BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

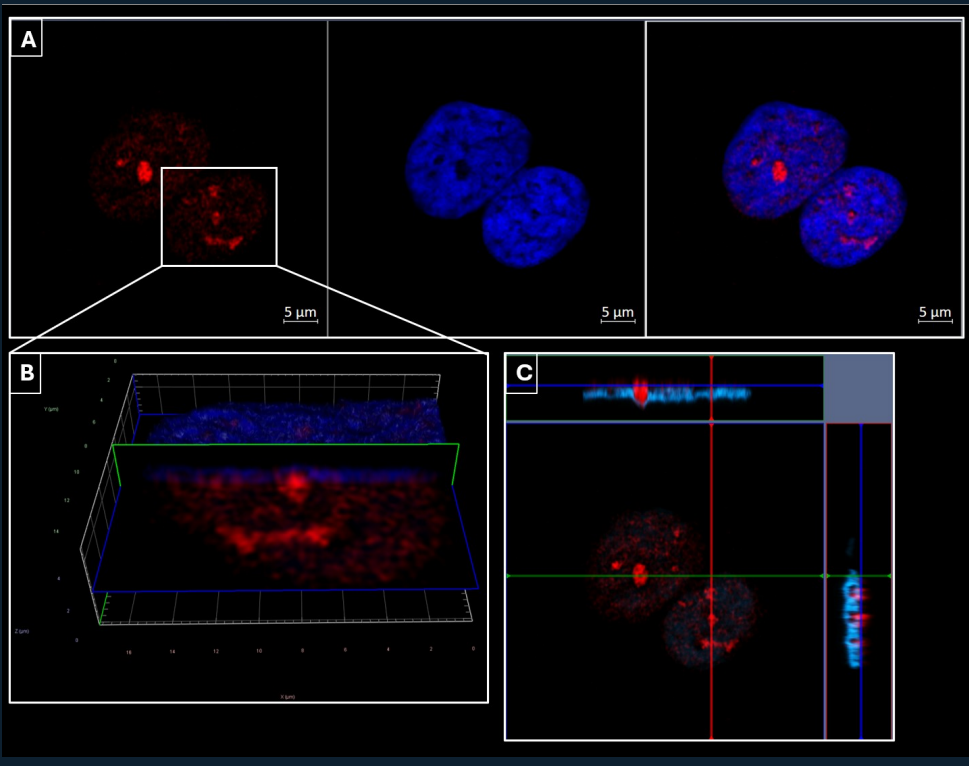
PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750

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

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A – This panel presents a split view of the 3-dimensional image acquisition, utilizing z-plane 81 of the total 161 acquired. On the left, we have the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750 direct conjugate (dark red). In the middle, DAPI is used for nuclear staining (blue), and on the right, we see the merged image from all channels. Panel A features a Region of Interest (ROI) highlighting one of the two nuclei captured in this acquisition.

Panel B – This panel provides a volume projection view of the ROI image presented in Panel A, incorporating all 181 z-planes collected. Additionally, a wedge view is employed in the X, Y, and X, Z axes approximately halfway through the nucleus to demonstrate the localization of the PARP1 750 direct conjugate within the nuclear space.

Panel C – This panel presents a maximum projection orthogonal view of the same image presented in Panel A at z-plane 81, the same plane used for the split view in Panel A. Here, we further support the localization shown in Panel B in the Z plane, top and right within the nuclear volume.

We note that, by continuing our step-down process, it is only when we reach confocal resolutions, specifically with the Zeiss LSM 980 equipped with an NIR laser, that we begin to visualize the solid aggregates seen in diffraction-limited images, such as widefield and widefield SIM, as more than a solid foci or aggregate. As we progress into super-resolution imaging of PARP1, we observe the complex structures that were previously obscured. We also note that this is as far as our applications allow us to visualize the 750 wavelength, as Airyscan and Super Resolution SIM currently do not provide applications for this wavelength.

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

DC-000033 | 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
- 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

BENNETT ASSESSMENT

The preserved DC-000033 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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-000033 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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 4 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	750	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 750	2; AF750; 49 DAPI; 672 - 748; 335 - 383; 765 - 855; 420 - 470; 752
Widefield SIM — Apotome	405/DAPI; 750	2; AF750; 49 DAPI; 672 - 748; 335 - 383; 765 - 855; 420 - 470; 752
Confocal	405/DAPI; 750	2; None; 730 nm @2.40%; 405 nm @0.2%; 752; 353; 779; 465

MODALITY ASSESSMENT / PART 1 OF 4

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 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

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 4

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 700ms; 4 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @70.00%; X-Cite Xylis Lamp Intensity @10.43%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 30.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 555; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 4

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 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 21 Slices (2 µm); Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm X 0.100 µm; Image size (Pixels): 382 X 379; Image Size (Pixels): 382 X 379; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 600 ms; 4 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @70.00%; X-Cite Xylis Lamp Intensity @10.43%. 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: 45.

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; 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

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 4

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: 161 Slices (4.8 µm); 181 Slices (5.40 µm) - Panel B; Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 794 X 794; 295 X 241; Image Size (Pixels): 794 X 794; 295 X 241; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.42 µs; Frame Time: 2.64 s. Illumination: Laser Wavelength: 730 nm @2.40%; 405 nm @0.2%. Optical/scan: Pinhole: 1.00AU / 79 µm; 1.00 AU / 43 µm; Scan Zoom: 2.8; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 6.8 (3D, Auto); 6.1 (3D, Auto). Structured source metadata values: 57.

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; 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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000033 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal. 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 - 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

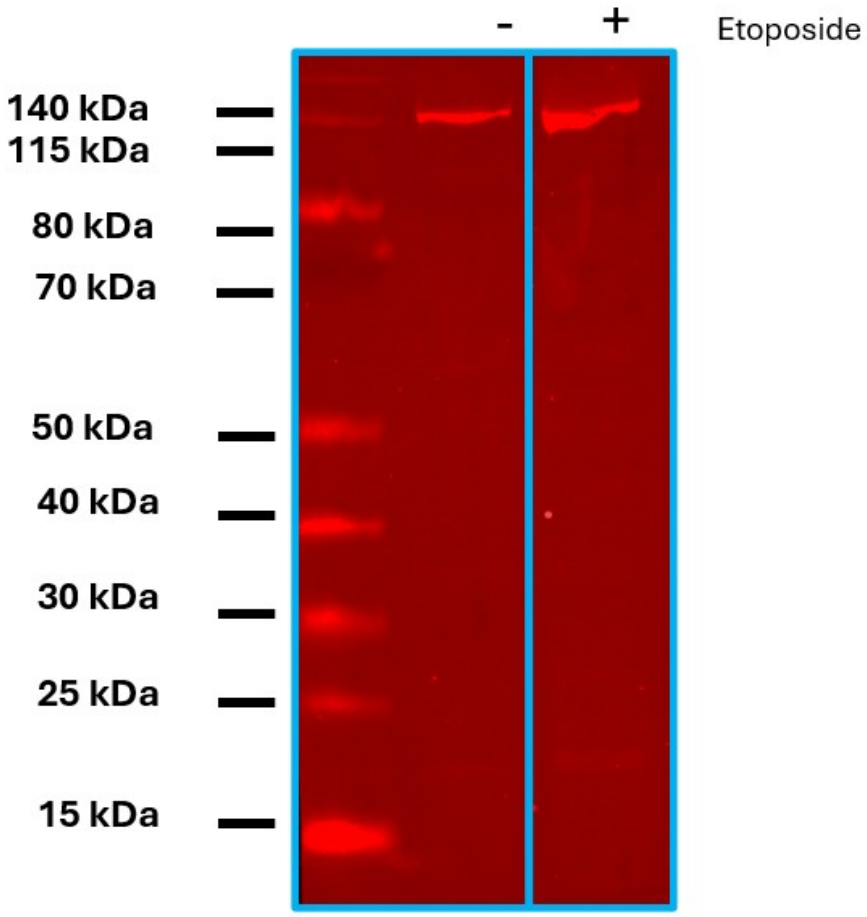
This dossier preserves the complete available evidence progression for DC-000033. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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

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	750
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	730nm
Emission Filter	829±62 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™ 750, IgG - (BLR018M)
Cat ID# - DC 000003

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 then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, 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 = 730nm
Emission Filter = 829±62 nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

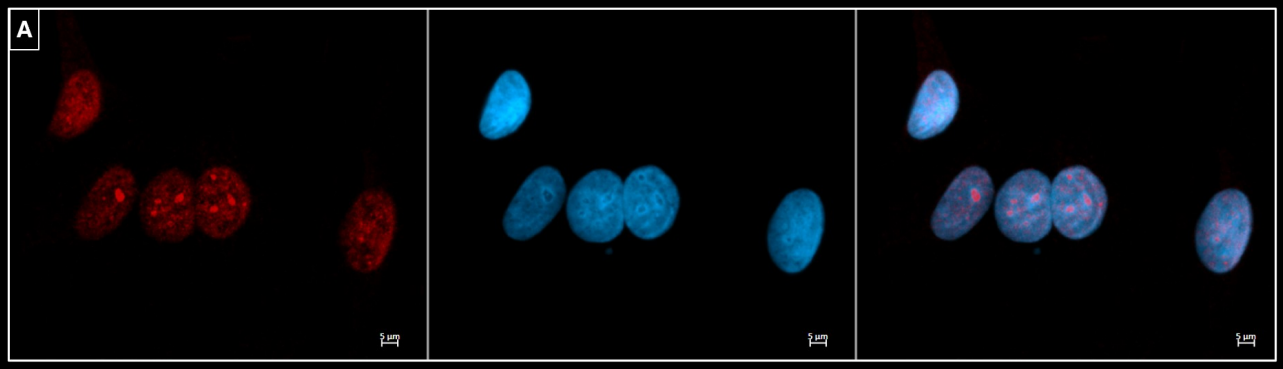
None

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

SOURCE PROVENANCE

Catalog	DC-000033
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	9CC9249A8703AE235DB158F0DD608F09A7D5E14A68365F8ABC8BBB8317194789
Method PDF SHA-256	53675051AB437CD75BDFCE73E039E020CEF49D644324923FF2C7ABEE7745D768

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View demonstrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750, IgG, Direct Conjugate. The left is PARP1 750 (dark red), the middle is DAPI (blue), and the right is the merged image of the two channels.

Notes - PARP1 750 Direct Conjugate continues to demonstrate aggregate localization and a highly specific signal. When used in the simplest of applications, such as wide-field microscopy, deploying an LED lamp and the AF750-49007 filter yields a bright and easily captured signal.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 750
METADATA VALUES	30

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 µm X 112.59 µm
Bit Depth	16 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	AF750 | 49 DAPI
Beam Splitter	760 | 395
Filter Excitation Wavelength	672 - 748 | 335 - 383
Filter Emission Wavelength	765 - 855 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @70.00% | X-Cite Xylis Lamp Intensity @10.43%
Exposure Time	700ms | 4 ms
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.25
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.51 µm | 0.90 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™ 750, IgG - (BLR018M)
Cat ID# - DC 000033

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

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 555, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 μm X 0.110 μm
Image size (Pixels) = 1216 X 1028
Image Size (Scaled) = 133.18 μm X 112.59 μm
Bit Depth = 16 Bit
Image Center Position = X: 0.00 μm, Y: 0.00 μm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

750 -

Reflector = AF750
Beam Splitter = 760
Filter Excitation Wavelength = 672 - 748
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @70.00%
Exposure Time = 700ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.25
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.51 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 @10.43%
Exposure Time = 4 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Split View - Panel A

The left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M) after DNA damage, the middle panel features DAPI nuclear staining, and the right panel is the merged image of all channels.

Summary

Panel A - Split View demonstrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M). The left is PARP1 750 (dark red), the middle is DAPI (blue), and the right is the merged image of the two channels.

Notes - PARP1 750 Direct Conjugate continues to demonstrate aggregate localization and a highly specific signal. When used in the simplest of applications, such as widefield microscopy, deploying an LED lamp and the AF750-49007 filter yields a bright and easily captured signal.

Image Correction

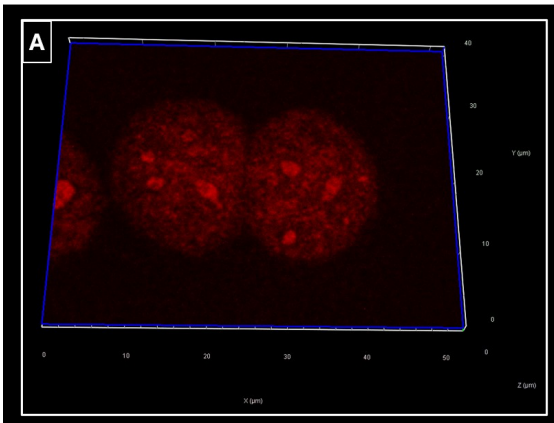
None

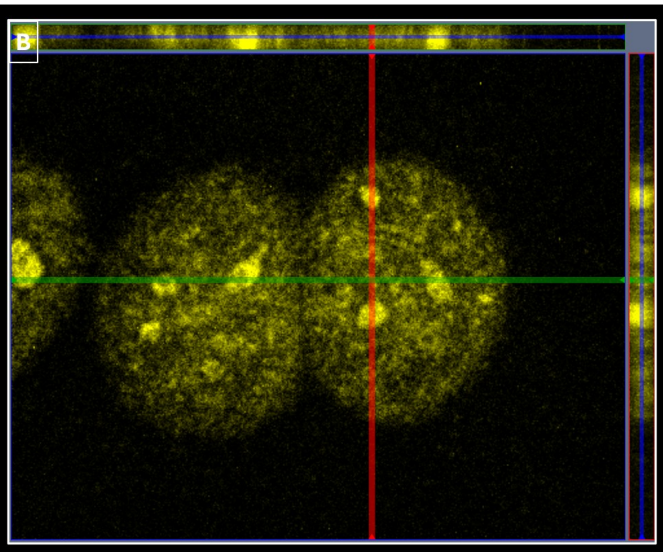
Image by E.F. Simon
Image Analysis by B.T. Bennett
Data Presentation by P.D. Amistoso

SOURCE PROVENANCE

Catalog	DC-000033
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	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D28B1DCB96D8FF79C88F7C5467A211DA96A883AB70330ABF0A7917C70E95FB4F
Method PDF SHA-256	226324E3594E44C44E7F019E9E6F735AB792C16B471CF3C5838B136E28853F7D

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME





Panel A – This panel presents a 3-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750. It uses an X, Y cut plane to highlight the structural details of PARP1 aggregates available to research while employing widefield SIM. The cut plane is indicated with a blue border.

Panel B – This panel features an orthogonal view of the same image, showcasing the X, Y, and Z axes in relation to the structural details at this resolution. Note that the PARP1 750 direct conjugate color was changed from the default dark red in Zeiss Zen to yellow for improved visualization.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	45

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 705, 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	21 Slices (2 µm)
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm X 0.100 µm
Image Size (Pixels)	382 X 379
Image Size (Scaled)	41.84 µm X 41.51 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	AF750 | 49 DAPI
Beam Splitter	760 | 395
Filter Excitation Wavelength	672 - 748 | 335 - 383
Filter Emission Wavelength	765 - 855 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @70.00% | X-Cite Xylis Lamp Intensity @10.43%
Exposure Time	600 ms | 4 ms
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.25
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1051 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
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)
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.

FIELD	SOURCE-DOCUMENTED VALUE
Position of Clip	-6%
Clip X	0°
Clip Y	0°
Cut Lines	X 128, Y 176, Z 11
Line Width	5 for all axes
Cut Line Opacity	50

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M)
Cat ID# - DC 000033

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™ 750, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 21 Slices (2 μm)

Channels = 2
Scaling (Per Pixel) = 0.110 μm X 0.110 μm X 0.100 μm
Image Size (Pixels) = 382 X 379
Image Size (Scaled) = 41.84 μm X 41.51 μm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 μm, Y: 0.00 μm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

750 -

Reflector = AF750
Beam Splitter = 760
Filter Excitation Wavelength = 672 - 748
Filter Emission Wavelength = 765 - 855
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @70.00%
Exposure Time = 600 ms
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.25
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1051 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 @10.43%
Exposure Time = 4 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 705
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 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)

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 = -6%
Clip X = 0°
Clip Y = 0°

Orthogonal View - Panel B

Cut Lines = X 128, Y 176, Z 11
Line Width = 5 for all axes
Cut Line Opacity = 50
Maximum Intensity Projection NOT selected
Measure 3D Distance NOT selected

Summary

Panel A - This panel presents a 3-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750. It uses an X, Y cut plane to highlight the structural details of PARP1 aggregates available to research while employing widefield SIM. The cut plane is indicated with a blue border.

Panel B - This panel features an orthogonal view of the same image, showcasing the X, Y, and Z axes in relation to the structural details at this resolution. Note that the PARP1 750 direct conjugate color was changed from the default dark red in Zeiss Zen to yellow for improved visualization.

Image Correction

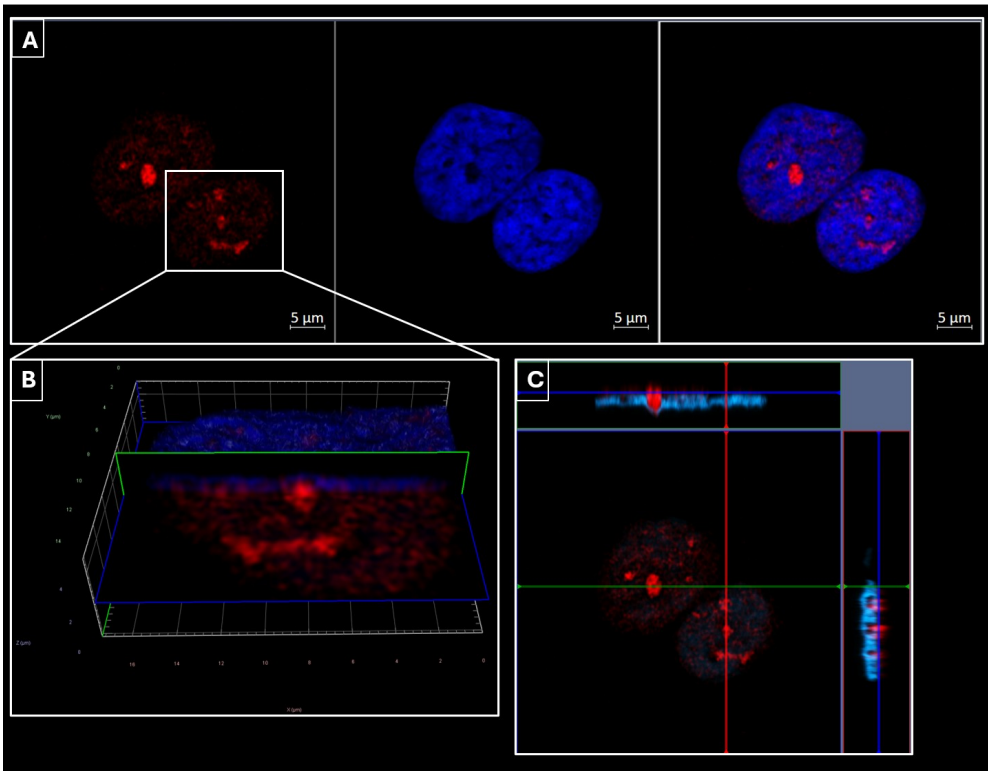
None

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

SOURCE PROVENANCE

Catalog	DC-000033
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 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6C9AC1D1AD758F586027C1C080D102DF330F7ED69FA3368ED2A54BFDFE845B30
Method PDF SHA-256	24AB89D62B6F2134CF49196C4BC1F3CA92CA1EAC59D475287A6695121498D5DA

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A – This panel presents a split view of the 3-dimensional image acquisition, utilizing z-plane 81 of the total 161 acquired. On the left, we have the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 750 direct conjugate (dark red). In the middle, DAPI is used for nuclear staining (blue), and on the right, we see the merged image from all channels. Panel A features a Region of Interest (ROI) highlighting one of the two nuclei captured in this acquisition.

Panel B – This panel provides a volume projection view of the ROI image presented in Panel A, incorporating all 181 z-planes collected. Additionally, a wedge view is employed in the X, Y, and X, Z axes approximately halfway through the nucleus to demonstrate the localization of the PARP1 750 direct conjugate within the nuclear space.

Panel C – This panel presents a maximum projection orthogonal view of the same image presented in Panel A at z-plane 81, the same plane used for the split view in Panel A. Here, we further support the localization shown in Panel B in the Z plane, top and right within the nuclear volume.

We note that, by continuing our step-down process, it is only when we reach confocal resolutions, specifically with the Zeiss LSM 980 equipped with an NIR laser, that we begin to visualize the solid aggregates seen in diffraction-limited images, such as widefield and widefield SIM, as more than a solid foci or aggregate. As we progress into super-resolution imaging of PARP1, we observe the complex structures that were previously obscured. We also note that this is as far as our applications allow us to visualize the 750 wavelength, as Airyscan and Super Resolution SIM currently do not provide applications for this wavelength.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 750
METADATA VALUES	57

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	161 Slices (4.8 µm) | 181 Slices (5.40 µm) - Panel B
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	794 X 794 | 295 X 241
Image Size (Scaled)	46.69 µm X 46.69 µm | 17.35 µm X 14.17 µm
Bit Depth	16 Bit
Image Center Position	X: 71.71 mm, Y: 51.42 mm
Stage Position	X: 71.71 mm, Y: 51.42 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 79 µm | 1.00 AU / 43 µm
LSM MBS	Plate MBS 405/730
LSM SBS	SBS LP 740 | Mirror
Laser Wavelength	730 nm @2.40% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.8
Rotation	0°
Sampling	1.20
Pixel Time	0.42 µs
Frame Time	2.64 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	752 | 353
Emission Wavelength	779 | 465
Effective NA	1.4
Detection Wavelength	755-900 | 435-470
Imaging Device	NIR Ch2 | GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAs-PMT | GaAsP-PMT
Detector Gain	750 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	6.8 (3D, Auto) | 6.1 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% 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)
Cut Lines	X 514, Y 383, Z 75
Line Width	5 for all axes
Cut Line Opacity	50

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M)
Cat ID# - DC 000033

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™ 750, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 161 Slices (4.8 μm)

Channels = 2
Scaling (Per Pixel) = 0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels) = 794 X 794
Image Size (Scaled) = 46.69 μm X 46.69 μm
Bit Depth = 16 Bit
Image Center Position = X: 71.71 mm, Y: 51.42 mm
Stage Position = X: 71.71 mm, Y: 51.42 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z-Stack = 181 Slices (5.40 µm) - Panel B

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 295 X 241
Image Size (Scaled) = 17.35 µm X 14.17 µm
Bit Depth = 16 Bit
Image Center Position = X: 71.71 mm, Y: 51.42 mm
Stage Position = X: 71.71 mm, Y: 51.42 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

750 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 79 µm
LSM MBS = Plate MBS 405/730
LSM SBS = SBS LP 740
Laser Wavelength = 730 nm @2.40%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.42 µs
Frame Time = 2.64 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 752
Emission Wavelength = 779
Effective NA = 1.4
Detection Wavelength = 755-900
Imaging Device = NIR Ch2
Detector Type = GaAs-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

DAPI -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 43 µm
LSM MBS = Plate MBS 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.42 µs
Frame Time = 2.64 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 = 435-470
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.1 (3D, Auto)

Split View - Panel A

The left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M) after DNA damage, the middle panel features DAPI nuclear staining, and the 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 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

Orthogonal View - Panel C

Cut Lines = X 514, Y 383, Z 75
Line Width = 5 for all axes
Cut Line Opacity = 50
Maximum Intensity Projection (MIP) NOT selected
Measure 3D Distance NOT selected

Summary

Panel A - This panel presents a split view of the 3-dimensional image acquisition, utilizing z-plane 81 of the total 161 acquired. On the left, we have the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 750, IgG - (BLR018M) (dark red). In the middle, DAPI is used for nuclear staining (blue), and on the right, we see the merged image from all channels. Panel A features a Region of Interest (ROI) highlighting one of the two nuclei captured in this acquisition.

Panel B - This panel provides a volume projection view of the ROI image presented in Panel A, incorporating all 181 z-planes collected. Additionally, a wedge view is employed in the X, Y, and X, Z axes approximately halfway through the nucleus to demonstrate the localization of the PARP1 750 direct conjugate within the nuclear space.

Panel C - This panel presents a maximum projection orthogonal view of the same image presented in Panel A at z-plane 81, the same plane used for the split View in Panel A. Here, we further support the localization shown in Panel B in the Z plane, top and right within the nuclear volume.

We note that, by continuing our step-down process, it is only when we reach confocal resolutions, specifically with the Zeiss LSM 980 equipped with an NIR laser, that we begin to visualize the solid aggregates seen in diffraction-limited images, such as widefield and widefield SIM, as more than a solid foci or aggregate. As we progress into super-resolution imaging of PARP1, we observe the complex structures that were previously obscured. We also note that this is as far as our applications allow us to visualize the 750 wavelength, as Airyscan and Super Resolution SIM currently do not provide applications for this wavelength.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	DC-000033
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:

SOURCE PROVENANCE

Structured source metadata values	57
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7346496186BE71853CC45087CAA9621C19EA3590084AE75068C1C4A1E7DF8306
Method PDF SHA-256	A0D0B043AABB477BE71ABF0B5D2B65E9B540343FA5DB67B6FCF4A8395FD833B9