BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

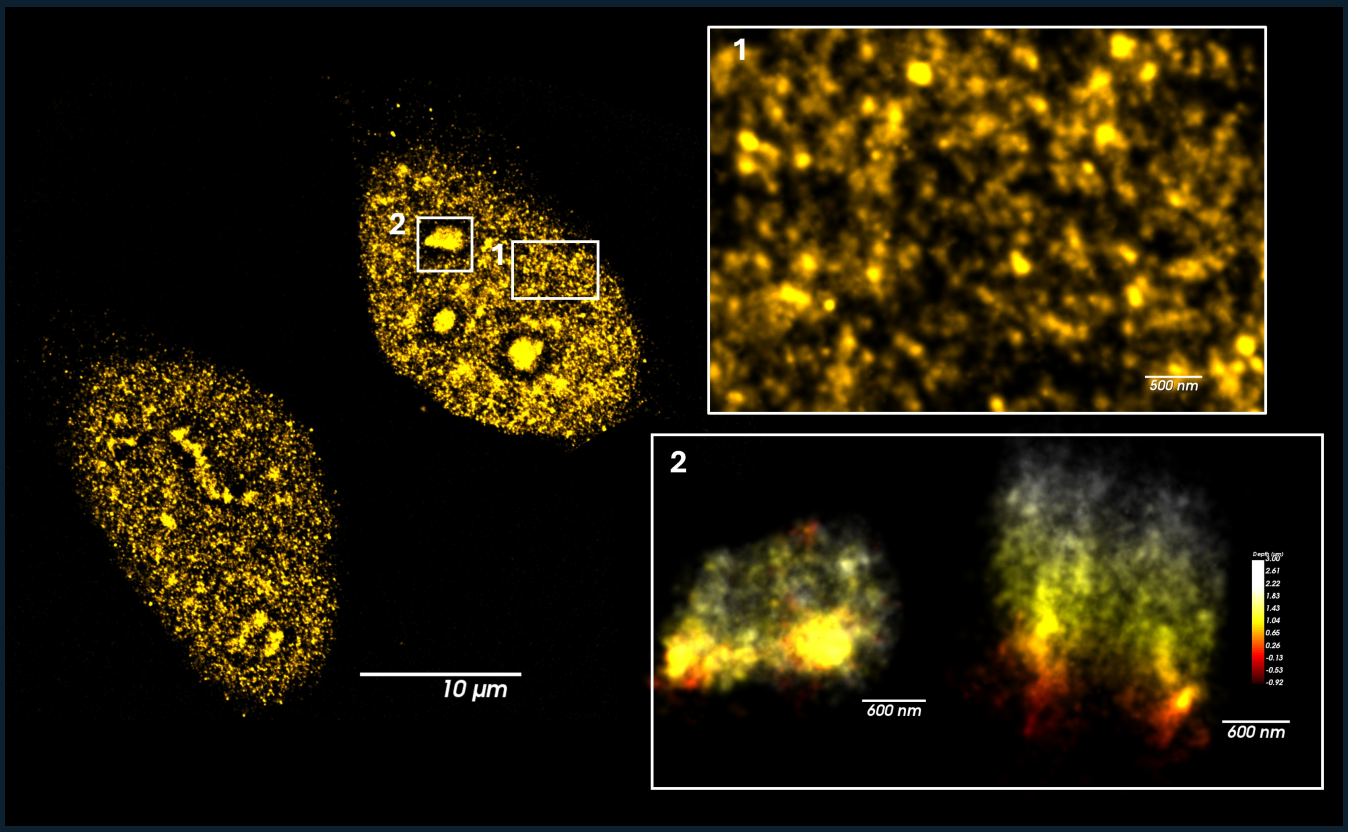
PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000003 | 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
- Single-molecule STORM presentation workflow
- Preserved control experiment
- 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 -> Single-molecule STORM -> Control

BENNETT ASSESSMENT

The preserved PA-RR-000003 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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 / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RR-000003 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 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

A true preserved control record is present and appears last.

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	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 680	3; 50 Cy5; 45 Texas Red; 49 DAPI; 625 - 655; 540 - 580; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 38 HE eGFP; 350 Cy5; 49 DAPI; 450 - 490; 625 - 665; 335-383; 500 - 550
Confocal	405/DAPI; 488; 555; 680; 750	3; None; 561nm @0.1%; 639nm @0.3%; 405nm @0.2%; 553; 679; 353
Airyscan	405/DAPI; 488; 594	2; None; 561nm @0.4%; 488nm @0.1%; 590; 493; 618; 517
SIM	405/DAPI; 488; 647	3; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR
SIM²	405/DAPI; 488; 555; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	3; 38 HE eGFP; 350 Cy5; 49 DAPI; 450 - 490; 625 - 665; 335-383; 500 - 550

MODALITY ASSESSMENT / PART 1 OF 9

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 488.

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 9

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 mono, 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 mono. Timing: Exposure Time: 400ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @12%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

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; 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 9

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: Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm x 100µm; Image size (Pixels): 751 X 885; Image Size (Pixels): 751 X 885; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono; Axiocam 807. Timing: Exposure Time: 150ms; 80ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.0%; X-Cite Xylis Lamp Intensity @6.0%. 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: 50.

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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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 9

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: 146 Slices (4.35µm); Channels: 3; Scaling (Per Pixel): 0.030µm X 0.030µm X 0.030µm; Image size (Pixels): 1276 X 1276; Image Size (Pixels): 1276 X 1276; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt 3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.26µs; Frame Time: 2.10s. Illumination: Laser Wavelength: 561nm @0.1%; 639nm @0.3%. Optical/scan: Pinhole: 1.00AU / 55µm; 1.00AU / 68µm; Scan Zoom: 3.5; 1.4; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 7.4 (3D, Auto); 7.5 (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; 555; 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 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 160 Slices (4.70µm); Channels: 2; Scaling (Per Pixel): 30.18nm X 30.18nm X 30.00nm; Image size (Pixels): 1096 X 1096; 405 X 423; Image Size (Pixels): 1096 X 1096; 405 X 423; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.69µs; Frame Time: 4.07s. Illumination: Laser Wavelength: 561nm @0.4%; 488nm @0.1%. Optical/scan: Pinhole: 5.00 AU / 257µm; 5.00AU / 217µm; Scan Zoom: 3.0; LSM Scan Speed: 8; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 50°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.4 (3D, Auto). Structured source metadata values: 52.

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 900, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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 9

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: 132 Slices (3.93µm); 47 Slices (2.3µm) - The Gallery tool was used to reduce the z-stack to provide clarity; Channels: 3; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 1162 X 1078; Image Size (Pixels): 5056 X 5056; 1162 X 1078; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 640nm @1.0%; 488nm @1.0%; 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: 68.

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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

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: 132 Slices (3.93µm); Channels: 3; Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm; Image size (Pixels): 5056 X 5056; 744 X 828; Image Size (Pixels): 5056 X 5056; 744 X 828; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 640nm @1.0%; 488nm @1.0%; 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: 63.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 647.

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.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 71.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / 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: Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 971 X 773; 930 X 813; Image Size (Pixels): 971 X 773; 930 X 813; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono; Axiocam 807. Timing: Exposure Time: 150ms; 80ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.0%; X-Cite Xylis Lamp Intensity @6.0%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Control 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 PA-RR-000003 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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): 30.18nm X 30.18nm X 30.00nm -> 0.03018 µm X 0.03018 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)=1096 X 1096; Image Size (Scaled)=33.08µm X 33.08µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm -> 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)=66.72µm X 66.72µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.03%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 30.00nm -> 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)=66.72µm X 66.72µm; 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.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"], "Control": ["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

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.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

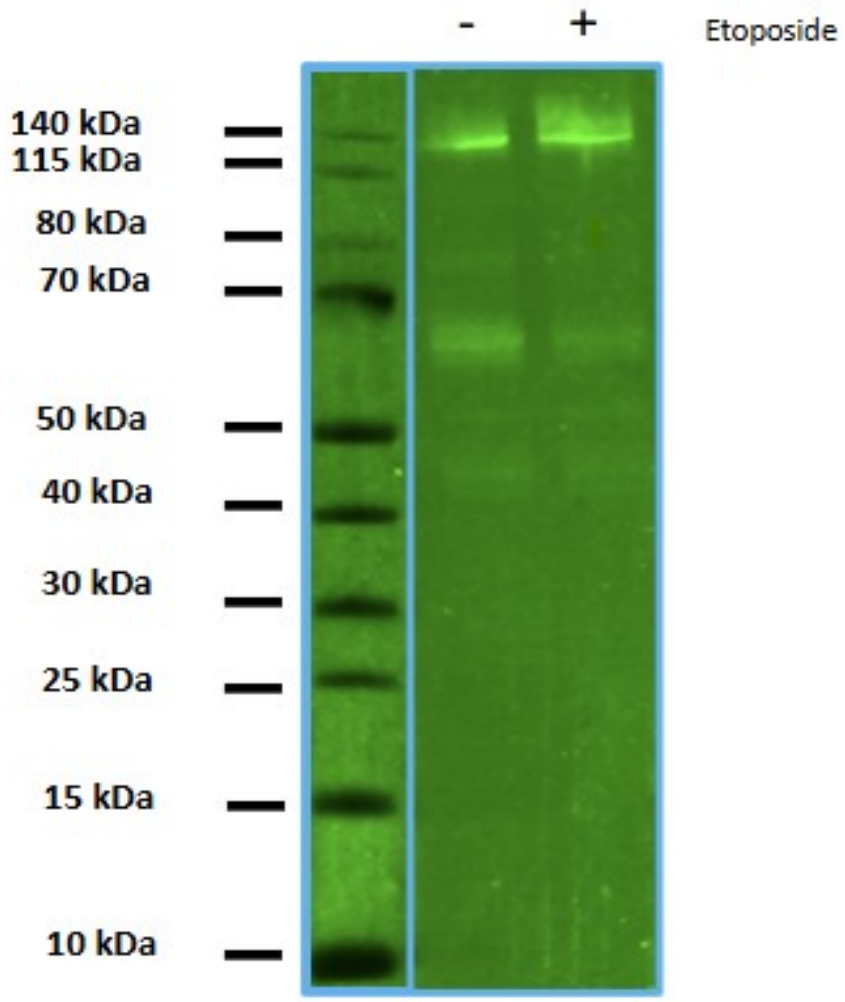
This dossier preserves the complete available evidence progression for PA-RR-000003. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	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	488
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	488nm
Emission Filter	513±17nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Kyle 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 - (BLR018M)
Cat ID# - PA-RR 000003

Expected Molecular Weight: 116 kDa

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.

This antibody was produced against Synthetic Peptides Corresponding to Variable Regions within Human PARP1. While we see a clear DNA damage response, we also note the presence of native PARP1, albeit at a lower concentration in the non-damage lane.

Method -

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, IgG for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with 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 = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

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

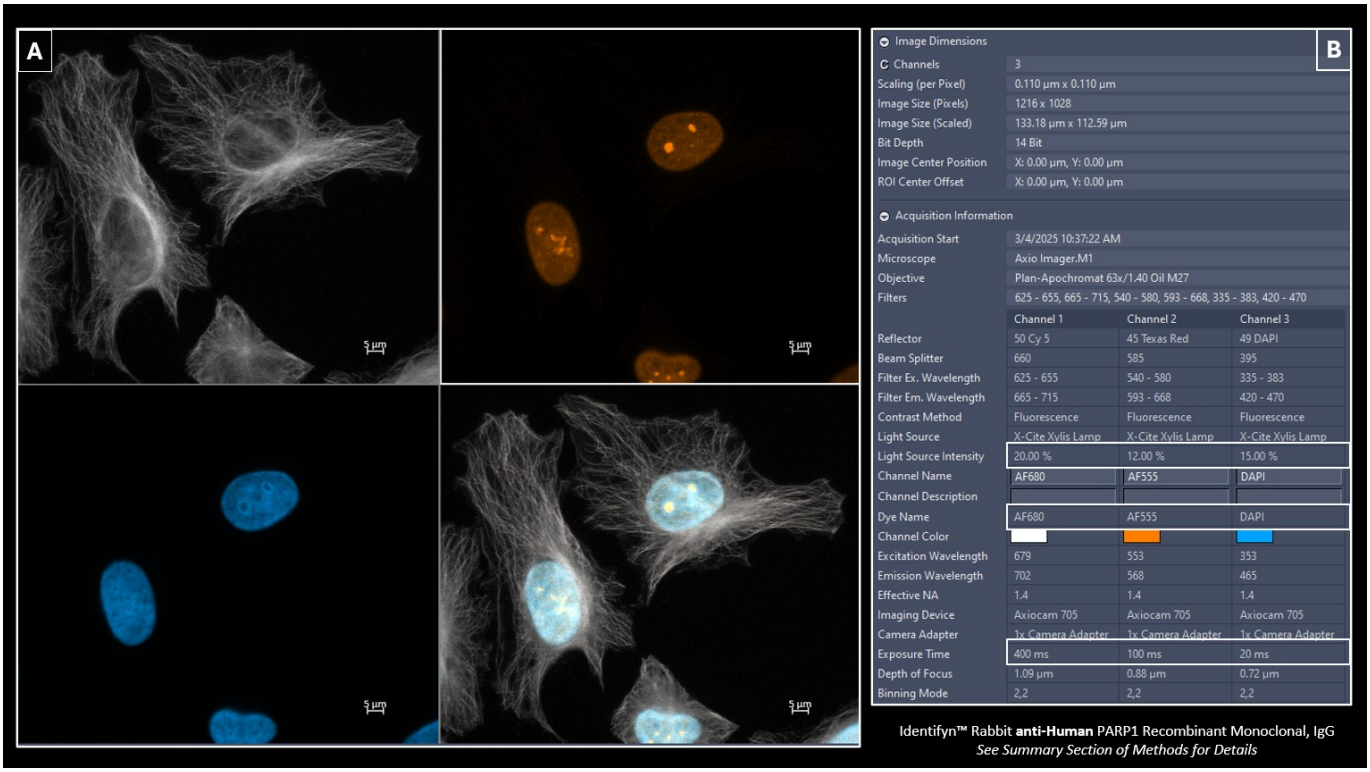
SOURCE PROVENANCE

Catalog	PA-RR-000003
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

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HEK293
Image SHA-256	749BED204F67D14B3F91D6363B7F9A327BE847B9B53188082FB37F43BC1670AB
Method PDF SHA-256	62AF4039551CAB9CD3F3557E57F5B052BA448609B49D67FEE357AD804AE6E521

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 680
METADATA VALUES	40

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 mono, 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 Cy5 | 45 Texas Red | 49 DAPI
Beam Splitter	660 | 585 | 395
Filter Excitation Wavelength	625 - 655 | 540 - 580 | 335 - 383
Filter Emission Wavelength	665 - 715 | 593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @12% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	400ms | 100ms | 20ms
Excitation Wavelength	679 | 553 | 353
Emission Wavelength	702 | 568 | 465
Effective NA	1.4
Imaging Device	Axiocam 705 mono
Camera Adapter	1X
Depth of Focus	1.09µm | 0.88µm | 0.72µm
Binning Mode	2,2
The image is presented in a split-view format	Panel A shows PARP1 staining in the upper left corner, alpha-tubulin staining in the upper right panel, DAPI staining in the lower left, and the merged image of all channels in the lower right. The split view is crucial for accurately visualizing localization within the nuclear compartment. In widefield applications, accurate localization can aid in studies of both population and individual nuclei.

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, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000035

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000070

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 680 secondary antibody 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.

Microscope

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

Single Plane (2D) - Split View Panel A

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 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 400ms
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 1.09µm
Binning Mode = 2,2

555 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @12%
Exposure Time = 100ms
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.88µm
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 @15%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Post Processing

None

Summary

This data point presents a widefield image of the Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal IgG. The imaging methods highlight that the PARP1 antibody was visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit IgG (H + L), Cat ID# SA 000035. This was followed by primary staining against alpha-tubulin, using Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse IgG (H + L), Cat ID# SA 000070. The nucleus was stained with DAPI following etoposide-induced DNA damage.

The PARP1 anti-Human Rabbit Recombinant antibody was produced against synthetic peptides corresponding to variable regions within human PARP1. The Western Blot data demonstrate that the antibody effectively recognizes the DNA damage response of the population we chose to image.

The image is presented in a split-view format: Panel A shows PARP1 staining in the upper left corner, alpha-tubulin staining in the upper right panel, DAPI staining in the lower left, and the merged image of all channels in the lower right. The split view is crucial for accurately visualizing localization within the nuclear compartment. In widefield applications, accurate localization can aid in studies of both population and individual nuclei.

Panel B features a clipped image directly from the Zeiss Zen software, providing transparency regarding the image acquisition. This ensures clarity in demonstrating the acquisition settings and highlights the robust signal produced by the combinatorial use of the Identifyn® PARP1 primary antibody and the Identifyn® SRM Alexa Fluor™ secondary conjugates, which exhibit excellent brightness with minimal LED input and exposure times.

Image Correction

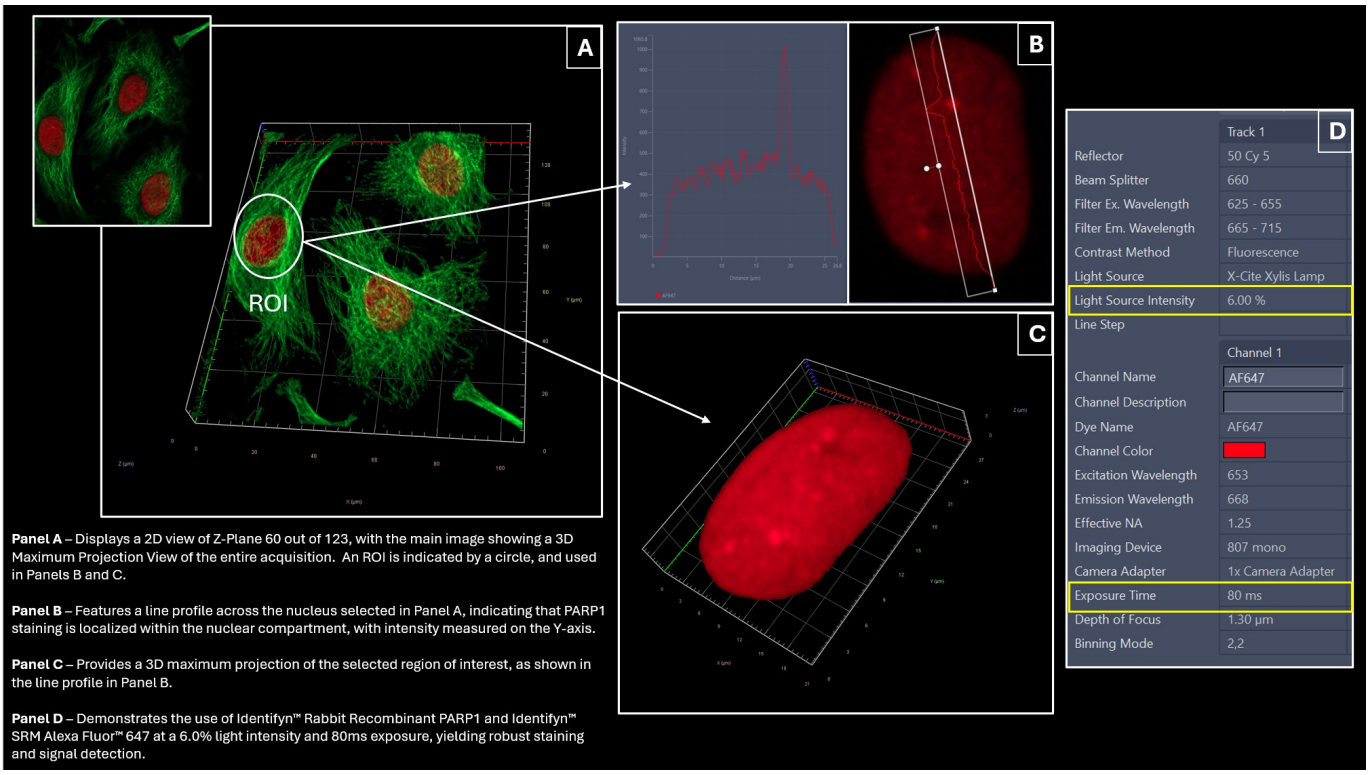
Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L), Cat ID# - SA 000070 was psuedo colored in ZEN software from the default color, dark red, to white to provide better visual contrast.

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	PA-RR-000003
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 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	361C47B0911173A2344122CBDCC58302130A41571EA3579ED668853877A67E62
Method PDF SHA-256	EA971BDCC64C1EE537A61BF331BCD01C4EB6C99F1548F0A5D36470C6512ED8AC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A – Displays a 2D view of Z-Plane 60 out of 123, with the main image showing a 3D Maximum Projection View of the entire acquisition. An ROI is indicated by a circle, and used in Panels B and C.

Panel B – Features a line profile across the nucleus selected in Panel A, indicating that PARP1 staining is localized within the nuclear compartment, with intensity measured on the Y-axis.

Panel C – Provides a 3D maximum projection of the selected region of interest, as shown in the line profile in Panel B.

Panel D – Demonstrates the use of Identifyn™ Rabbit Recombinant PARP1 and Identifyn™ SRM Alexa Fluor™ 647 at a 6.0% light intensity and 80ms exposure, yielding robust staining and signal detection.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	50

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	123 Slices (12.2µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm x 100µm
Image Size (Pixels)	751 X 885
Image Size (Scaled)	107.29µm X 126.43µm
Bit Depth	14 Bit
Image Center Position	X: 62.38mm, Y: 50.80mm
Stage Position	X: 62.38mm, Y: 50.80mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	38 HE eGFP | 350 Cy5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 665 | 335-383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.0% | X-Cite Xylis Lamp Intensity @6.0% | X-Cite Xylis Lamp Intensity 6.0%%
Exposure Time	150ms | 80ms | 18ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono | Axiocam 807
Camera Adapter	1X
Depth of Focus	1.00µm | 1.30µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background	Black
Light	Brightness 100%, Tone Mapping was NOT 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)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1

FIELD	SOURCE-DOCUMENTED VALUE
Profile View	X and Y axes were set to Auto - X (Min 0.00 and Max 18.6), Y (Min 2 and Max 4340)

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, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 488 secondary antibody 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.

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)

Z - Stack = 123 Slices (12.2µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm
Image Size (Pixels) = 751 X 885
Image Size (Scaled) = 107.29µm X 126.43µm
Bit Depth = 14 Bit
Image Center Position = X: 62.38mm, Y: 50.80mm
Stage Position = X: 62.38mm, Y: 50.80mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.0%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

647 -

Reflector = 350 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 665
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.0%
Exposure Time = 80ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
Binning Mode = 2,2

DAPI - (Not Shown but stained in mounting media)

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 6.0%%
Exposure Time = 18ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

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 - Panels A and C

3D Maximum Projection = Precise
Appearance = Threshold 13% for all channels, Ramp 0% for all channels, and Maximum 100% for all channels.
Background = Black
Light = Brightness 100%, Tone Mapping was NOT 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)

Profile View Display - Panel B

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

Profile View = X and Y axes were set to Auto - X (Min 0.00 and Max 18.6), Y (Min 2 and Max 4340)

Summary

Panel A - Displays a 2D view of Z-Plane 60 out of 123, with the main image showing a 3D Maximum Projection View of the entire acquisition. A circle indicates an ROI and is used in Panels B and C.

Panel B - Features a line profile across the nucleus selected in Panel A, indicating that PARP1 staining is localized within the nuclear compartment, with intensity measured on the Y-axis.

Panel C - Provides a 3D maximum projection of the selected region of interest, as shown in the line profile in Panel B.

Panel D - Demonstrates the use of Identifyn® Rabbit Recombinant PARP1 and Identifyn® SRM Alexa Fluor™ 647 at a 6.0% light intensity and 80 ms exposure, yielding robust staining and signal detection.

Image Corrections

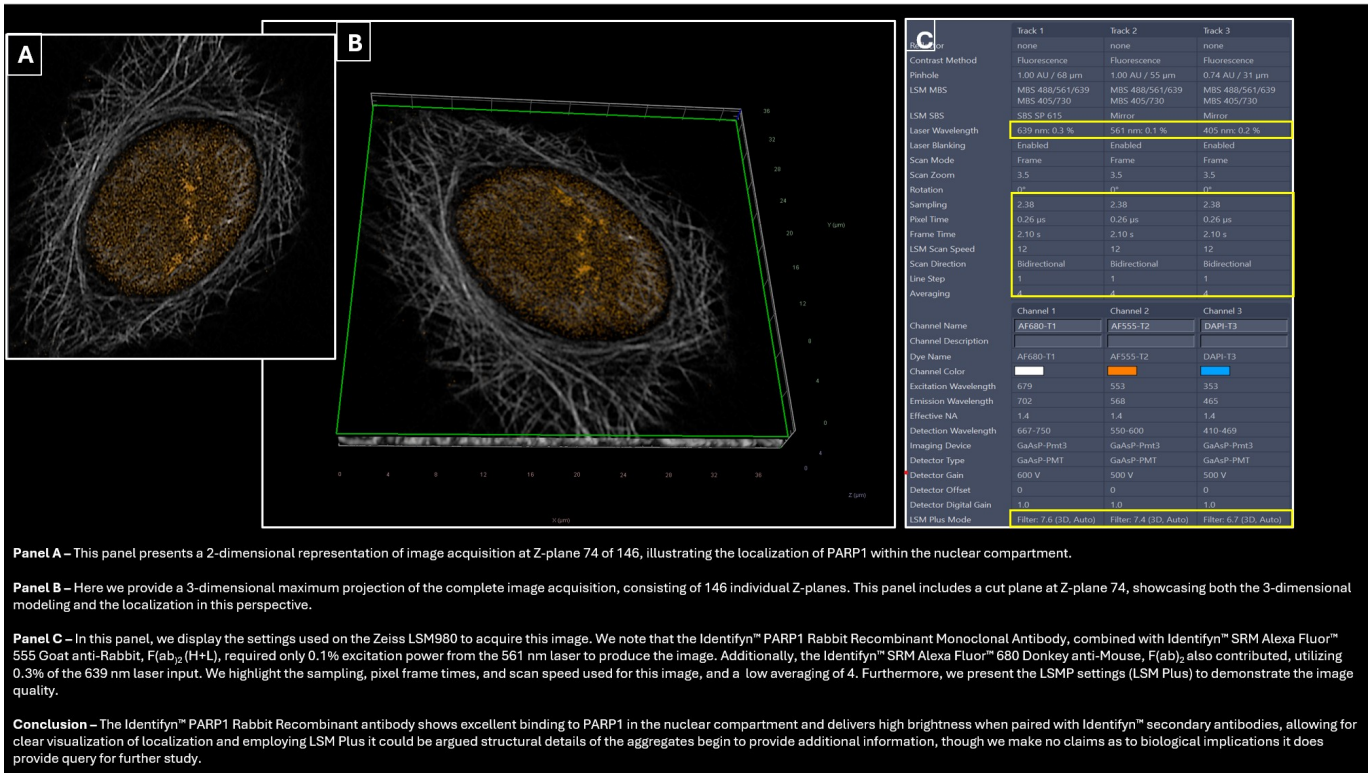
None

Image by E.F. Simon
Analysis and Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000003
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BEA39AF39063F3E42AE965B6F534DA0BF8210F5A0F36BFF47F228FE70EEF3C80
Method PDF SHA-256	C2F1551A50DAC0FC720F26CA4526CAF583F33F176A1869D20E05208B9B4D9CA5

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A – This panel presents a 2-dimensional representation of image acquisition at Z-plane 74 of 146, illustrating the localization of PARP1 within the nuclear compartment.

Panel B – Here we provide a 3-dimensional maximum projection of the complete image acquisition, consisting of 146 individual Z-planes. This panel includes a cut plane at Z-plane 74, showcasing both the 3-dimensional modeling and the localization in this perspective.

Panel C – In this panel, we display the settings used on the Zeiss LSM980 to acquire this image. We note that the Identifyn™ PARP1 Rabbit Recombinant Monoclonal Antibody, combined with Identifyn™ SRM Alexa Fluor™ 555 Goat anti-Rabbit, $F(ab_{12}(H+L)$, required only 0.1% excitation power from the 561 nm laser to produce the image. Additionally, the Identifyn™ SRM Alexa Fluor™ 680 Donkey anti-Mouse, $F(ab)_2$ also contributed, utilizing 0.3% of the 639 nm laser input. We highlight the sampling, pixel frame times, and scan speed used for this image, and a low averaging of 4. Furthermore, we present the LSMP settings (LSM Plus) to demonstrate the image quality.

Conclusion – The Identifyn™ PARP1 Rabbit Recombinant antibody shows excellent binding to PARP1 in the nuclear compartment and delivers high brightness when paired with Identifyn™ secondary antibodies, allowing for clear visualization of localization and employing LSM Plus it could be argued structural details of the aggregates begin to provide additional information, though we make no claims as to biological implications it does provide query for further study.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 680; 750
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	146 Slices (4.35µm)
Channels	3
Scaling (Per Pixel)	0.030µm X 0.030µm X 0.030µm
Image Size (Pixels)	1276 X 1276
Image Size (Scaled)	37.77µm X 37.77µm
Bit Depth	16 Bit
Image Center Position	X: 88.97mm, Y: 49.88mm
Stage Position	X: 88.97mm, Y: 49.88mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 55µm | 1.00AU / 68µm | 0.74 AU / 31µm
LMS MBS	MBS 488/561/639, 405/730
LSM SBS	Mirror | SBS SP 615
Laser Wavelength	561nm @0.1% | 639nm @0.3% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.5 | 1.4
Rotation	0°
Sampling	2.38
Pixel Time	0.26µs
Frame Time	2.10s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	553 | 679 | 353
Emission Wavelength	568 | 702 | 465
Effective NA	1.4
Detection Wavelength	550 - 600 | 667 - 750 | 410 - 469
Imaging Device	GaAsP-Pmt 3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V | 600 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	7.4 (3D, Auto) | 7.5 (3D, Auto) | 6.7 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness100%, Tone Mapping NOT 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)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Y	Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. The cut plane was used to show the specificity, localization, and brightness of the Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG (BLR018M) combined with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, IgG (H + L)Cat ID# - SA 000035.
Position of Clip	-20%
Clip X	0°
Clip Y	0°

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000041

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000075

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 680 secondary antibody 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.

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)

Z-Stack = 146 Slices (4.35µm)

Channels = 3
Scaling (Per Pixel) = 0.030µm X 0.030µm X 0.030µm
Image Size (Pixels) = 1276 X 1276
Image Size (Scaled) = 37.77µm X 37.77µm
Bit Depth = 16 Bit
Image Center Position = X: 88.97mm, Y: 49.88mm
Stage Position = X: 88.97mm, Y: 49.88mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 55µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 561nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.5
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 550 - 600
Imaging Device = GaAsP-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.4 (3D, Auto)

680 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 68µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.5
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 679
Emission Wavelength = 702
Effective NA = 1.4
Detection Wavelength = 667 - 750
Imaging Device = GaAsP-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.5 (3D, Auto)

DAPI - (Not Shown, in mounting medium)

Reflector - None
Contrast Method - Fluorescence
Pinhole = 0.74 AU / 31µm
LMS MBS = MBS 488/561/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Scan Zoom = 3.5
Rotation = 0°
Sampling = 2.38
Pixel Time = 0.26µs
Frame Time = 2.10s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 469
Imaging Device = GaAsP-Pmt 3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.7 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background = Black
Light = Brightness100%, Tone Mapping NOT 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)

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

X-Y = Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z-Stack of the cell from the image, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected. The cut plane was used to show the specificity, localization, and brightness of the Identifyn® Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG (BLR018M) combined with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Rabbit, IgG (H + L)Cat ID# - SA 000035.

Position of Clip = -20%
Clip X = 0°
Clip Y =0°

Summary -

Panel A - This panel presents a 2-dimensional representation of image acquisition at Z-plane 74 of 146, illustrating the localization of PARP1 within the nuclear compartment.

Panel B - Here, we provide a 3-dimensional maximum projection of the complete image acquisition, consisting of 146 individual Z-planes. This panel features a cut plane at Z-plane 74, showcasing both the 3D modeling and localization in this perspective.

Panel C - In this panel, we display the settings used on the Zeiss LSM980 to acquire this image. We note that the Identifyn® PARP1 Rabbit Recombinant Monoclonal Antibody, combined with Identifyn® SRM Alexa Fluor™ 555 Goat anti-Rabbit, F(ab)2 (H+L), required only 0.1% excitation power from the 561 nm laser to produce the image. Additionally, the Identifyn® SRM Alexa Fluor™ 680 Donkey anti-Mouse, F(ab')2 also contributed, utilizing 0.3% of the 639 nm laser input. We highlight the sampling, pixel frame times, and scan speed used for this image, along with a low averaging of 4. Furthermore, we present the LSMP settings (LSM Plus) to demonstrate the image quality.

Conclusion - The Identifyn® PARP1 Rabbit Recombinant antibody exhibits excellent binding to PARP1 in the nuclear compartment and delivers high brightness when paired with Identifyn® secondary antibodies, enabling clear visualization of localization. Employing LSM Plus, it can be argued that the structural details of the aggregates begin to provide additional information. However, we make no claims regarding biological implications; it does, however, provide a query for further study.

Image Corrections

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Mouse, IgG (H + L), Cat ID# - SA 000070 was psuedo colored in ZEN software from the default color, dark red, to white to provide better visual contrast.

Image by J.K. Bennett
Analysis and Presentation by B.T. Bennett

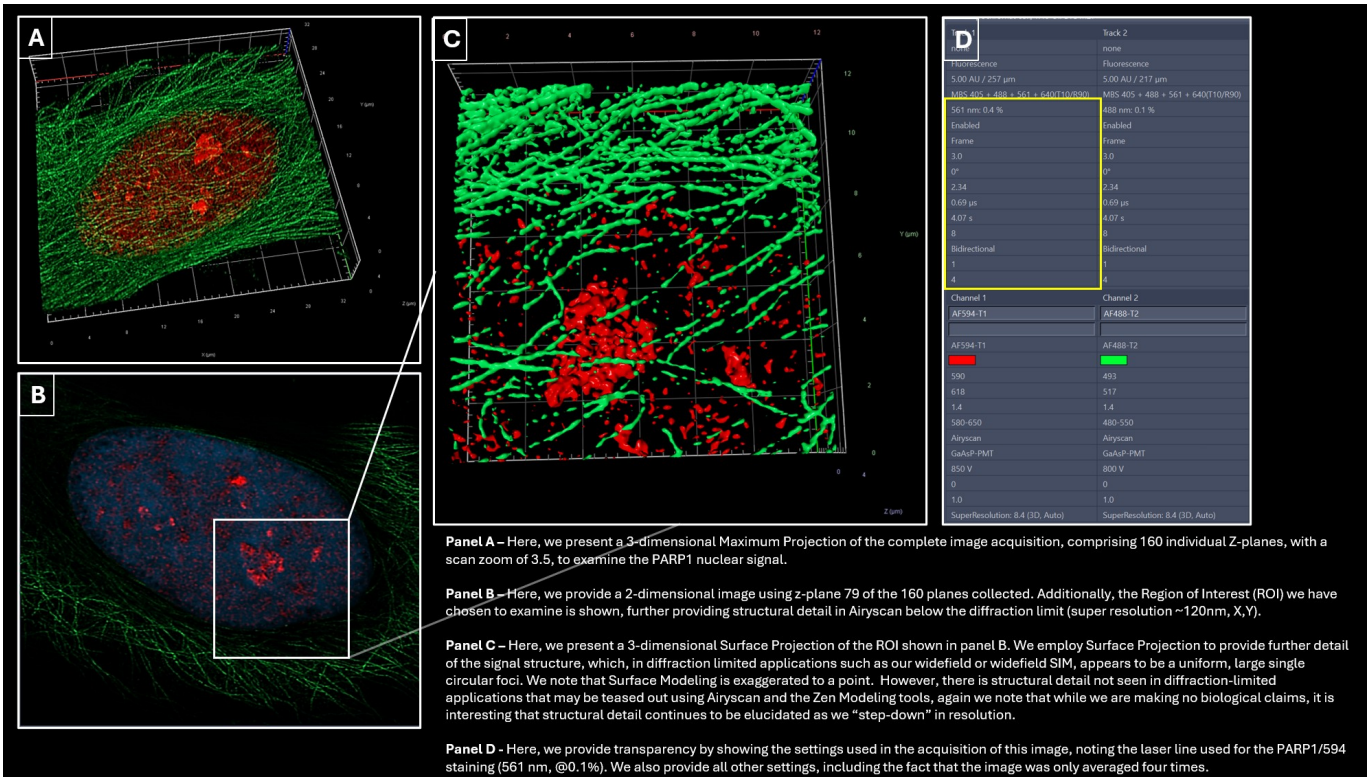
SOURCE PROVENANCE

Catalog	PA-RR-000003
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	30E07BD9E67A4D6D02BFD9D11CE90AD277AA64B0EC90F4A015B59B328985CA6E

SOURCE PROVENANCE

Method PDF SHA-256	696D8DBEF66D9BE6873358C42AE1FF68BF965D433BFDA0959A1DB9B33A8788AB

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A – Here, we present a 3-dimensional Maximum Projection of the complete image acquisition, comprising 160 individual Z-planes, with a scan zoom of 3.5, to examine the PARP1 nuclear signal.

Panel B – Here, we provide a 2-dimensional image using z-plane 79 of the 160 planes collected. Additionally, the Region of Interest (ROI) we have chosen to examine is shown, further providing structural detail in Airyscan below the diffraction limit (super resolution ~120nm, X,Y).

Panel C – Here, we present a 3-dimensional Surface Projection of the ROI shown in panel B. We employ Surface Projection to provide further detail of the signal structure, which, in diffraction limited applications such as our widefield or widefield SIM, appears to be a uniform, large single circular foci. We note that Surface Modeling is exaggerated to a point. However, there is structural detail not seen in diffraction-limited applications that may be teased out using Airyscan and the Zen Modeling tools, again we note that while we are making no biological claims, it is interesting that structural detail continues to be elucidated as we "step-down" in resolution.

Panel D - Here, we provide transparency by showing the settings used in the acquisition of this image, noting the laser line used for the PARP1/594 staining (561 nm, @0.1%). We also provide all other settings, including the fact that the image was only averaged four times.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	52

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 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	160 Slices (4.70µm)
Channels	2
Scaling (Per Pixel)	0.03018 µm X 0.03018 µm X 0.03000 µm
Image Size (Pixels)	1096 X 1096 | 405 X 423
Image Size (Scaled)	33.08µm X 33.08µm | 12.22µm X 12.77µm
Bit Depth	16 Bit
Image Center Position	X: 78.66mm, Y: 51.92mm
Stage Position	X: 78.66mm, Y: 51.92mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 257µm | 5.00AU / 217µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.4% | 488nm @0.1%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	3.0
Rotation	0°
Sampling	2.34
Pixel Time	0.69µs
Frame Time	4.07s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 493
Emission Wavelength	618 | 517
Effective NA	1.4
Detection Wavelength	580-650 | 480 - 550
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.4 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (594), Threshold 1% (488), Ramp 0% all channels, Maximum 100% all channels | Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 55% of all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Azimuth 42°, Elongation -86° , Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) | View Angle 50°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000052

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 488 secondary antibody 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.

Microscope

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with 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 = 160 Slices (4.70µm)

Channels = 2
Scaling (Per Pixel) = 30.18nm X 30.18nm X 30.00nm
Image Size (Pixels) = 1096 X 1096
Image Size (Scaled) = 33.08µm X 33.08µm
Bit Depth = 16 Bit
Image Center Position = X: 78.66mm, Y: 51.92mm
Stage Position = X: 78.66mm, Y: 51.92mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Region of Interest Panel C

Z-Stack = 160 Slices (4.70µm)

Channels = 2
Scaling (Per Pixel) = 30.18nm X 30.18nm X 30.00nm
Image Size (Pixels) = 405 X 423
Image Size (Scaled) = 12.22µm X 12.77µm
Bit Depth = 16 Bit
Image Center Position = X: 78.66mm, Y: 51.92mm
Stage Position = X: 78.66mm, Y: 51.92mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 257µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 4.07s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 580-650
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 217µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 3.0
Rotation = 0°
Sampling = 2.34
Pixel Time = 0.69µs
Frame Time = 4.07s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480 - 550
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.4 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (594), Threshold 1% (488), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise
Appearance = Threshold 20% of all channels, Ambient Light 0% of all channels, Specular Light 100% of all channels, Shininess 55% of all channels
Background = Black
Light = Azimuth 42°, Elongation -86° , Enabled Tone Mapping
Projection = View Angle 50°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary -

Panel A - Here, we present a 3-dimensional Maximum Projection of the complete image acquisition, comprising 160 individual Z-planes, with a scan zoom of 3.5, to examine the PARP1 nuclear signal.

Panel B - Here, we provide a 2-dimensional image using z-plane 79 of the 160 planes collected. Additionally, the Region of Interest (ROI) we have chosen to examine is shown, further providing structural detail in Airyscan below the diffraction limit.

Panel C - Here, we present a 3-dimensional Surface Projection of the ROI shown in panel B. We employ Surface Projection to provide further detail of the signal structure, which, in sub-diffraction applications such as our widefield or widefield SIM, appears to be a uniform, large single circular foci. We note that Surface Modeling is exaggerated to a point. However, there is a structural detail not visible in diffraction-limited applications that may be teased out using Airyscan and the Zen Modeling tools; again, we note that while we are making no biological claims, it is interesting that structural detail continues to be elucidated as we “step down” in resolution.

Panel D - Here, we provide transparency by showing the settings used in the acquisition of this image, noting the laser line used for the PARP1/594 staining (561 nm, @0.1%). We also provide all other settings, including the fact that the image was only averaged four times.

Image Corrections

None

Image by J.K. Bennett
Analysis and Presentation by B.T. Bennett

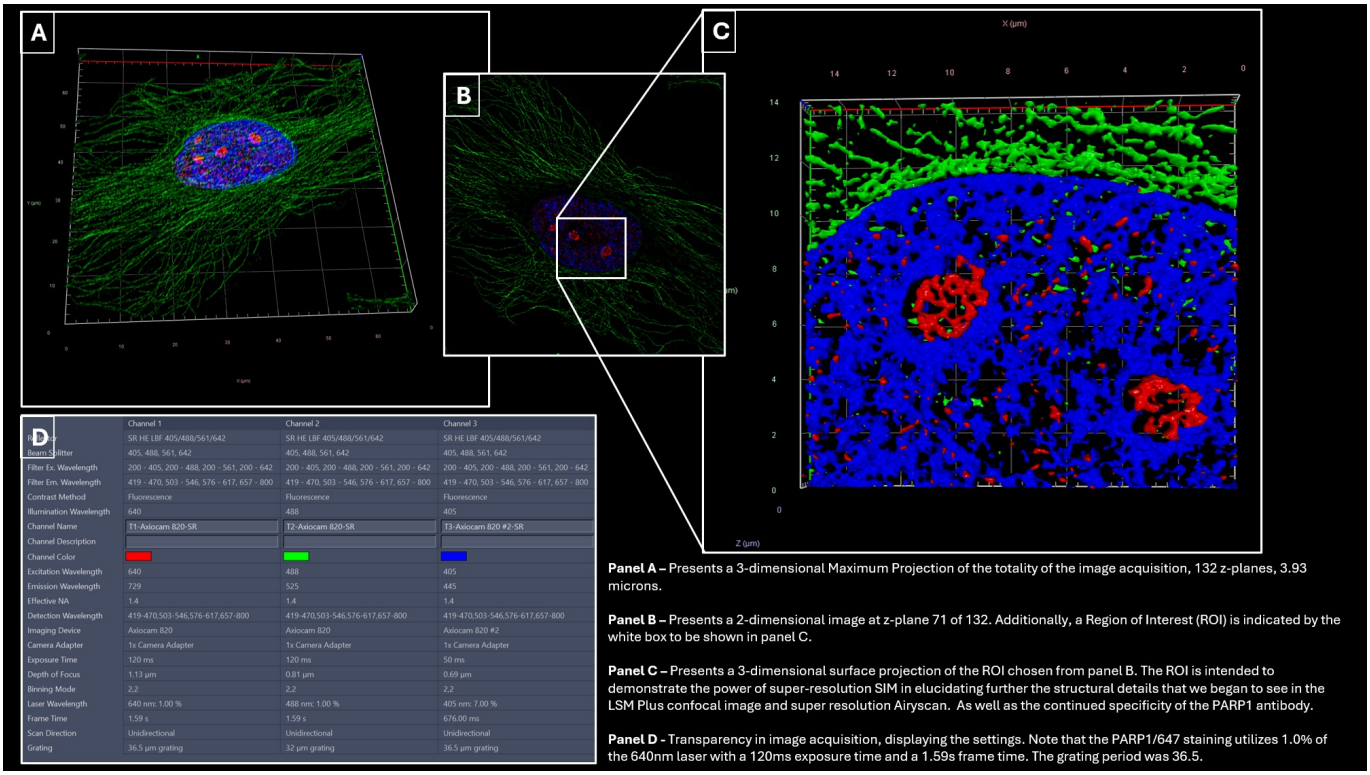
SOURCE PROVENANCE

Catalog	PA-RR-000003
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	B0BBB51CED0BADB39372790E03B11D84DF9163A5D41D8E88165FB7324DD1F2EE
Method PDF SHA-256	9C5A28A05B32DF9E5AD1B294FA5BCE53FBAC5E5956C9DB8B17BEBA1B5C7B2D97

ORIGINAL IMAGE EVIDENCE / SIM



Panel A – Presents a 3-dimensional Maximum Projection of the totality of the image acquisition, 132 z-planes, 3.93 microns.

Panel B – Presents a 2-dimensional image at z-plane 71 of 132. Additionally, a Region of Interest (ROI) is indicated by the white box to be shown in panel C.

Panel C – Presents a 3-dimensional surface projection of the ROI chosen from panel B. The ROI is intended to demonstrate the power of super-resolution SIM in elucidating further the structural details that we began to see in the LSM Plus confocal image and super resolution Airyscan. As well as the continued specificity of the PARP1 antibody.

Panel D - Transparency in image acquisition, displaying the settings. Note that the PARP1/647 staining utilizes 1.0% of the 640nm laser with a 120ms exposure time and a 1.59s frame time. The grating period was 36.5.

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	68

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	132 Slices (3.93µm) | 47 Slices (2.3µm) - The Gallery tool was used to reduce the z-stack to provide clarity
Channels	3 | 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1162 X 1078
Image Size (Scaled)	66.72µm X 66.72µm | 15.33µm X 14.22µm
Bit Depth	16 Bit
Image Center Position	X: 62.07mm, Y: 35.57mm
Stage Position	X: 62.07mm, Y: 35.57mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
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
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @1.0% | 488nm @1.0% | 405nm @7.0%
Frame Time	1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.4461
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (488), Threshold 1% DAPI, Ramp 0% all channels, Maximum 100% all channels | Threshold 20% all channels, Ambient Light 0% all channels, Specular Light 100% all channels, Shininess 57% of all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Azimuth 80°, Elongation -130° , 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 Surface Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 488 secondary antibody 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.

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) - Panel A, Total Image Acquisition

Z-Stack = 132 Slices (3.93µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel C, Region of Interest

Z-Stack = 47 Slices (2.3µm) - The Gallery tool was used to reduce the z-stack to provide clarity

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 1162 X 1078
Image Size (Scaled) = 15.33µm X 14.22µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

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 = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @1.0%
Frame Time = 1.59s
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 = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.0%
Frame Time = 1.59s
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 = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @7.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 2
Channels = 1
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.4461
Input SNR = N/A
Regularization Weight = N/A
Iterations = N/A
Filter = N/A
Fitting = N/A
Normalization = Automatic
Experimental PSF = N/A

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 1% (647), Threshold 1% (488), Threshold 1% DAPI, 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 Surface Projection = Precise
Appearance = Threshold 20% all channels, Ambient Light 0% all channels, Specular Light 100% all channels, Shininess 57% of all channels
Background = Black
Light = Azimuth 80°, Elongation -130° , Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary -

Panel A - Presents a 3-dimensional Maximum Projection of the totality of the image acquisition, 132 z-planes, 3.93 microns.

Panel B - Presents a 2-dimensional image at z-plane 71 of 132. Additionally, a Region of Interest (ROI) is indicated by the white box to be shown in panel C.

Panel C - Presents a 3-dimensional surface projection of the ROI chosen from panel B. The ROI is intended to demonstrate the power of super-resolution SIM in elucidating further the structural details that we began to see in the LSM Plus confocal image and super resolution Airyscan.

Panel D - Transparency in image acquisition, displaying the settings. Note that the PARP1/647 staining utilizes 1.0% of the 640 nm laser with a 120 ms exposure time and a 1.59 s frame time. The grating period was 36.5.

Image Corrections

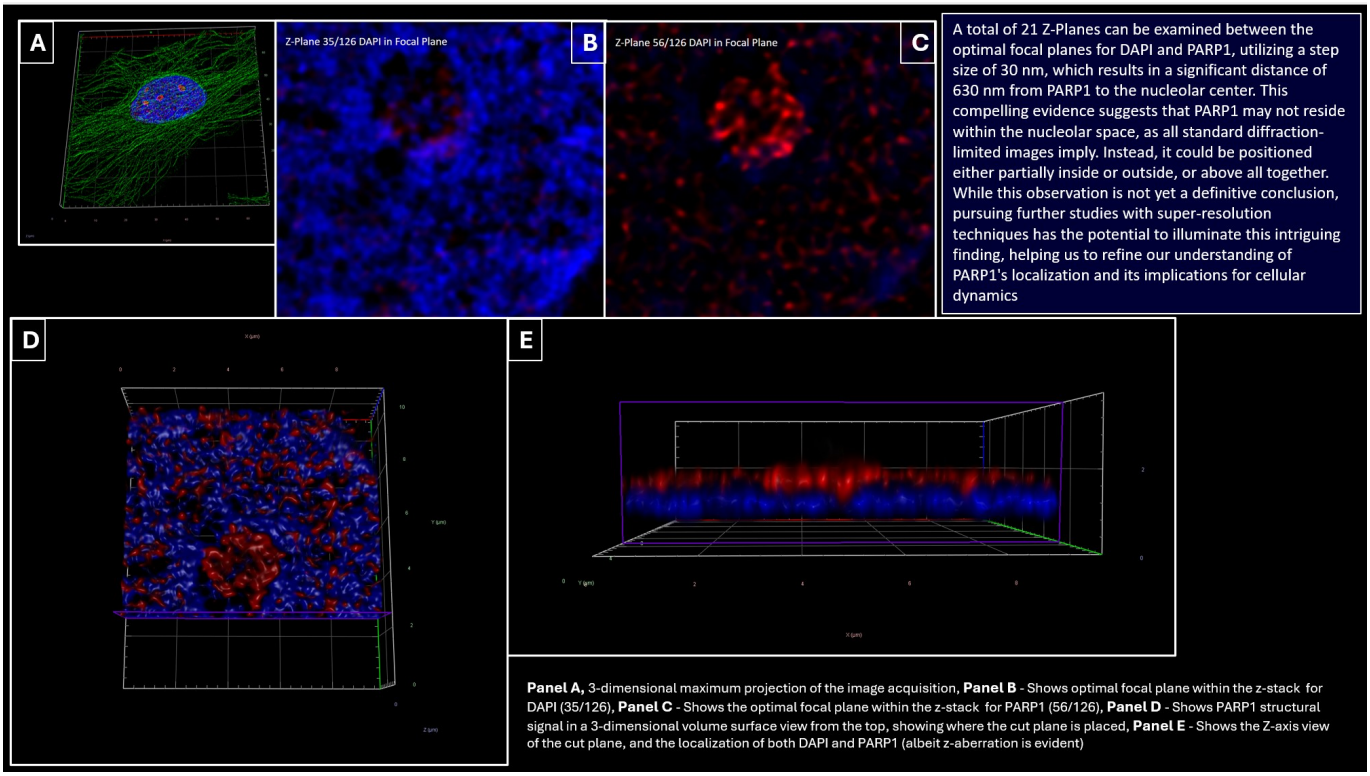
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000003
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	68
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	15DE7D96B9C8B22AB9B084290111D6683D1CF68AA9658CE1CFDCC5D084E74839
Method PDF SHA-256	8E51F67DAD22F1EBBBB45470F7435D5D67880049953423F0A9F49145CED7F76E

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	63

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	132 Slices (3.93µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 744 X 828
Image Size (Scaled)	66.72µm X 66.72µm | 9.82µm X 10.93µm
Bit Depth	16 Bit
Image Center Position	X: 62.07mm, Y: 35.57mm
Stage Position	X: 62.07mm, Y: 35.57mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
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
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800 | 419-470, 503-546, 576-617, 657-800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @1.0% | 488nm @1.0% | 405nm @7.0%
Frame Time	1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
Sharpness Setting	Standard
Auto Sharpness	On
Sharpness	8.8687 (647), 9.4576 (555), 8.0281 (DAPI)
Order Combination	Weiner Filter
Regularization	None
Regularization Weight	0.000
Iterations	3
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Volume Projection	Precise
Appearance	Threshold 2% (647), Threshold 2% (488), Threshold 2% (405), 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	Active was selected, and invisible was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment and both the DAPI and PARP1 signals. A purple outline of the clipped perimeter was shown, and the clip back was selected, while the 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, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody 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.

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) - Panel A, Total Image Acquisition

Z-Stack = 132 Slices (3.93µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 66.72µm X 66.72µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels D/E, Region of Interest

Z-Stack = 132 Slices (3.93µm)

Channels = 3
Scaling (Per Pixel) = 13.20nm X 13.20nm X 30.00nm
Image Size (Pixels) = 744 X 828
Image Size (Scaled) = 9.82µm X 10.93µm
Bit Depth = 16 Bit
Image Center Position = X: 62.07mm, Y: 35.57mm
Stage Position = X: 62.07mm, Y: 35.57mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

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 = 120ms
Depth of Focus = 1.13µm
Binning Mode = 2,2
Laser Wavelength = 640nm @1.0%
Frame Time = 1.59s
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 = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @1.0%
Frame Time = 1.59s
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 = 525
Effective NA = 1.4
Detection Wavelength = 419-470, 503-546, 576-617, 657-800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @7.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM2
Sharpness Setting = Standard
Auto Sharpness = On
Sharpness = 8.8687 (647), 9.4576 (555), 8.0281 (DAPI)
Order Combination = Weiner Filter
Regularization = None
Regularization Weight = 0.000
Iterations = 3
Filter - No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

3D Image Processing - Panels D/E

3D Volume Projection = Precise
Appearance = Threshold 2% (647), Threshold 2% (488), Threshold 2% (405), 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 - Panels D/E

The Clipping plane is introduced to demonstrate signal specificity and localization.

X-Y = Active was selected, and invisible was chosen to remove the obstruction from the image cut plane and highlight the nuclear compartment and both the DAPI and PARP1 signals. A purple outline of the clipped perimeter was shown, and the clip back was selected, while the clip front was NOT selected.

Summary -

Panel A -3-dimensional maximum projection of the image acquisition

Panel B - Shows optimal focal plane within the z-stack for DAPI (35/126)

Panel C - Shows the optimal focal plane within the z-stack for PARP1 (56/126)

Panel D - Shows PARP1 structural signal in a 3-dimensional volume surface view from the top, showing where the cut plane is placed.

Panel E - Shows the Z-axis view of the cut plane and the localization of both DAPI and PARP1 (albeit z-aberration is evident)

Evidentiary Conclusion -

21 Z-Planes exist between optimal focal plane for DAPI vs. PARP1, using a 30nm step size = 630nm between PARP1 and nucleolar center - suggest that super resolution may with some degree of confidence that the PARP1 is not actually with nuclear space as all diffraction limited images show, but somewhere either partially in or out. There are 21 Z-Planes available between the optimal focal plane for DAPI and PARP1, with a step size of 30 nm, resulting in a distance of 630 nm from PARP1 to the nucleolar center. This suggests that, with a certain degree of confidence, PARP1 may not be located within the nuclear space as indicated by all diffraction-limited images. Instead, it may be situated either partially inside or outside of it. While this does not constitute a definitive conclusion, further studies utilizing super-resolution techniques could provide more clarity on this finding. This certainly does not represent fact, but additional studies of localization using super resolution could “shine more light” on this finding.

Image Corrections

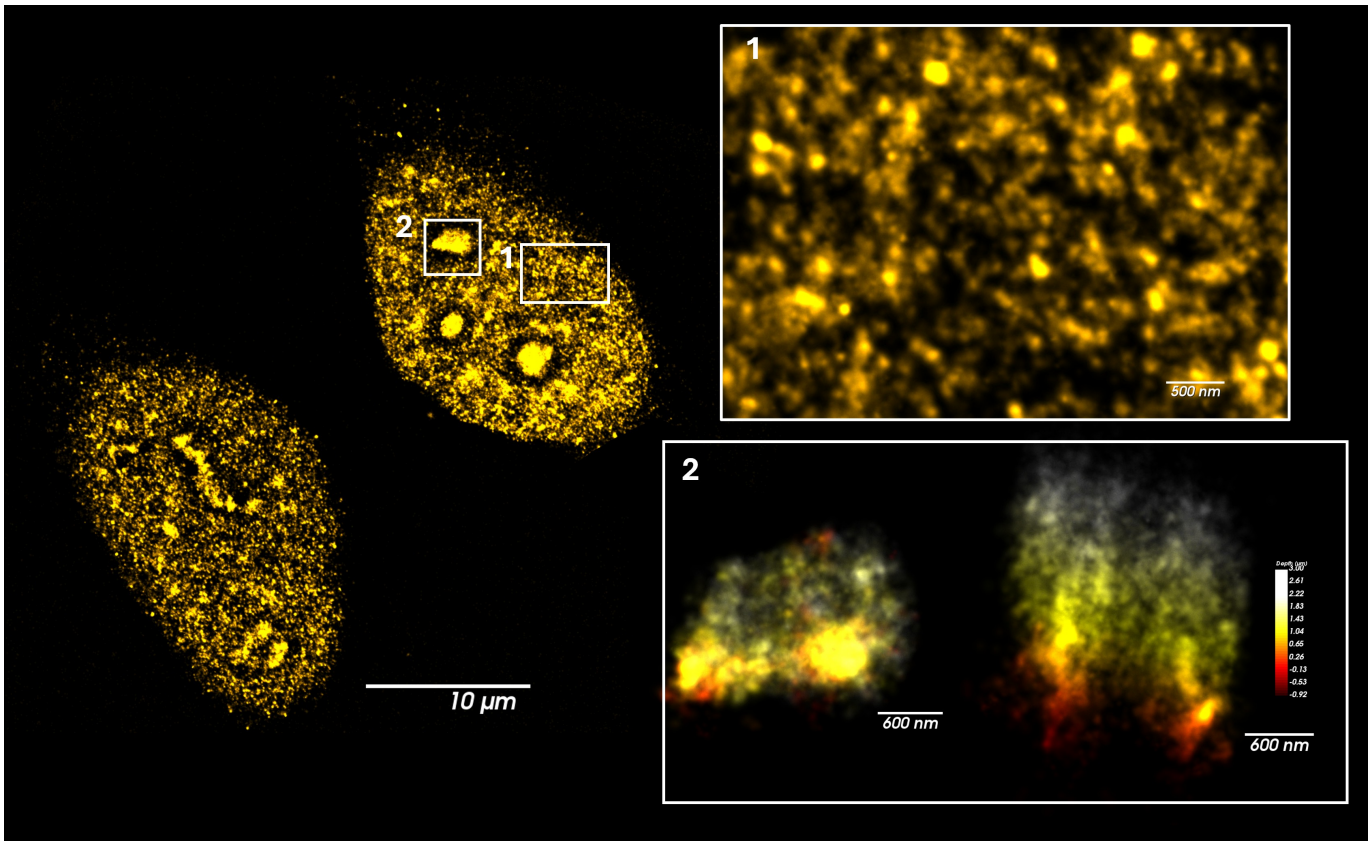
None

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

SOURCE PROVENANCE

Catalog	PA-RR-000003
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	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	46B9A44AD46FB390989E80203B06EF29923BE5F16F6DE5D86E5BE732153785B8
Method PDF SHA-256	6CDD42031BDC28364B4744FCEF5C853965CEA41ACBE02FCA7E7ACC8BEE877EEE

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	179.8 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 175.5; End: 179.1
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	18 using Steps: 0.2µm (200nm)
Cycles	10
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	20%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Radial Precision
Particle size	4nm
Color by	Constant
Color Table	Single
Opacity Mode	Constant; Opacity 1: 03
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	7 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	30
Axial precision	70

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, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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 in an µ-Slide 8 Well high Glass-Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 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, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:50. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn's Calibration Standard Operating Procedure, which can be found here.

https://identifyn.com/storm-calibration

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, 'Record' is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1
Number of Simultaneous Probes: 1
Number of Reference Probes: 1
Camera: Both Cameras
Color Channel: Both Channels
Probe Capture Mode: Interleaved
Z -Stack Capture Mode: Interleaved
Multiple Location Capture: Not selected
Post Record Reference: Not Selected
Fluidics: Not Selected
Autofocus Drift Correction: On

Microscope Configuration

Biplane Module: 635nm Dichroic
Objective: Silicon Selected
Stage Insert: Rectangular Selected
Course Focus Position: 8-Well Chamber Selected
Dichroic: SuperRes
PSF: (Red, Orange)
Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%
Widefield Camera Exposure Time: 100ms
Super Resolution Camera Exposure Time: 20ms
Widefield Camera Binning: 1
Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200µm
Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization - The red-bordered box identifies the area of the SuperRes view and defines a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.
Piezo Current: 179.8 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100ms
Default values are maintained unless otherwise noted.
Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position" -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50µm is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - Positioning of the target Cell or Subsection of the Target Cell is optimized and optimally focused.

Piezo Record: Begin: 175.5; End: 179.1

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control 640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 18 using Steps: 0.2µm (200nm)
Cycles: 10
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (640 nm Dichroic)

Laser Power: 20%

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to their default values.

Localization

Cutout Width: 16px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1px
Fiducial at Max Accumulation: Not selected
Max Particles Per Frame: No Max
Background SLPE Removal: Not Selected
Cutout/Background Removal: Not Selected
Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:
Probe1: BG threshold: 15

Region of Interest -

Not selected

Display -

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Radial Precision
Particle size: 4nm
Color by: Constant
Color Table: Single
Opacity Mode: Constant; Opacity 1: 03
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 7 Intervals
Pixel Size: 50nm
Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

PSF Z offset: -600 to +600
Radial precision: 30
Axial precision: 70
The other parameters were not applied.

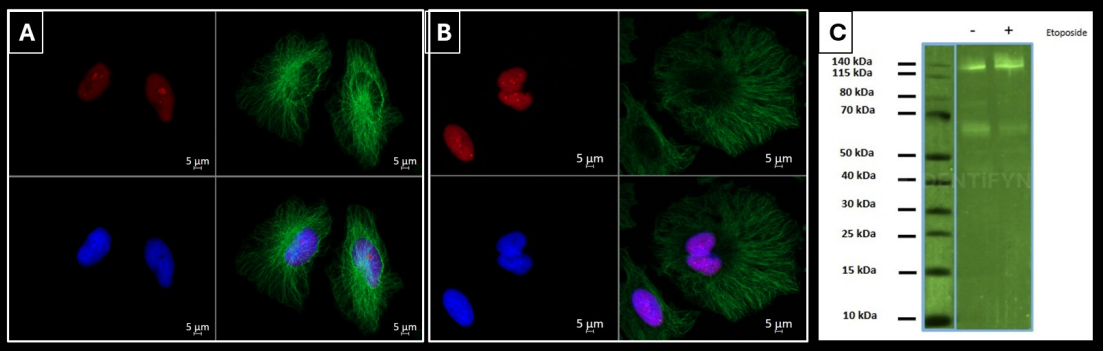
Statistical analysis - NA

Image by S. Thakur
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000003
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	651B34C0542889C04555103C744A52337856DC661B2580629B56E39D29733487
Method PDF SHA-256	D22B1C4BBF504B66E90F2386B6592056A756A47FCF5CF5C4E93E064433214DBA

ORIGINAL IMAGE EVIDENCE / CONTROL



Panel A – Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) in the absence of Etoposide

Panel B – Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) treated with Etoposide

Panel C – Western Blot, minus/plus etoposide-induced DNA damage.

Note - The settings for both images in Panels A and B were the same, using a Zeiss Axio Observer.Z1/7 Apotome SIM with an EC Plan-Neofluar 63X/1.25 oil M27 objective, an Axiocam 807, and an X-Cite Xylis Lamp.

As observed in the Western Blot, the appearance of PARP1 in both the undamaged and damaged lanes increases in response to the presence of Etoposide. Similar results are seen in the control images.

EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / CONTROL

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
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	971 X 773 | 930 X 813
Image Size (Scaled)	138.71µm X 110.43µm | 132.86µm X 116.14µm
Bit Depth	14 Bit
Image Center Position	X: 52.81mm, Y: 47.64mm | X: 60.90mm, Y: 54.88mm
Stage Position	X: 52.81mm, Y: 47.64mm | X: 60.90mm, Y: 54.88mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	38 HE eGFP | 350 Cy5 | 49 DAPI
Beam Splitter	495 | 660 | 395
Filter Excitation Wavelength	450 - 490 | 625 - 665 | 335-383
Filter Emission Wavelength	500 - 550 | 665 - 715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.0% | X-Cite Xylis Lamp Intensity @6.0% | X-Cite Xylis Lamp Intensity 6.0%%
Exposure Time	150ms | 80ms | 18ms
Excitation Wavelength	493 | 653 | 353
Emission Wavelength	517 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono | Axiocam 807
Camera Adapter	1X
Depth of Focus	1.00µm | 1.30µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction 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, IgG - (BLR018M)
Cat ID# - PA-RR 000003

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

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, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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® 488 secondary antibody 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.

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:

Single Plane (2D) - Panel A
Channels = 3
Scaling (Per Pixel) = 0.143μm X 0.143μm
Image Size (Pixels) = 971 X 773
Image Size (Scaled) = 138.71μm X 110.43μm
Bit Depth = 14 Bit
Image Center Position = X: 52.81mm, Y: 47.64mm
Stage Position = X: 52.81mm, Y: 47.64mm
ROI Center Offset = X: 1.14μm, Y: 0.00μm

Single Plane (2D) - Panel B
Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 930 X 813
Image Size (Scaled) = 132.86µm X 116.14µm
Bit Depth = 14 Bit
Image Center Position = X: 60.90mm, Y: 54.88mm
Stage Position = X: 60.90mm, Y: 54.88mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.0%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

647 -

Reflector = 350 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 665
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.0%
Exposure Time = 80ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
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 6.0%%
Exposure Time = 18ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

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.

Summary

Panel A - Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) in the absence of Etoposide.

Panel B - Rabbit anti-Human PARP1 Recombinant Monoclonal, IgG - (BLR018M) treated with Etoposide.

Panel C - Western Blot, minus/plus etoposide-induced DNA damage.

Note - The settings for both images in Panels A and B were the same, using a Zeiss Axio Observer.Z1/7 Apotome SIM with an EC Plan-Neofluar 63X/1.25 oil M27 objective, an Axiocam 807, and an X-Cite Xylis Lamp.
As observed in the Western Blot, the appearance of PARP1 in both the undamaged and damaged lanes increases in response to the presence of Etoposide. Similar results are seen in the control images.

Image Corrections

None

Image by E.F. Simon
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000003
Stage	Control
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:

SOURCE PROVENANCE

Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	077E1279F5FBE589C92487F0534EF6E0E3B11C17009403CC24265ADE639F26E2
Method PDF SHA-256	A97C4DEA1473182506EFAA7B349EE7E42DACA9CEBFC907BB669043DD079E0281