-Z planes to illustrate the localization of PARP1 aggregates within the nuclear compartment.

It is important to note that, as we have demonstrated multiple times, while confocal resolution provides more information than LED microscopes such as Widefield (SIM), it does not resolve the PARP1 aggregates in detail until we employ super resolution techniques.

Image Corrections

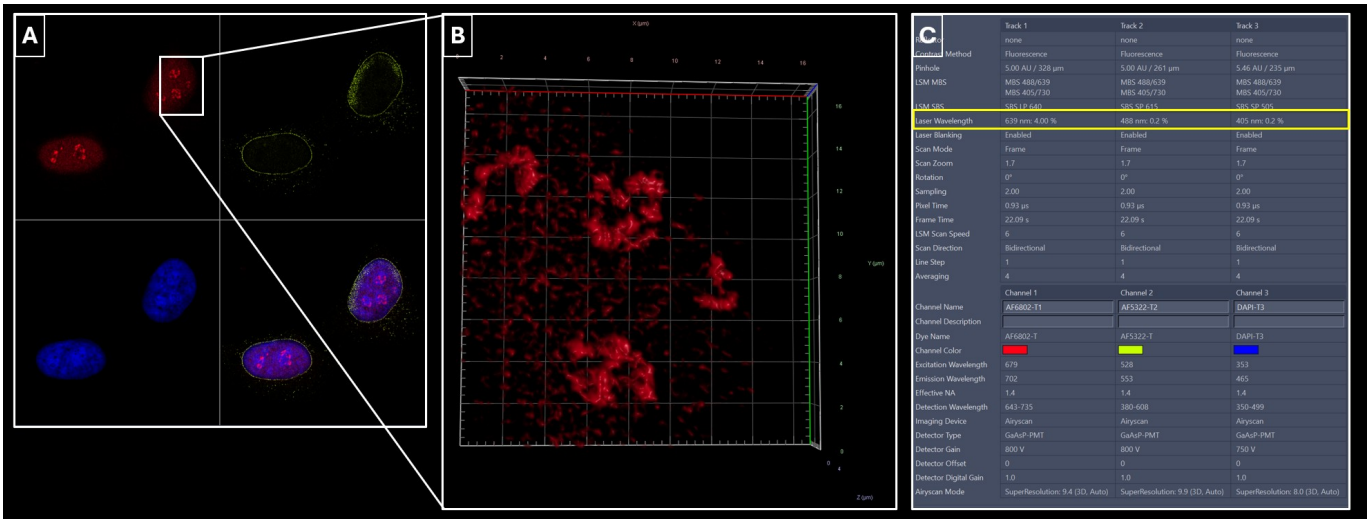
None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
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	67
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	70B6AC3AB272F3685274DDC515B03EE5F4447CC4E969C5207F7C58326B3C42E1
Method PDF SHA-256	EF814D779BA746E6EED19D75BA5901073C88BDCBF00CCED56EE93E7A3778FDF1

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A – This panel presents a split view of the z-stack acquired at z-plane 60 of 141. In the upper right, we see the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate (shown in red). Upper right, is a Nuclear Pore Complex primary antibody counterstained with Identifyn™ SRM Alexa Fluor™ 532 secondary (in yellow). The lower left displays DAPI nuclear staining (in blue), while the lower right shows a merged image of all three channels. The white box indicates the Region of Interest (ROI) we have selected to investigate the PARP1 60 DC-labeled aggregate, which begins to reveal structural details in super-resolution, specifically using Airyscan.

Panel B – This panel illustrates a three-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate (in red). The Airyscan super-resolution, achieved at ~100-120 nm in the X and Y dimensions, demonstrates that the previously observed large circular non-informational signal, evident in diffraction-limited techniques such as widefield, widefield SIM, and even confocal microscopy, may now provides valuable structural information.

Panel C – This panel features a clip from Zeiss Zen software showing the image acquisition of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate. It was visualized over the course of 141 z-planes with a laser power of 4.0% at 639 nm, revealing structural details. This confirms the PARP1 680 DC is both bright and specific.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 680; 750
METADATA VALUES	59

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	141 Slices (4.2 µm)
Channels	3
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.03000 µm
Image Size (Pixels)	2221 X 2221 | 476 X 486
Image Size (Scaled)	78.38 µm X 78.38 µm | 16.80 µm X 17.15 µm
Bit Depth	16 Bit
Image Center Position	X: 89.19 mm, Y: 53.98 mm
Stage Position	X: 89.19 mm, Y: 53.98 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 261 µm | 5.46 AU / 235 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 640 | SBS SP 615 | SBS SP 505
Laser Wavelength	639 nm @4.00% | 488 nm @0.2% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	2.0
Pixel Time	0.93 µs | 93 µs
Frame Time	22.09 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	679 | 528 | 353
Emission Wavelength	702 | 553 | 465
Effective NA	1.4
Detection Wavelength	643-735 | 380-608 | 350-465
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 9.4 (3D, Auto) | SuperResolution 9.9 (3D, Auto) | SuperResolution 8.0 (3D, Auto)
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 50%, 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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000029

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™ 680, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250.

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™ 532 Affinity Purified Goat Anti-Mouse, F(a'')□ (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 = 141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 2221 X 2221
Image Size (Scaled) = 78.38 µm X 78.38 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.19 mm, Y: 53.98 mm
Stage Position = X: 89.19 mm, Y: 53.98 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 141 Slices (4.2 µm)

Channels = 3
Scaling (Per Pixel) = 35.29 nm X 35.29 nm X 30.00 nm
Image Size (Pixels) = 476 X 486
Image Size (Scaled) = 16.80 µm X 17.15 µm
Bit Depth = 16 Bit
Image Center Position = X: 89.19 mm, Y: 53.98 mm
Stage Position = X: 89.19 mm, Y: 53.98 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @4.00%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.0
Pixel Time = 0.93 µs
Frame Time = 22.09 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.4 (3D, Auto)

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 261 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 488 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.0
Pixel Time = 93 µs
Frame Time = 22.09 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 380-608
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.46 AU / 235 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.0
Pixel Time = 0.93 µs
Frame Time = 22.09 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 = 350-465
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.0 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

Split View - Panel A

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR018M) after DNA damage, the top-right panel features Nuclear Pore Complexes visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, F(a'')□ (H + L), the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

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 50%, 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 z-stack acquired at z-plane 60 of 141. In the upper right, we see the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate (shown in red). Upper right is a Nuclear Pore Complex primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 532 secondary (in yellow). The lower left displays DAPI nuclear staining (in blue), while the lower right shows a merged image of all three channels. The white box indicates the Region of Interest (ROI) we have selected to investigate the PARP1 60 DC-labeled aggregate, which begins to reveal structural details in super-resolution, specifically using Airyscan.

Panel B - This panel illustrates a three-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate (in red). The Airyscan super resolution, achieved at ~100-120 nm in the X and Y dimensions, demonstrates that the previously observed significant circular non-informational signal, evident in diffraction-limited techniques such as widefield, widefield SIM, and even confocal microscopy, may now provide valuable structural information.

Panel C - This panel features a clip from Zeiss Zen software showing the image acquisition of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate. It was visualized over the course of 141 z-planes with a laser power of 4.0% at 639 nm, revealing structural details. This confirms the PARP1 680 DC is both bright and specific.

Image Corrections

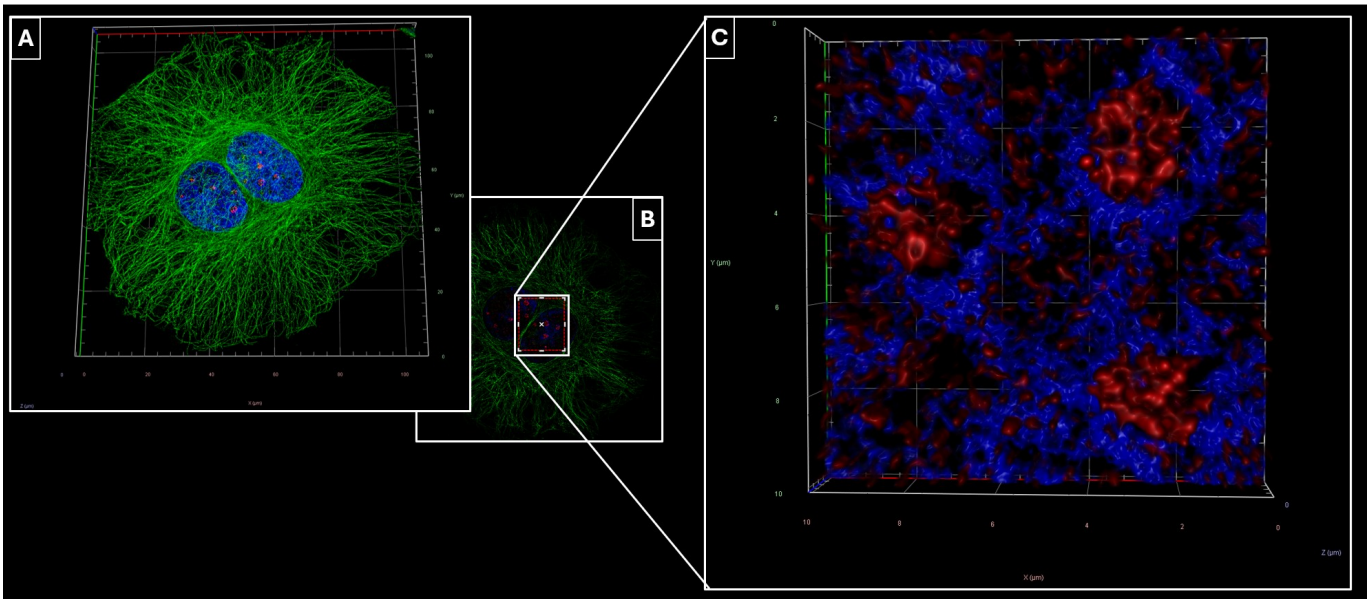
None

Image by J.K. Bennett
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
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	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	FB12425223DCC5B0EEE984EF1F164B16A086639F9C83687680070AC91222EC23
Method PDF SHA-256	88A64BBA0F16E85B576EBCAFF6CA3075AF34A5E60F36D1FF8CE2345318CBEEE1

ORIGINAL IMAGE EVIDENCE / SIM



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™ 680 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 illustrates a single z-plane at position 52 of the 178 planes collected, including a white box denoting the Region of Interest (ROI) we selected to investigate further the PARP1 680 direct conjugate structural details available using super resolution SIM, which provides ~ 60 - 80nm in the X, and Y planes.

Panel C – This panel presents a 3-dimensional volume projection of the z-stack acquired using only the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate channel. We note, as has been repeated throughout our image acquisition and analysis, that as we "step down" in resolution, we gain more detailed structural information.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	65

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	178 Slices (5.31 µm) | 53 Slices (1.56 µm)
Channels	3
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm | 0.021 µm X 0.021 µm X 0.030 µm
Image Size (Pixels)	5056 X 5056 | 478 X 478
Image Size (Scaled)	106.75 µm X 106.75 µm | 10.09 µm X 10.09 µm
Bit Depth	16 Bit
Image Center Position	X: 57.58 mm, Y: 37.14 mm
Stage Position	X: 57.58 mm, Y: 37.14 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	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150 ms | 100 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @5.0% | 488 nm @1.50% | 405 nm 4.0%
Frame Time	1.98 s | 1.33 s | 676.00 ms
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	9.3768
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% 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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (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™ 680, 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')□ (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, 1X Tubelens, was used with the following parameters for each dye:

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

Z-Stack = 178 Slices (5.31 µm)

Channels = 3
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 106.75 µm X 106.75 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.58 mm, Y: 37.14 mm
Stage Position = X: 57.58 mm, Y: 37.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 53 Slices (1.56 µm)

Channels = 3
Scaling (Per Pixel) = 0.021 µm X 0.021 µm X 0.030 µm
Image Size (Pixels) = 478 X 478
Image Size (Scaled) = 10.09 µm X 10.09 µm
Bit Depth = 16 Bit
Image Center Position = X: 57.58 mm, Y: 37.14 mm
Stage Position = X: 57.58 mm, Y: 37.14 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @5.0%
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 = 100 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm 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 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm 4.0%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = 2
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.3768

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% 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 - This panel presents a 3-dimensional maximum projection of the z-stack acquired Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 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 illustrates a single z-plane at position 52 of the 178 planes collected, including a white box denoting the Region of Interest (ROI) we selected to investigate further the PARP1 680 direct conjugate structural details available using super resolution SIM, which provides ~ 60 - 80nm in the X and Y planes.

Panel C - This panel presents a 3-dimensional volume projection of the z-stack acquired using only the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate channel. We note, as has been repeated throughout our image acquisition and analysis, that as we "step down" in resolution, we gain more detailed structural information.

Image Corrections

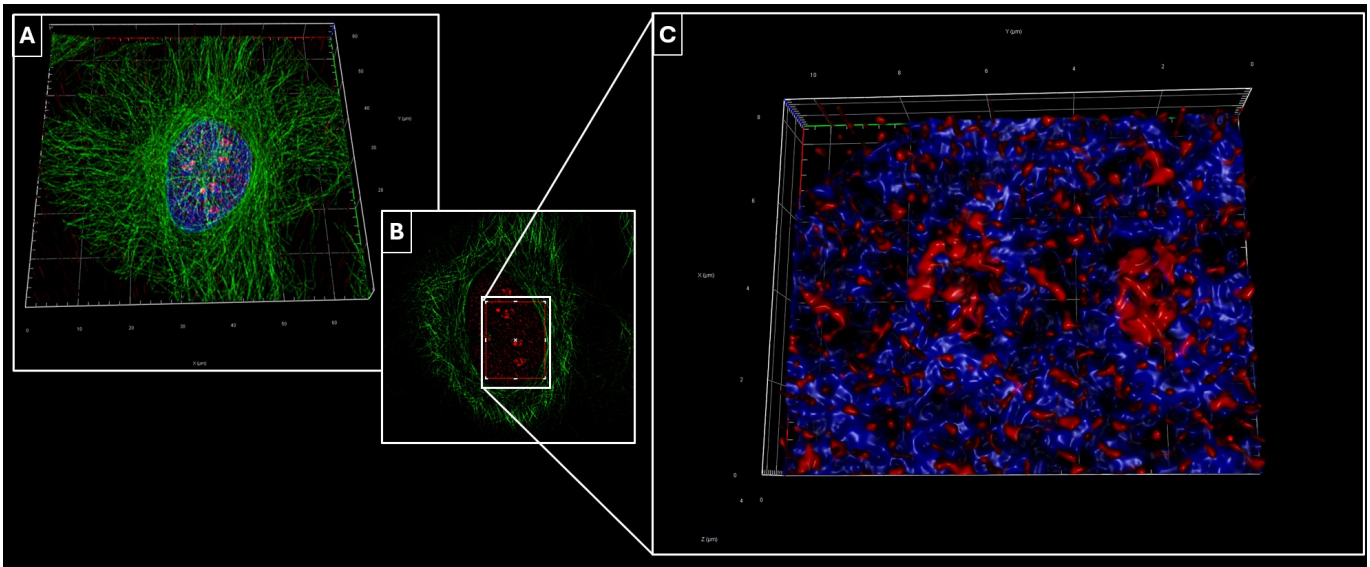
None

Image by P. Raut
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5BDD509E9F9D3F8E6D090806D4651F6D4218587503CB9F985AD1EBA4CE583781
Method PDF SHA-256	FEA5FE8F66B1AC1ADA1D164E1E6686BB6C0BED506C7DDF8BD9247EF8741DD109

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™ 680 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 illustrates a single z-plane at position 40 of the 164 planes collected, including a white box denoting the Region of Interest (ROI) we selected to investigate further the PARP1 680 direct conjugate structural details available using super resolution SIM2, which provides ~ 40 – 60nm in the X, and Y planes.

Panel C – This panel presents a 3-dimensional volume projection of the z-stack acquired using only the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 680 direct conjugate channel. We note, as has been repeated throughout our image acquisition and analysis, that as we "step down" in resolution, we gain more detailed structural information. However, this structure is relatively large at scale, and the difference between super resolution SIM and SIM2 is left to the investigator.

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	64

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	164 Slices (4.89 µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 643 X 810
Image Size (Scaled)	66.72 µm X 66.72 µm | 8.48 µm X 10.69 µm
Bit Depth	16 Bit
Image Center Position	X: 60.23 mm, Y: 36.70 mm
Stage Position	X: 60.23 mm, Y: 36.70 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	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150 ms | 120 ms | 50 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @5.00% | 488 nm @1.50% | 405 nm @6.00%
Frame Time	1.98 s | 1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	36.5µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Algorithm	SIM2
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.9570
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% 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™ 680, IgG - (BLR018M)
Cat ID# - DC 000024

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (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™ 680, 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')□ (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, 1X Tubelens, was used with the following parameters for each dye:

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

Z-Stack = 164 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72 µm X 66.72 µm
Bit Depth = 16 Bit
Image Center Position = X: 60.23 mm, Y: 36.70 mm
Stage Position = X: 60.23 mm, Y: 36.70 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 164 Slices (4.89 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 643 X 810
Image Size (Scaled) = 8.48 µm X 10.69 µm
Bit Depth = 16 Bit
Image Center Position = X: 60.23 mm, Y: 36.70 mm
Stage Position = X: 60.23 mm, Y: 36.70 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

680 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @5.00%
Frame Time = 1.98 s
Scan Direction = Unidirectional
Grating = 36.5µm 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 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @6.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 36.5µm grating

Post Processing - SIM2 Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 8.9570
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 C

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all 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 - This panel presents a 3-dimensional maximum projection of the z-stack acquired Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 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 illustrates a single z-plane at position 40 of the 164 planes collected, including a white box denoting the Region of Interest (ROI) we selected to investigate further the PARP1 680 direct conjugate structural details available using super resolution SIM2, which provides ~ 40 - 60nm in the X and Y planes.

Panel C - This panel presents a 3-dimensional volume projection of the z-stack acquired using only the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680 direct conjugate channel. We note, as has been repeated throughout our image acquisition and analysis, that as we "step down" in resolution, we gain more detailed structural information. However, this structure is relatively large at scale, and the difference between super resolution SIM and SIM2 is left to the investigator.

Image Corrections

None

Image by P. Raut
Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000024
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9F557D63C759D24F4FCC1C2E27BC36D23D1643E1463151039E3E532318B162B4
Method PDF SHA-256	AFA448AB4244E9DE57BC1E49C77440F1D60B452C1C12B3BB758B5350F867DA30