

IDENTIFYN RESOLUTION STEPDOWN

PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 790

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

4

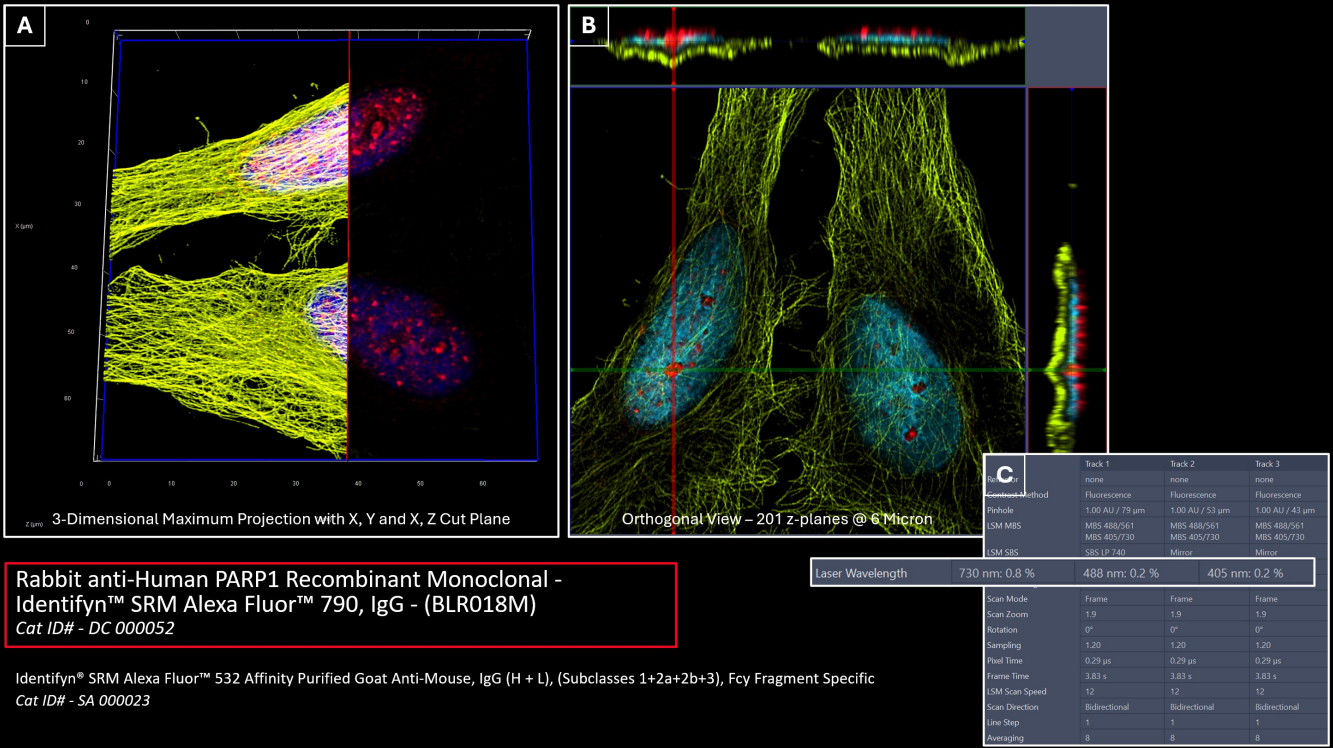
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / CONFOCAL

DC-000052 | 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-000052 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-000052 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	790	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 790	3; AF750-49007; AF532-49014; 49 DAPI; 672 - 748; 515 - 545; 335 - 383; 765 - 855
Widefield SIM — Apotome	405/DAPI; 532; 555; 790	2; AF750-49007; AF532-49014; 672 - 748; 515 - 545; 765 - 855; 555 - 595; 752
Confocal	405/DAPI; 488; 532; 790	3; None; 730 nm @ 0.8%; 488 nm @0.2%; 405 nm @0.2%; 789; 528; 353

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 790.

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 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, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.073 μm X 0.073 μm ; Image size (Pixels): 3153 X 2211; Image Size (Pixels): 3153 X 2211; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 39.

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 Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 532; 555; 790.

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, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 89 Slices (8.8 μm); Channels: 2; Scaling (Per Pixel): 0.073 μm X 0.073 μm X 0.100 μm ; Image size (Pixels): 1772 X 1567; Image Size (Pixels): 1772 X 1567; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Hamamatsu ORCA-Quest. Timing: Exposure Time: 300 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @30.00%; X-Cite Xylis Lamp Intensity @15.00%. 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: 37.

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; 532; 555; 790.

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: 201 Slices (6.0 μm); Channels: 3; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 1181 X 1162; Image Size (Pixels): 1181 X 1162; Bit Depth: 16 Bit. Detector/camera: Imaging Device: NIR Ch2; GaAsP-Pmt3; Detector Type: GaAs-PMT; GaAsP-PMT. Timing: Pixel Time: 0.29 μs ; Frame Time: 3.83 s. Illumination: Laser Wavelength: 730 nm @ 0.8%; 488 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 79 μm ; 1.00 AU / 53 μm ; Scan Zoom: 1.9; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.4 (3D, Auto); 7.2(3D, Auto). Structured source metadata values: 61.

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; 790.

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-000052 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 μ M; 2 μ M -> Etoposide = 2 μ M. The governing scientist identified every 200 μ M occurrence as a technician transcription error. The canonical historical concentration is 2 μ M. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

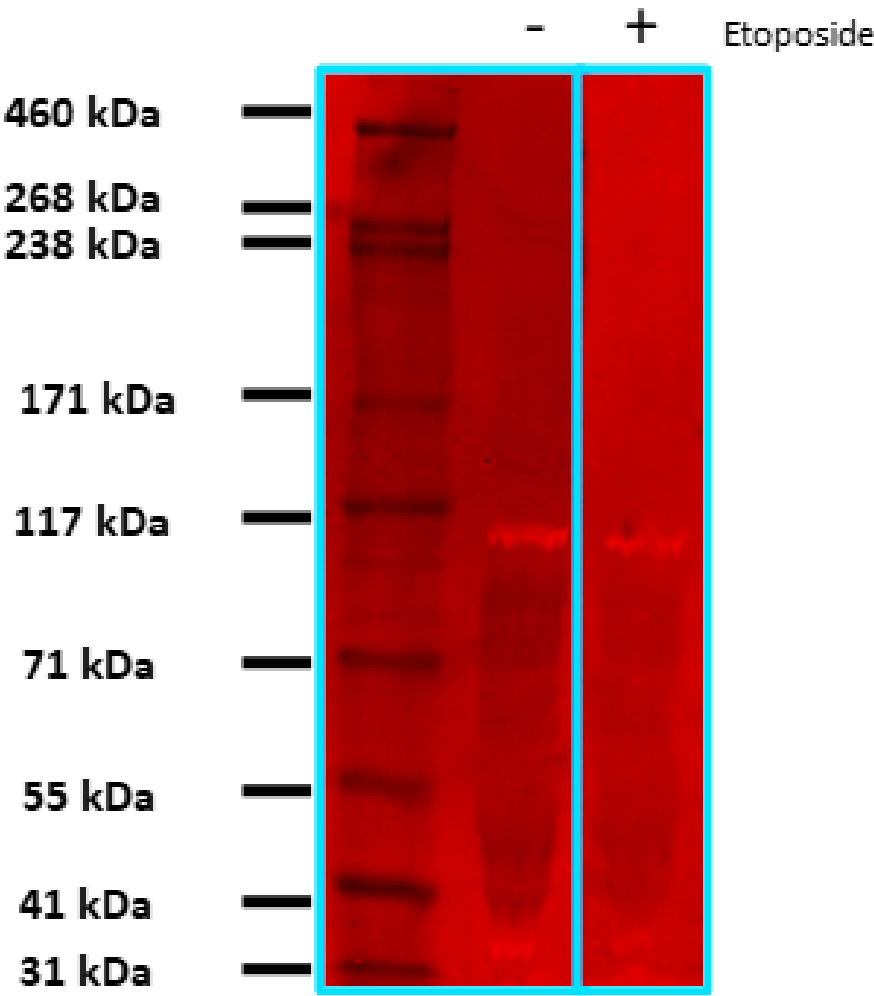
This dossier preserves the complete available evidence progression for DC-000052. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Observer.Z1/7 Apotome	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	HeLa
DETECTED DYES	790
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	790nm
Emission Filter	829±62nm
Detector	PMT
Intensity	3
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™ 790, IgG - (BLR018M)
Cat ID# - DC 000052

Expected Molecular Weight: 116 kDa

HELA cells were treated with 200 µM etoposide for 20 hours to induce DNA damage. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm in a Mini Gel Tank. The Thermo Fisher's HiMark™ Pre-stained Protein Standard 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™ 790, IgG - (BLR018M) for 2 hours, followed by five washes in 1X PBS. 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 = 790nm

Emission Filter = 829±62nm

Detector = PMT

Intensity = 3

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

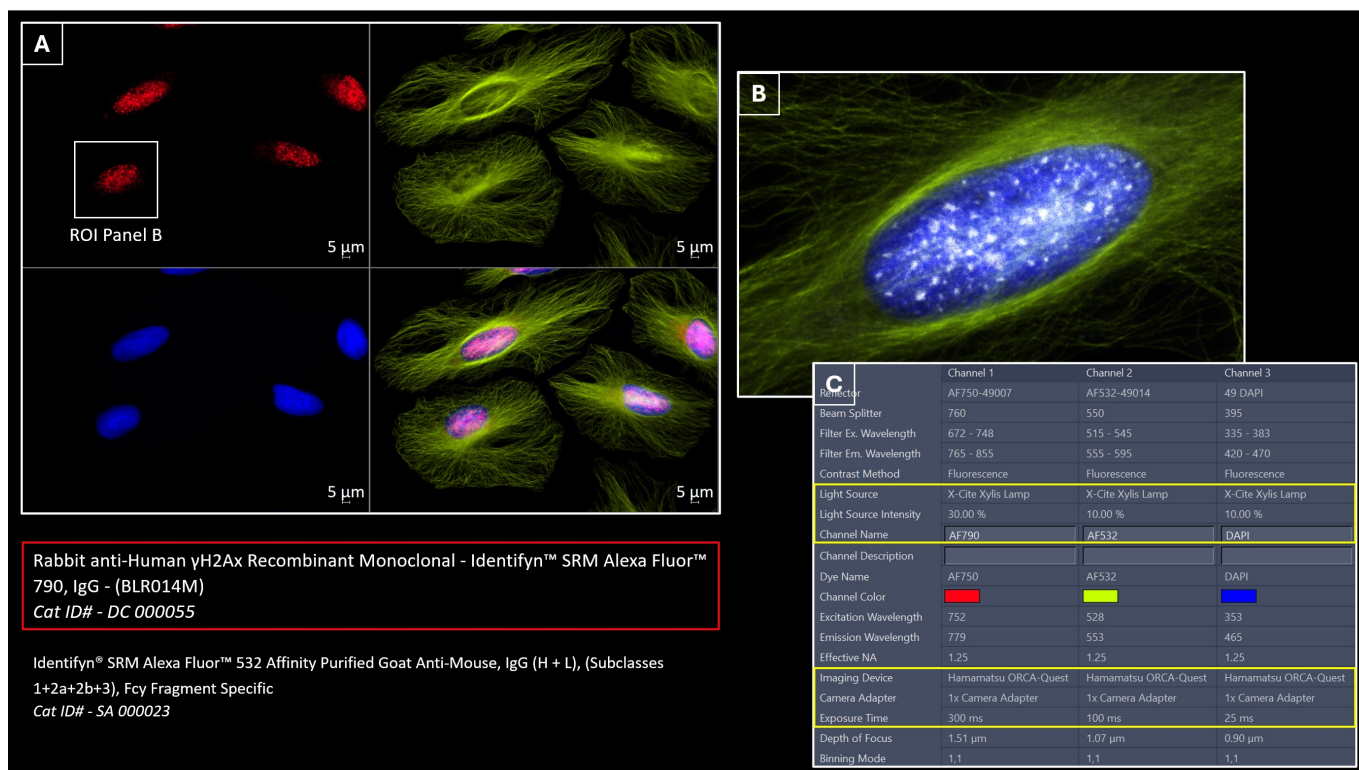
Image: K. Small

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SOURCE PROVENANCE

Catalog	DC-000052
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	HeLa
Image SHA-256	97349E1F80CBC7C40BFE0C97282BCB3DB958E3EE3629E135F2909BEE3007DE76
Method PDF SHA-256	75E7083BADC766E4CAB6F34F670DACD72923AD005BDAEBE0E90D94D5AC052F23

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 790
METADATA VALUES	39

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 Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	3
Scaling (Per Pixel)	0.073 μ m X 0.073 μ m
Image size (Pixels)	3153 X 2211
Image Size (Scaled)	230.22 μ m X 161.44 μ m
Bit Depth	16 Bit
Image Center Position	X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflector	AF750-49007 AF532-49014 49 DAPI
Beam Splitter	760 550 395
Filter Excitation Wavelength	672 - 748 515 - 545 335 - 383
Filter Emission Wavelength	765 - 855 555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms 100 ms 25 ms
Excitation Wavelength	752 528 353
Emission Wavelength	779 553 465
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 μ m 1.07 μ m 0.90 μ m
Binning Mode	1,1 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™ 790, IgG - (BLR018M)
Cat ID# - DC 000052

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000023

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™ 790, 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 Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.073 µm X 0.073 µm

Image size (Pixels) = 3153 X 2211

Image Size (Scaled) = 230.22 µm X 161.44 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

790 -

Reflector = AF750-49007
 Beam Splitter = 760
 Filter Excitation Wavelength = 672 - 748
 Filter Emission Wavelength = 765 - 855
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @30.00%
 Exposure Time = 300 ms
 Excitation Wavelength = 752
 Emission Wavelength = 779
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 1.51 μ m
 Binning Mode = 1,1

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 = 100 ms
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 1.07 μ m
 Binning Mode = 1,1

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 = 25 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 0.90 μ m
 Binning Mode = 2,2

Split View - Panel A

The upper left features Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 790 - Direct Conjugate - (BLR018M) the upper right panel features alpha tubulin visualized with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, the lower left panel shows DAPI nuclear staining, and the lower right shows the merged image of all channels.

Region of Interest Image - Panel B

Region of Interest shown in Panel A is displayed to demonstrate better visualization of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 790 - Direct Conjugate - (BLR018M) within the nuclear compartment. Note that the PARP1 790 Direct Conjugate was changed from its default red in Zeiss Zen to white to enhance this visualization.

Zeiss Zen Settings - Panel C, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings, the PARP1 790 Direct Conjugate. We note that widefield NIR is possible in these settings, using the filter shown, AF750-49007 (Chroma), and we also deploy a Hamamatsu ORCA-Quest camera, which is compatible with any widefield scope. We note that the X-Cite LED light input at 30% and the camera exposure time of 300ms were sufficient to capture this far-red NIR direct conjugate with high brightness. a

Image Correction - Panel B

PARP1 790 Direct Conjugate was changed from its default red in Zeiss Zen to white to enhance this visualization.

Image by E.F. Simon

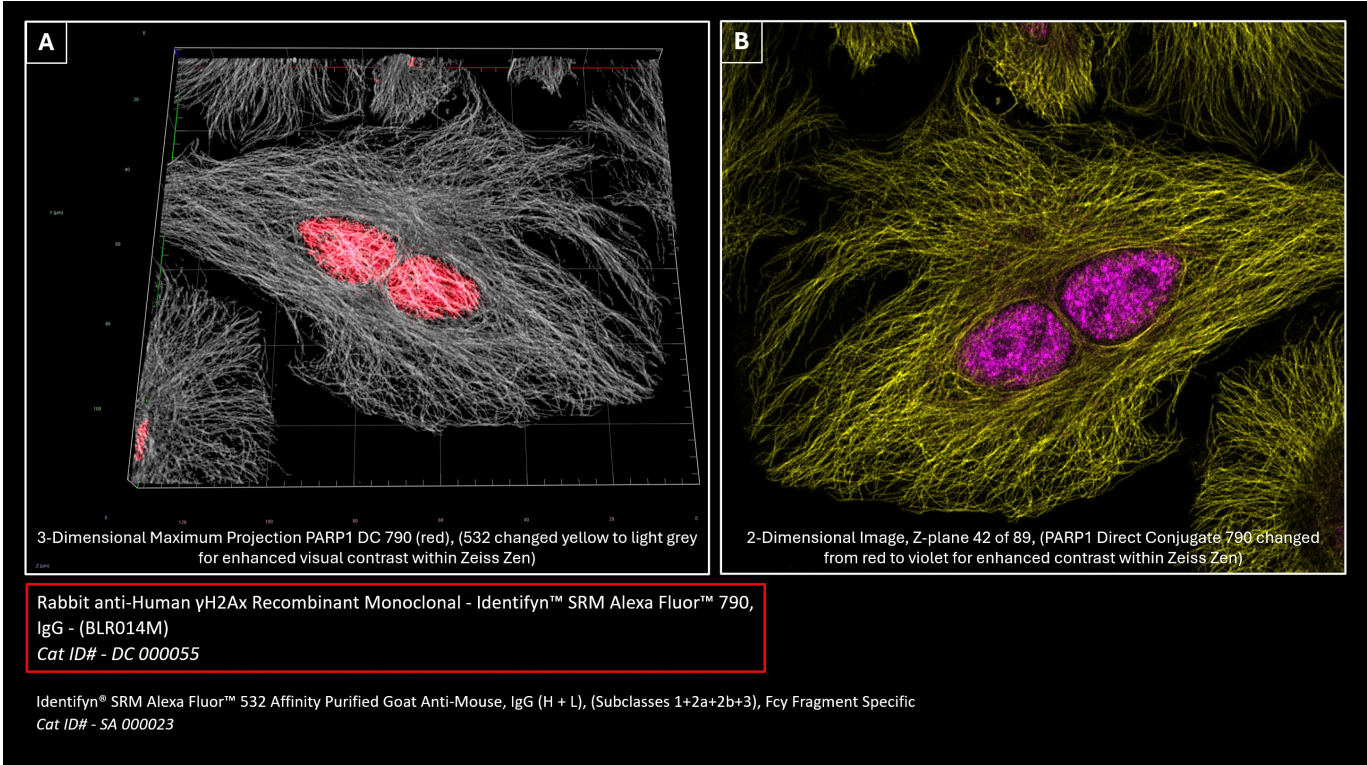
Image Analysis and Data Presentation B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000052
Stage	Widefield
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, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5827AC15F10E360AE244E3845411AAA2D665833B102D9628F2465B512AE2CD90
Method PDF SHA-256	DE5FDC4BF288FE75B747CCFED1784E7221305F06F2407F6C0CD8E055DBE4B02C

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 790
METADATA VALUES	37

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, Hamamatsu ORCA-Quest, 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	89 Slices (8.8 μm)
Channels	2
Scaling (Per Pixel)	0.073 μm X 0.073 μm X 0.100 μm
Image Size (Pixels)	1772 X 1567
Image Size (Scaled)	129.38 μm X 114.42 μm
Bit Depth	16 Bit
Image Center Position	X: 0.00 mm, Y: 0.00 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	AF750-49007 AF532-49014
Beam Splitter	760 550
Filter Excitation Wavelength	672 - 748 515 - 545
Filter Emission Wavelength	765 - 855 555 - 595
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @30.00% X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	300 ms 150 ms
Excitation Wavelength	752 528
Emission Wavelength	779 553
Effective NA	1.25
Imaging Device	Hamamatsu ORCA-Quest
Camera Adapter	1X
Depth of Focus	1.51 μm 1.07 μm
Binning Mode	1,1
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%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 790, IgG - (BLR018M)
Cat ID# - DC 000052

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000023

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™ 790, 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 Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 89 Slices (8.8 µm)

Channels = 2

Scaling (Per Pixel) = 0.073 µm X 0.073 µm X 0.100 µm

Image Size (Pixels) = 1772 X 1567

Image Size (Scaled) = 129.38 µm X 114.42 µm

Bit Depth = 16 Bit

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

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

Single Plane (2D) - Z-plane 42 of 89 total - Panel B

Channels = 2

Scaling (Per Pixel) = 0.073 µm X 0.073 µm X 0.100 µm

Image Size (Pixels) = 1772 X 1567

Image Size (Scaled) = 129.38 µm X 114.42 µm

Bit Depth = 16 Bit

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

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

790 -

Reflector = AF750-49007

Beam Splitter = 760

Filter Excitation Wavelength = 672 - 748

Filter Emission Wavelength = 765 - 855

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @30.00%

Exposure Time = 300 ms

Excitation Wavelength = 752

Emission Wavelength = 779

Effective NA = 1.25

Imaging Device = Hamamatsu ORCA-Quest

Camera Adapter = 1X

Depth of Focus = 1.51 µm

Binning Mode = 1,1

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 @15.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.25
 Imaging Device = Hamamatsu ORCA-Quest
 Camera Adapter = 1X
 Depth of Focus = 1.07 μ m
 Binning Mode = 1,1

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.
 Normalization, Phase Corrector, 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%, 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)

Image Correction

Panel A - Alpha Tubulin countersained with Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was changed from its default color, yellow, to light grey in Zeiss Zen to provide for better visual contrast.

Panel B - Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 790 - Direct Conjugate - (BLR018M) was changed from its default color red to violet in Zeiss Zen to provide for better visual contrast.

Image by E.F. Simon

Image Analysis and Data Presentation by B.T. Bennett

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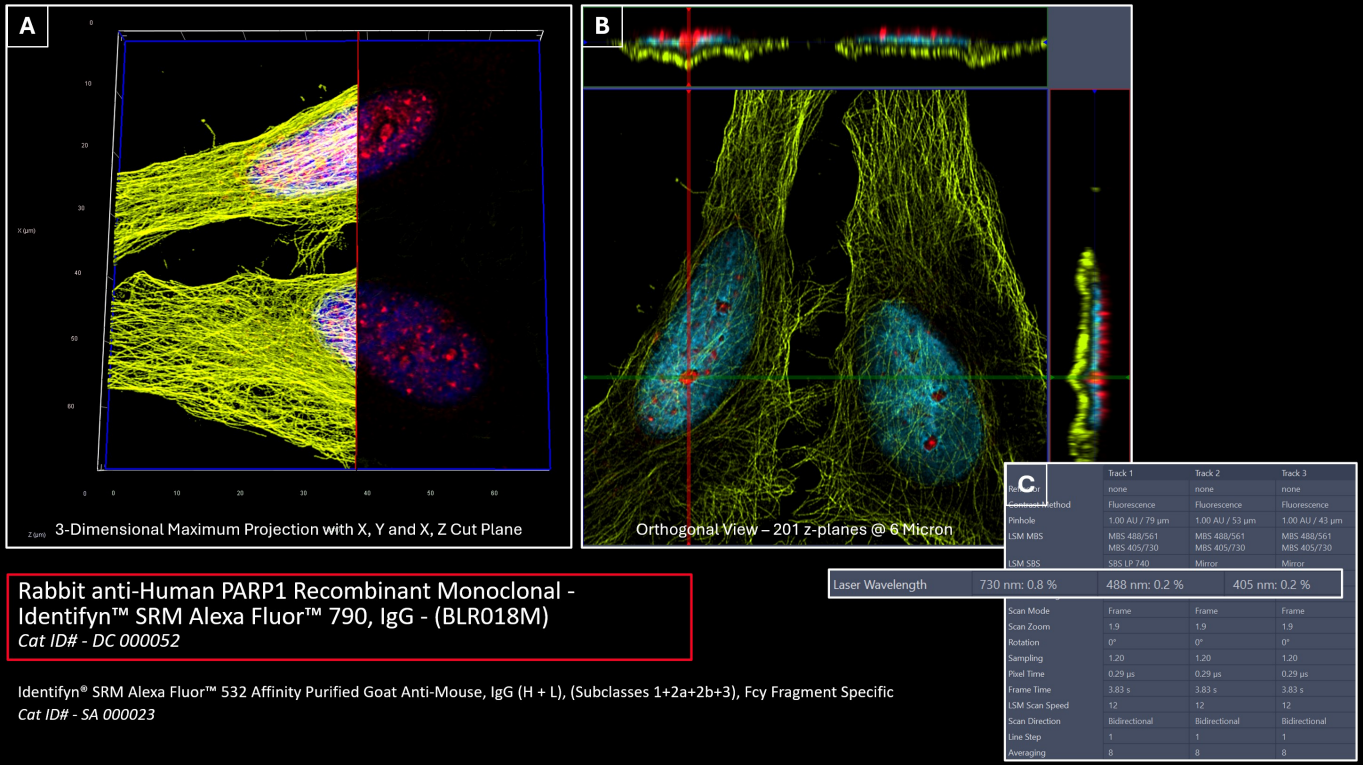
SOURCE PROVENANCE

Catalog	DC-000052
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome

SOURCE PROVENANCE

Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Hamamatsu ORCA-Quest, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6E68077AC512409C0EC528230AEE643F65D29C189C9B7E13503DC93EB8C9F565
Method PDF SHA-256	A1DF42E32EF9EF83C5001946020FDD5D4FE4189253D80B9C624AA0DDBFC03C50

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 790
METADATA VALUES	61

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	201 Slices (6.0 μm)
Channels	3
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	1181 X 1162
Image Size (Scaled)	69.48 μm X 68.36 μm
Bit Depth	16 Bit
Image Center Position	X: 62.80 mm, Y: 51.50 mm
Stage Position	X: 62.80 mm, Y: 51.50 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 79 μm 1.00 AU / 53 μm 1.00 AU / 43 μm
LSM MBS	MBS 488/561, 405/730
LSM SBS	SBS LP 740 Mirror
Laser Wavelength	730 nm @ 0.8% 488 nm @0.2% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.9
Rotation	0°
Sampling	1.20
Pixel Time	0.29 μs
Frame Time	3.83 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	789 528 353
Emission Wavelength	794 553 465
Effective NA	1.4
Detection Wavelength	755-900 530-580 430-470
Imaging Device	NIR Ch2 GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAs-PMT GaAsP-PMT
Detector Gain	700 V 550 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.4 (3D, Auto) 7.2(3D, Auto) 6.8(3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 91%, 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 269, Y 734, Z 89
Line Width	10 for the X and Y axes, and 1 for the Z axis
Cut Line Opacity	42

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™ 790, IgG - (BLR018M)
Cat ID# - DC 000052

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000023

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™ 790, 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 Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific 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) - Panels A (Volume Projection) & Panel B (Orthogonal View)

Z-Stack = 201 Slices (6.0 µm)

Channels = 3

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

Image Size (Pixels) = 1181 X 1162

Image Size (Scaled) = 69.48 µm X 68.36 µm

Bit Depth = 16 Bit

Image Center Position = X: 62.80 mm, Y: 51.50 mm

Stage Position = X: 62.80 mm, Y: 51.50 mm

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

790 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00 AU / 79 µm

LSM MBS = MBS 488/561, 405/730

LSM SBS = SBS LP 740

Laser Wavelength = 730 nm @ 0.8%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.9

Rotation = 0°

Sampling = 1.20

Pixel Time = 0.29 µs

Frame Time = 3.83 s

LSM Scan Speed = 12

Scan Direction = Bidirectional

Line Step = 1

Averaging = 8

Excitation Wavelength = 789

Emission Wavelength = 794

Effective NA = 1.4

Detection Wavelength = 755-900

Imaging Device = NIR Ch2

Detector Type = GaAs-PMT

Detector Gain = 700 V

Detector Offset = 0

Detector Digital Gain = 1.0

LSM Plus Mode = 7.4 (3D, Auto)

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 53 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 488 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.9
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.29 μ s
 Frame Time = 3.83 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 = 530-580
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 7.2(3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 43 μ m
 LSM MBS = MBS 488/561, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.9
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.29 μ s
 Frame Time = 3.83 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 = 430-470
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.8(3D, Auto)

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 91%, 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 - Panel A

X-Y - Position -28%, X and Y are at 0°, shown using a blue outline
 X-Z - Position 10%, X and Y are at 0°, shown using a red outline

Orthogonal View - Panel B

Cut Lines = X 269, Y 734, Z 89
 Line Width = 10 for the X and Y axes, and 1 for the Z axis
 Cut Line Opacity = 42
 Maximum Intensity Projection (MIP) NOT selected
 Measure 3D Distance NOT selected

Zeiss Zen Settings - Panel C, Laser Power Input

A clipped image from Zeiss Zen image acquisition settings for this z-stack comprising 181 planes. We note that the 730 nm laser power is set at 0.8% to obtain maximal imaging of the direct conjugate. Indicating high brightness and stability over the course of the z-stack comprised of 201 z-planes.

Image Corrections

None

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	DC-000052
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	61
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F559E43C2012FFF18845E9AE9339AF8F7AFDA32FF47B16BAF9523B3BE09AA06C
Method PDF SHA-256	42405B063C11161288F8E624DDB5FAE9A23581081BE102052B64A7DE1C396F0A