BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

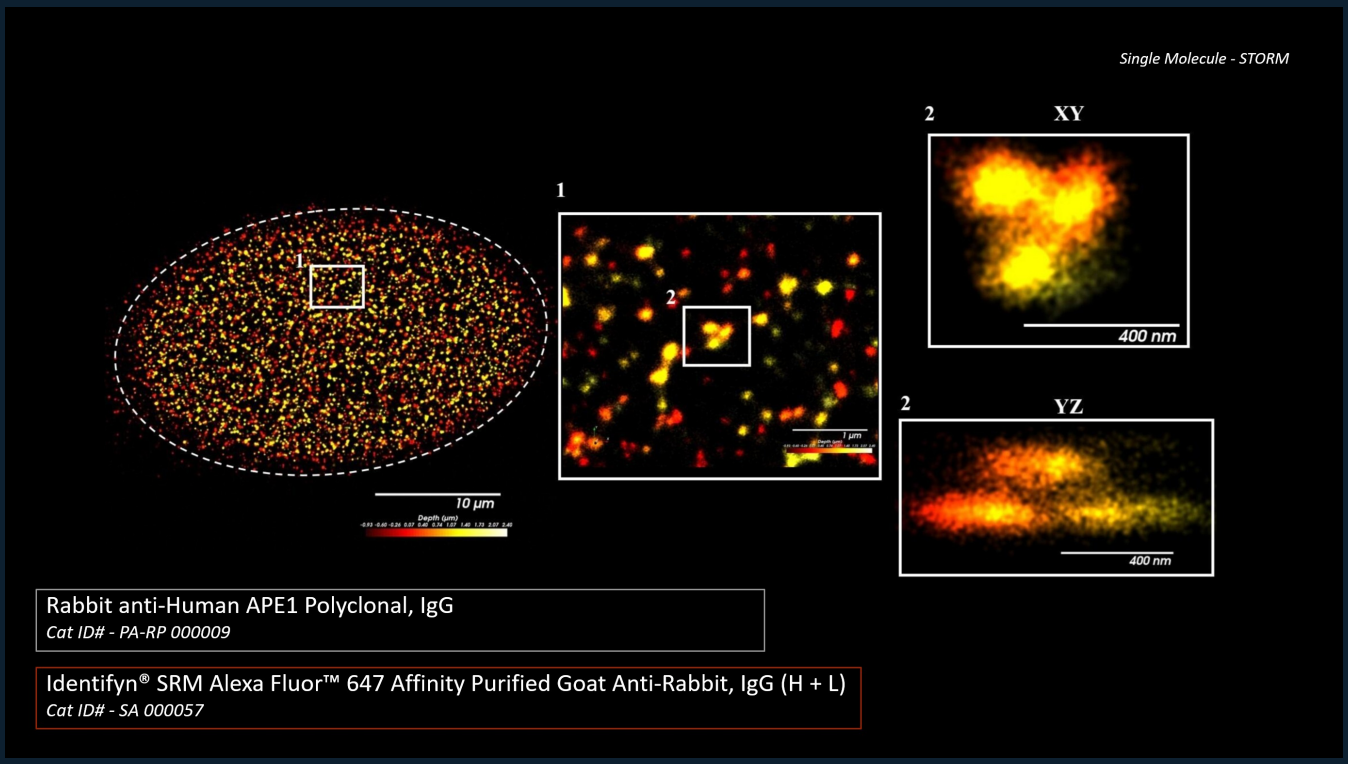
APE1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human APE1 Polyclonal

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-RP-000009 | 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 APE1, 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-RP-000009 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-RP-000009 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	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 647	2; 50 Cy 5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Widefield SIM — Apotome	405/DAPI; 555; 647	2; 50 Cy 5; AF532-49014; 625 - 655; 515 - 545; 665 - 715; 555 - 595; 653
Confocal	405/DAPI; 488; 555; 647	3; None; 640 nm @ 0.80%; 561 nm @ 0.20%; 405 nm @ 1.50%; 653; 553; 353
Airyscan	405/DAPI; 488; 555; 647	2; None; 640 nm @ 1.00%; 561 nm @ 2.40%; 653; 553; 668; 568
SIM	405/DAPI; 488; 555; 647	2; 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; 640; 561
SIM²	405/DAPI; 488; 555; 647	2; 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; 640; 561
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	2; 1; None; 640 nm @ 0.80%; 653; 668

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 Imaging. 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 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

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, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 1021 X 881; Image Size (Pixels): 1021 X 881; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705; Axiocam 807 Mono. Timing: Exposure Time: 200 ms; 30 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 20.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 32.

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; 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

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 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 77 Slices (7.6 µm); 81 Slices (8.0 µm); Channels: 2; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 946 X 782; 218 X 276; Image Size (Pixels): 946 X 782; 218 X 276; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 200 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 20.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 54.

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; 555; 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 900. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 141 Slices (6.0 µm); Channels: 3; Scaling (Per Pixel): 0.071 µm X 0.071 µm X 0.030 µm; Image size (Pixels): 397 X 457; Image Size (Pixels): 397 X 457; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt2; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.59 µs; Frame Time: 2.22 s. Illumination: Laser Wavelength: 640 nm @ 0.80%; 561 nm @ 0.20%. Optical/scan: Pinhole: 1.00 AU / 56 µm; 1.00 AU / 49 µm; Scan Zoom: 1.6; LSM Scan Speed: 9; 11; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Structured source metadata values: 50.

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 900, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 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

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: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 900 Confocal, 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: 171 Slices (6.0 µm); Channels: 2; Scaling (Per Pixel): 0.035 µm X 0.035 µm X 0.030 µm; Image size (Pixels): 722 X 943; 228 X 241; Image Size (Pixels): 722 X 943; 228 X 241; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.62 µs; Frame Time: 8.36 s. Illumination: Laser Wavelength: 640 nm @ 1.00%; 561 nm @ 2.40%. Optical/scan: Pinhole: 5.00 AU / 280 µm; 5.00 AU / 243 µm; Scan Zoom: 1.7; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution 8.9 (3D, Auto); SuperResolution 8.8 (3D, Auto). Structured source metadata values: 49.

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; 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

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 and 1.25X Tubelens. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 147 Slices (4.38 µm); Channels: 2; Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm; Image size (Pixels): 1550 X 1220; 251 X 245; Image Size (Pixels): 1550 X 1220; 251 X 245; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 120 ms; 150 ms; Frame Time: 1.33 s. Illumination: Laser Wavelength: 640 nm @ 5.0%; 561 nm @ 4.0%; Illumination Wavelength: 640; 555. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; 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: 52.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 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 and 1.25X Tubelens. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 147 Slices (4.38 µm); Channels: 2; Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm; Image size (Pixels): 1583 X 1446; 207 X 161; Image Size (Pixels): 1583 X 1446; 207 X 161; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 120 ms; 150 ms; Frame Time: 1.33 s. Illumination: Laser Wavelength: 640 nm @ 5.0%; 561 nm @ 4.0%; Illumination Wavelength: 640; 555. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 52.

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: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 73.

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 LSM 900. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 141 Slices (4.2 µm); 181 Slices (5.4 µm); Channels: 2; 1; Scaling (Per Pixel): 0.071 µm X 0.071 µm X 0.030 µm; Image size (Pixels): 898 X 898; 501 X 524; Image Size (Pixels): 898 X 898; 501 X 524; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt2; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.59 µs; 0.55 µs; Frame Time: 2.22 s; 3.26 s. Illumination: Laser Wavelength: 640 nm @ 0.80%. Optical/scan: Pinhole: 1.00 AU / 56 µm; Scan Zoom: 1.6; 1.8; LSM Scan Speed: 9; Scan Direction: Bidirectional; Averaging: 4; 8; Effective NA: 1.4. Structured source metadata values: 52.

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 LSM 900, 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-RP-000009 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

SIM / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02112 µm X 0.02110 µm X 0.02980 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)=251 X 245; Image Size (Scaled)=5.30 µm X 5.17 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02111 µm X 0.02111 µm X 0.02980 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)=1583 X 1446; Image Size (Scaled)=33.42 µm X 30.53 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Single-molecule STORM": ["48 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

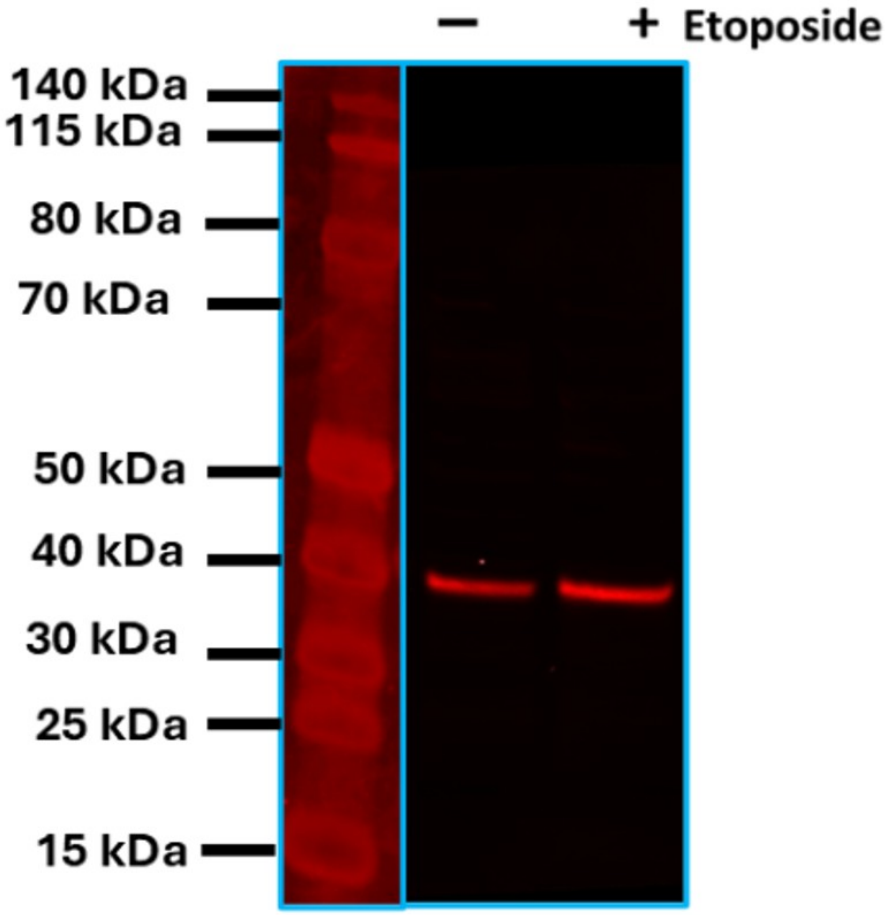
This dossier preserves the complete available evidence progression for PA-RP-000009. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 900	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	HeLa
DETECTED DYES	647
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 Imaging
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L3
Pixel size	100 µm
Scan Speed	Highest
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	S. Khanal

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Expected MW: 37 kDa

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear lysis buffer, then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis on NuPAGE™ 4-12% Bis-Tris, 1.0-1.5 mm Mini Protein Gels in a Mini Gel Tank. Thermo Fisher™ PageRuler™ Prestained Protein Ladder, 10-180 kDa, was used as a molecular weight marker.

Following electrophoresis, 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, then washed 5 times with 1X PBS. The blocked membrane was incubated with Rabbit anti-Human APE1 Polyclonal, IgG for 20 hours, followed by 5 washes with 1X PBS. Identifyn™ SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5 times with 1X PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L3
Pixel size = 100 µm
Scan Speed = Highest
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

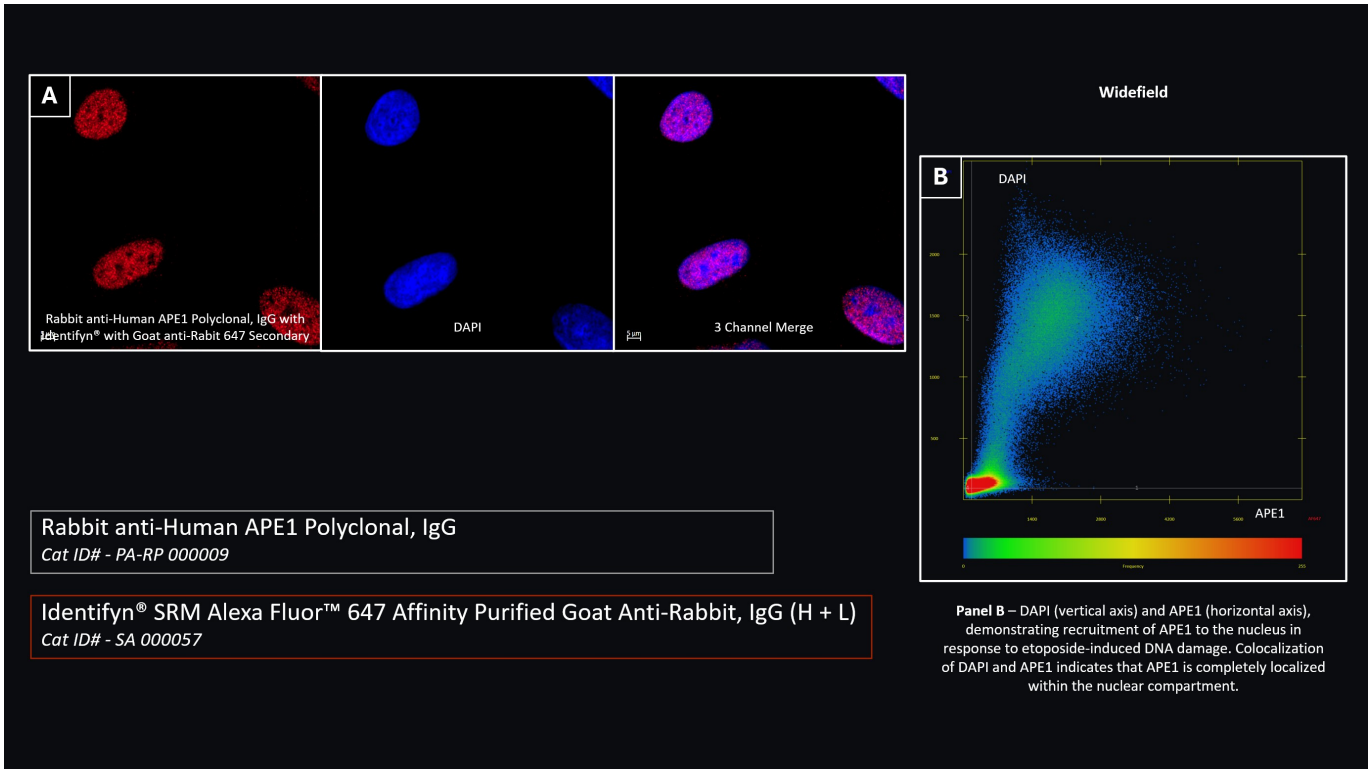
NONE

Image: S. Khanal

SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AF2EAFC1E8B878CAE66483A23DD9E8ECF89CA4DA191FEB209BEB7B4D09FC9DC0
Method PDF SHA-256	403288F4086247EC4A9BD74BB1AF87BC3B36C8A781BFBFE8023C5F2D31B58FA2

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image Size (Pixels)	1021 X 881
Image Size (Scaled)	111.82 µm X 96.49 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 49 DAPI
Beam Splitter	660 | 395
Filter Excitation Wavelength	625 - 655 | 335 - 383
Filter Emission Wavelength	665 - 715 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 20.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	200 ms | 30 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705 | Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.72 µm
Binning Mode	2,2
Image Measurement	Entire Field

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat#P36962).

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 1021 X 881
Image Size (Scaled) = 111.82 µm X 96.49 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 30 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L) secondary antibody. The middle panel shows DAPI staining, and the right panel shows the two-channel merged image.

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 183, Range Auto
Vertical Axis - DAPI, Threshold 94, Range Auto
Image Measurement = Entire Field

Demonstrates recruitment of APE1 to the nucleus in response to etoposide-induced DNA damage. Colocalization of DAPI and APE1 indicates that APE1 is localized within the nuclear compartment.

Summary

The left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), demonstrating a strong nuclear-associated fluorescence signal following etoposide-induced DNA damage. The middle panel shows DAPI nuclear counterstaining, while the right panel presents the merged image demonstrating predominant nuclear localization of APE1 with minimal background signal under widefield fluorescence microscopy conditions.

APE1 is a critical component of the base excision repair (BER) pathway and is responsible for processing apurinic/apyrimidinic (AP) sites generated during DNA damage repair. Following etoposide treatment, increased nuclear localization of APE1 is consistent with activation of DNA repair signaling and recruitment of BER-associated repair machinery.

The dataset demonstrates strong compatibility between Rabbit anti-Human APE1 Polyclonal, IgG and Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), supporting robust fluorescence signal generation, low-background staining characteristics, and preservation of biologically consistent nuclear localization patterns under standard widefield fluorescence microscopy acquisition conditions.

Image Correction

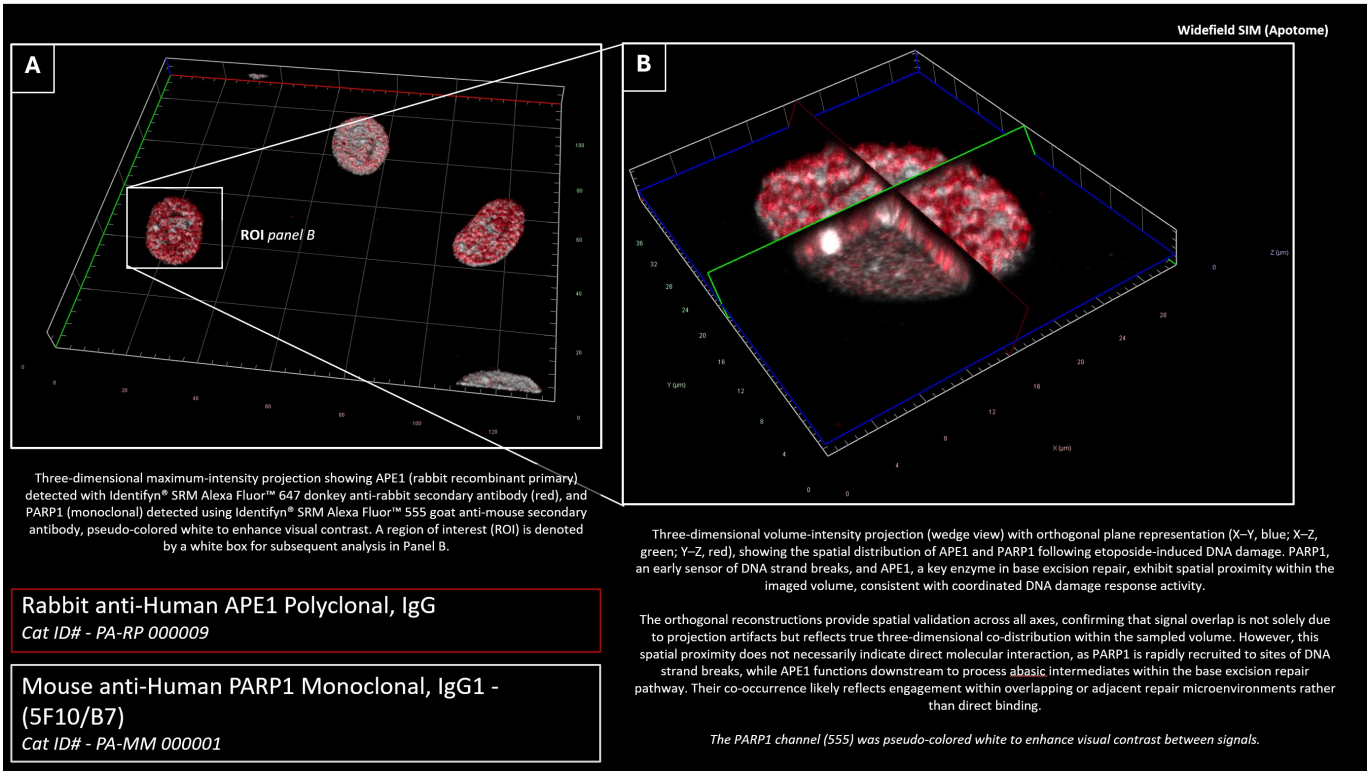
NONE

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

SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	32
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	97356A3D86C5811BA347BACF305DCBC9BFB41E29EFC94BBF9CCF427A2FF40E78
Method PDF SHA-256	9ECCF870A6F79AFEAD2C2B50FCCAE637F3FB53541780FCC494D4D5EA0F32DEAC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Three-dimensional maximum-intensity projection showing APE1 (rabbit recombinant primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) detected using Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudo-colored white to enhance visual contrast. A region of interest (ROI) is denoted by a white box for subsequent analysis in Panel B.

Rabbit anti-Human APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation (X–Y, blue; X–Z, green; Y–Z, red), showing the spatial distribution of APE1 and PARP1 following etoposide-induced DNA damage. PARP1, an early sensor of DNA strand breaks, and APE1, a key enzyme in base excision repair, exhibit spatial proximity within the imaged volume, consistent with coordinated DNA damage response activity.

The orthogonal reconstructions provide spatial validation across all axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this spatial proximity does not necessarily indicate direct molecular interaction, as PARP1 is rapidly recruited to sites of DNA strand breaks, while APE1 functions downstream to process abasic intermediates within the base excision repair pathway. Their co-occurrence likely reflects engagement within overlapping or adjacent repair microenvironments rather than direct binding.

The PARP1 channel (555) was pseudo-colored white to enhance visual contrast between signals.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 647
METADATA VALUES	54

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 Mono, 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	77 Slices (7.6 µm) | 81 Slices (8.0 µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	946 X 782 | 218 X 276
Image Size (Scaled)	135.14 µm X 111.71 µm | 31.14 µm X 39.43 µm
Bit Depth	14 Bit
Image Center Position	X: 58.78 mm, Y: 46.16 mm
Stage Position	X: 58.78 mm, Y: 46.16 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | AF532-49014
Beam Splitter	660 | 550
Filter Excitation Wavelength	625 - 655 | 515 - 545
Filter Emission Wavelength	665 - 715 | 555 - 595
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 20.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	200 ms | 100 ms
Excitation Wavelength	653 | 493
Emission Wavelength	668 | 517
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.10 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 90%, 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)

FIELD	SOURCE-DOCUMENTED VALUE
3D Volume Projection	Precise
X-Y	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-39% | -3% | 5%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	-30° | 30°
Y-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. 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 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 77 Slices (7.6 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 946 X 782
Image Size (Scaled) = 135.14 µm X 111.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 58.78 mm, Y: 46.16 mm
Stage Position = X: 58.78 mm, Y: 46.16 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI

Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 218 X 276
Image Size (Scaled) = 31.14 µm X 39.43 µm
Bit Depth = 14 Bit
Image Center Position = X: 58.78 mm, Y: 46.16 mm
Stage Position = X: 58.78 mm, Y: 46.16 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

555 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 100 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.10 µm
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

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 13% all channels, Ramp 50% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 90%, 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 - Panel X

Clipping planes are introduced in Panel B to highlight the APE1 Polyclonal and the PARP1 Mouse Monoclonal within the nuclear compartment by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -39%
Clip X = 0°
Clip Z = 0°

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -3%
Clip Y = -30°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 5%
Clip Y = 30°
Clip Z = 0°

Summary

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation (X-Y, blue; X-Z, green; Y-Z, violet), showing the spatial distribution of APE1 and PARP1 following etoposide-induced DNA damage. PARP1, an early sensor of DNA strand breaks, and APE1, a key enzyme in base excision repair, exhibit spatial proximity within the imaged volume, consistent with coordinated DNA damage response activity.

The orthogonal reconstructions provide spatial validation across all axes, confirming that signal overlap is not solely due to projection artifacts but reflects true three-dimensional co-distribution within the sampled volume. However, this

spatial proximity does not necessarily indicate direct molecular interaction, as PARP1 is rapidly recruited to sites of DNA strand breaks. At the same time, APE1 functions downstream to process abasic intermediates within the base excision repair pathway. Their co-occurrence likely reflects engagement within overlapping or adjacent repair microenvironments rather than direct binding.

Image Correction

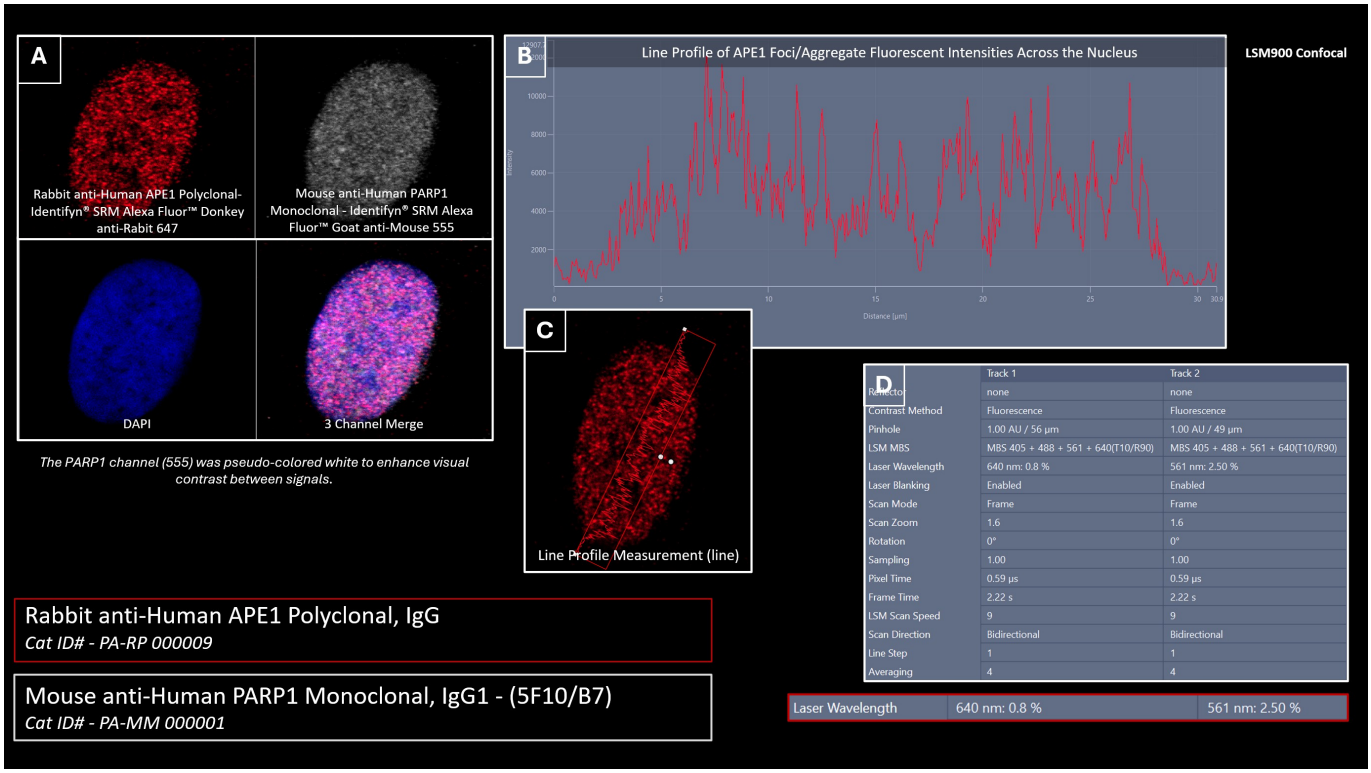
The PARP1 channel (555) was pseudocolored white to enhance structural contrast and improve visualization within the orthogonal projection workflow.

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

SOURCE PROVENANCE

Catalog	PA-RP-000009
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 Mono, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7671B151929CD3BB8C58F92609ABCAA7E32E0DC76582E278A775A7565DCDAE2D
Method PDF SHA-256	51B08A0D6D34428C0D9B7669D1858E47BA4FFD4E10E70BA26CB8919F44DE919A

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	50

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 900 Confocal, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	141 Slices (6.0 µm)
Channels	3
Scaling (Per Pixel)	0.071 µm X 0.071 µm X 0.030 µm
Image Size (Pixels)	397 X 457
Image Size (Scaled)	28.02 µm X 32.26 µm
Bit Depth	16 Bit
Image Center Position	X: 86.46 mm, Y: 46.80 mm
Stage Position	X: 86.64 mm, Y: 46.80 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 56 µm | 1.00 AU / 49 µm | 1.00 AU / 37 µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640 nm @ 0.80% | 561 nm @ 0.20% | 405 nm @ 1.50%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.00
Pixel Time	0.59 µs
Frame Time	2.22 s
LSM Scan Speed	9 | 11
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 553 | 353
Emission Wavelength	668 | 568 | 465
Effective NA	1.4
Detection Wavelength	630 - 700 | 557 - 603 | 410 - 470
Imaging Device	GaAsP-Pmt2 | Airyscan | GaAsP-Pmt1
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	600 V | 650 V
Detector Offset	0
Detector Digital Gain	1.0
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 30.9), Y (Min 0 and Max 12908)

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in methanol at -20 °C and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. 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 a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 69 of 141, Panels A, B, and C

Z-Stack = 141 Slices (6.0 µm)

Channels = 3
Scaling (Per Pixel) = 0.071 µm X 0.071 µm X 0.030 µm
Image Size (Pixels) = 397 X 457
Image Size (Scaled) = 28.02 µm X 32.26 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.46 mm, Y: 46.80 mm
Stage Position = X: 86.64 mm, Y: 46.80 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640 nm @ 0.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 49 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561 nm @ 0.20%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 557 - 603
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 37 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405 nm @ 1.50%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410 - 470
Imaging Device = GaAsP-Pmt1
Detector Type = GaAsP-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

Split View - Panel A

The top-left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 30.9), Y (Min 0 and Max 12908)

This panel quantifies APE1 foci, representing diffraction-limited signal aggregates, following etoposide-induced DNA damage. Line profile analysis reveals well-defined intensity peaks and spatial confinement, supporting localized enrichment of APE1 at damage-associated regions.

Maximum Peak - 12,293.00 A.U.

Zen Acquisition Info Tab - Panel D

Shown are the acquisition parameters for the 647 channel (APE1) and 555 channel (PARP1), collected using the 640 nm laser at 0.80% power and the 561 nm laser at 0.20% power, respectively. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Summary

The top-left panel shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L), demonstrating discrete nuclear-associated APE1 fluorescence following etoposide-induced DNA damage. The top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific, highlighting PARP1 distribution within the nuclear compartment. The lower-left panel shows DAPI nuclear counterstaining, while the bottom-right panel presents the three-channel merged image demonstrating overlapping nuclear-associated localization patterns between APE1 and PARP1 under confocal fluorescence microscopy conditions.

APE1 is a critical component of the base excision repair (BER) pathway and functions downstream of DNA damage recognition to process abasic intermediates generated during DNA repair. PARP1 serves as an early sensor of DNA strand breaks and is rapidly recruited to sites of genomic damage following etoposide treatment. The observed spatial proximity between APE1 and PARP1 is consistent with coordinated engagement of DNA damage response and BER-associated repair pathways within the nuclear compartment.

Line-profile analysis demonstrates discrete APE1 fluorescence intensity peaks and localized signal confinement, supporting the formation of resolution-limited repair-associated foci following DNA damage induction. The dataset further demonstrates strong compatibility between Rabbit anti-Human APE1 Polyclonal, IgG and Identifyn® SRM Alexa Fluor™ secondary antibody systems under confocal acquisition conditions, supporting robust fluorescence signal generation, low-background imaging characteristics, and preservation of biologically consistent nuclear localization patterns.

Image Correction

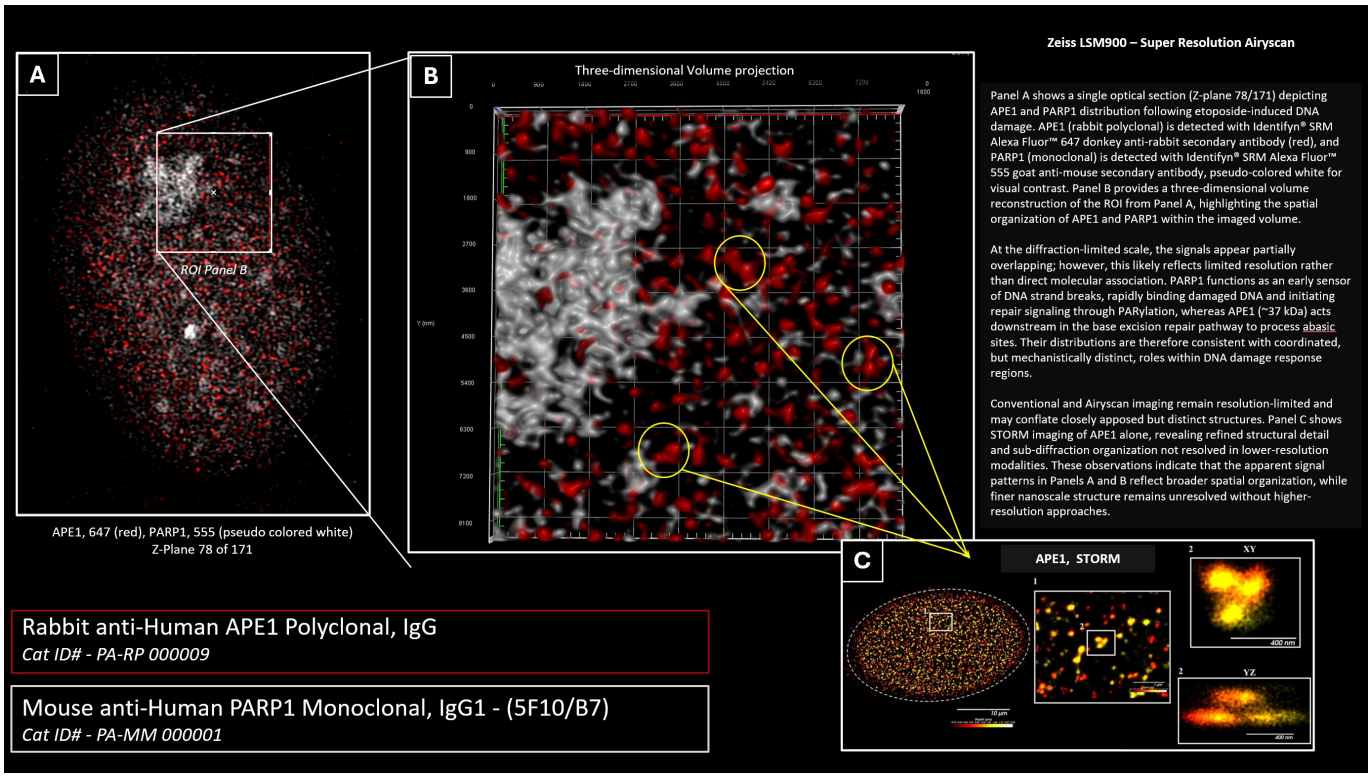
The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

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

SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, 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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	443BE0B20A899B700AD8C8C280AFDA543CB66CBBD8508F2B7AF678EA27F8E81A
Method PDF SHA-256	18A2BD2A9720C35DD1DE573B4773996454AD8F65025F4B7533728F7043F2B723

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	49

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 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	171 Slices (6.0 µm)
Channels	2
Scaling (Per Pixel)	0.035 µm X 0.035 µm X 0.030 µm
Image Size (Pixels)	722 X 943 | 228 X 241
Image Size (Scaled)	25.49 µm X 33.29 µm | 8.05 µm X 8.51 µm
Bit Depth	16 Bit
Image Center Position	X: 91.65 mm, Y: 49.86 mm
Stage Position	X: 91.65 mm, Y: 49.86 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 280 µm | 5.00 AU / 243 µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640 nm @ 1.00% | 561 nm @ 2.40%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	2.00
Pixel Time	0.62 µs
Frame Time	8.36 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 553
Emission Wavelength	668 | 568
Effective NA	1.4
Detection Wavelength	640 - 700 | 560 - 600
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	SuperResolution 8.9 (3D, Auto) | SuperResolution 8.8 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in methanol at -20 °C and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. 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 a Plan-Apochromat 63x/1.40 Oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 78 of 171, Panel A

Z-Stack = 171 Slices (6.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm
Image Size (Pixels) = 722 X 943
Image Size (Scaled) = 25.49 µm X 33.29 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.65 mm, Y: 49.86 mm
Stage Position = X: 91.65 mm, Y: 49.86 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 171 Slices (6.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.035 µm X 0.035 µm X 0.030 µm
Image Size (Pixels) = 228 X 241
Image Size (Scaled) = 8.05 µm X 8.51 µm
Bit Depth = 16 Bit
Image Center Position = X: 91.65 mm, Y: 49.86 mm
Stage Position = X: 91.65 mm, Y: 49.86 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 280 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640 nm @ 1.00%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.62 µs
Frame Time = 8.36 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 640 - 700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.9 (3D, Auto)

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 243 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561 nm @ 2.40%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.62 µs
Frame Time = 8.36 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 560 - 600
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.8 (3D, Auto)

2-Dimensional View- Panel A

Shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product data set to demonstrate the resolution limitations of Airyscan while also demonstrating improved localization and structural detail relative to conventional confocal imaging.

Summary

Panel A shows a single optical section (Z-plane 78/171) depicting APE1 and PARP1 distribution following etoposide-induced DNA damage. APE1 (rabbit polyclonal) is detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) is detected with Identifyn® SRM Alexa Fluor™ 555 goat anti-mouse secondary antibody, pseudocolored white for visual contrast. Panel B provides a three-dimensional volume reconstruction of the ROI from Panel A, highlighting the spatial organization of APE1 and PARP1 within the imaged volume.

At the diffraction-limited scale, the signals appear partially overlapping; however, this likely reflects limited resolution rather than direct molecular association. PARP1 functions as an early sensor of DNA strand breaks, rapidly binding damaged DNA and initiating repair signaling through PARylation, whereas APE1 (~37 kDa) acts downstream in the base excision repair pathway to process abasic sites. Their distributions are therefore consistent with coordinated, but mechanistically distinct, roles within DNA damage response regions.

Conventional and Airyscan imaging remain resolution-limited and may conflate closely apposed but distinct structures. Panel C shows STORM imaging of APE1 alone, revealing refined structural detail and sub-diffraction organization not resolved in lower-resolution modalities. These observations indicate that the apparent signal patterns in Panels A and B reflect broader spatial organization, while finer nanoscale structure remains unresolved without higher-resolution approaches.

Image Correction

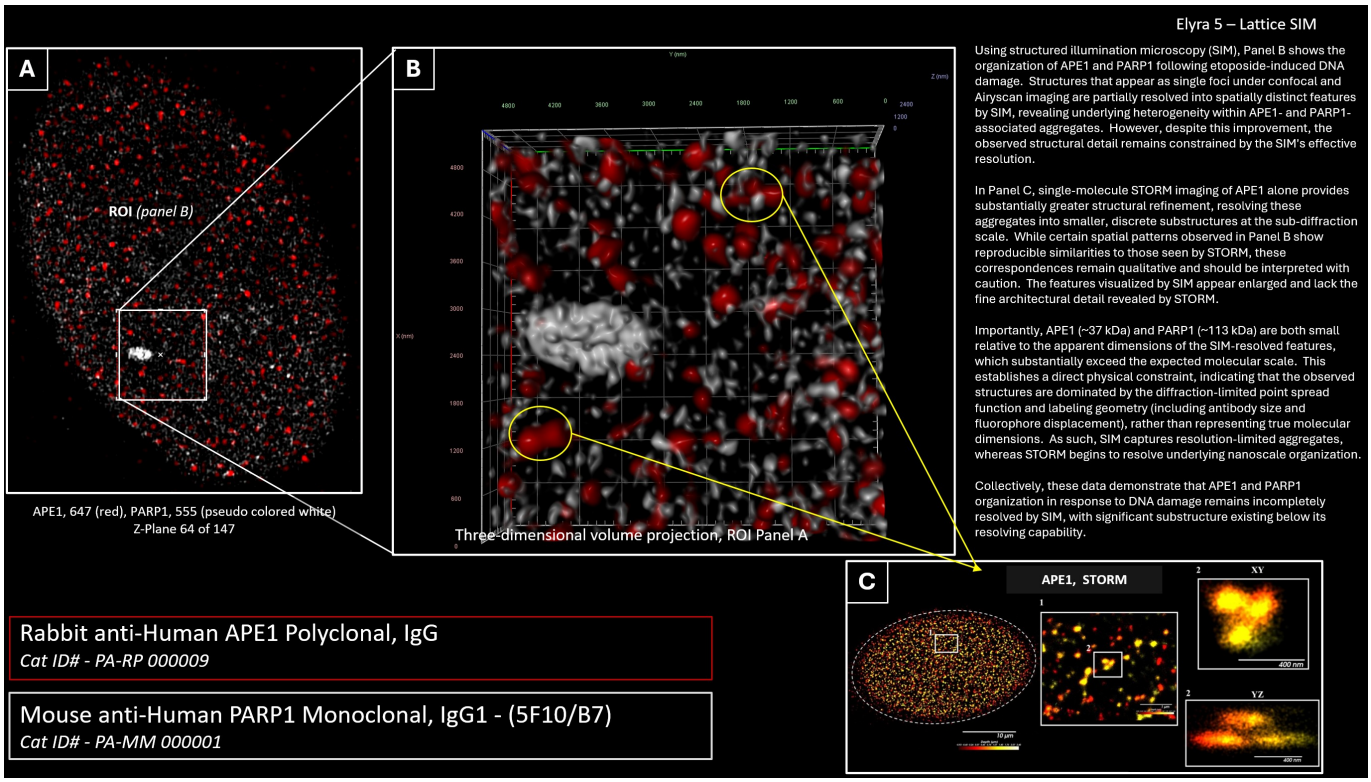
The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

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

SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, 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	49
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7D04B3A9224C2BD94226B4AEFE36956D28C6B3C8A4506E41B34387DBFE1497FE
Method PDF SHA-256	A64830B69A5E8861CBAD029FEA9A0BE9A0543B90F7C6C9300206B7A1079EA76D

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	52

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 a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens
Z-Stack	147 Slices (4.38 µm)
Channels	2
Scaling (Per Pixel)	0.02112 µm X 0.02110 µm X 0.02980 µm
Image Size (Pixels)	1550 X 1220 | 251 X 245
Image Size (Scaled)	35.72 µm X 25.76 µm | 5.30 µm X 5.17 µm
Bit Depth	16 Bit
Image Center Position	X: 63.80 mm, Y: 37.58 mm
Stage Position	X: 63.80 mm, Y: 37.58 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 555
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR
Excitation Wavelength	640 | 561
Emission Wavelength	729 | 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820
Camera Adapter	1X
Exposure Time	120 ms | 150 ms
Depth of Focus	1.13 µm | 0.92 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 5.0% | 561 nm @ 4.0%
Frame Time	1.33 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.9.77 (647), 11.2499 (555)
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates DNA repair, restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. 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 a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 64 of 147, Panel A

Z-Stack = 147 Slices (4.38 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image Size (Pixels) = 1550 X 1220
Image Size (Scaled) = 35.72 µm X 25.76 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.80 mm, Y: 37.58 mm
Stage Position = X: 63.80 mm, Y: 37.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume Projection, Panel B

Z-Stack = 147 Slices (4.38 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image Size (Pixels) = 251 X 245
Image Size (Scaled) = 5.30 µm X 5.17 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.80 mm, Y: 37.58 mm
Stage Position = X: 63.80 mm, Y: 37.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 5.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

555 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 555
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 4.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.9.77 (647), 11.2499 (555)
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View- Panel A

Shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

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

STORM - Panel C

Shown from the same product dataset to demonstrate the resolution limitations of Lattice SIM while also highlighting its improved localization and structural detail relative to Airyscan imaging. The observed signal distribution is consistent with the corresponding Airyscan dataset; however, the structures are more resolution-limited and less spatially refined than those observed with single-molecule localization microscopy by STORM.

Summary

Using structured illumination microscopy (SIM), Panel B shows the organization of APE1 and PARP1 following etoposide-induced DNA damage. Structures that appear as single foci under confocal and Airyscan imaging are partially resolved into spatially distinct features by SIM, revealing underlying heterogeneity within APE1- and PARP1-associated aggregates. However, despite this improvement, the observed structural detail remains constrained by the SIM's effective resolution.

In Panel C, single-molecule STORM imaging of APE1 alone provides substantially greater structural refinement, resolving these aggregates into smaller, discrete substructures at the sub-diffraction scale. While certain spatial patterns observed in Panel B show reproducible similarities to those seen by STORM, these correspondences remain qualitative and should be interpreted with caution. The features visualized by SIM appear enlarged and lack the fine architectural detail revealed by STORM.

Importantly, APE1 (~37 kDa) and PARP1 (~113 kDa) are both small relative to the apparent dimensions of the SIM-resolved features, which substantially exceed the expected molecular scale. This establishes a direct physical constraint, indicating that the observed structures are dominated by the diffraction-limited point spread function and labeling geometry (including antibody size and fluorophore displacement), rather than representing true molecular dimensions. As such, SIM captures resolution-limited aggregates, whereas STORM begins to resolve underlying nanoscale organization.

Collectively, these data demonstrate that the organization of APE1 and PARP1 in response to DNA damage remains incompletely resolved by SIM, with significant substructure below its resolving capability.

Image Correction

The PARP1 channel (555) was pseudocolored white to enhance visual contrast between signals.

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

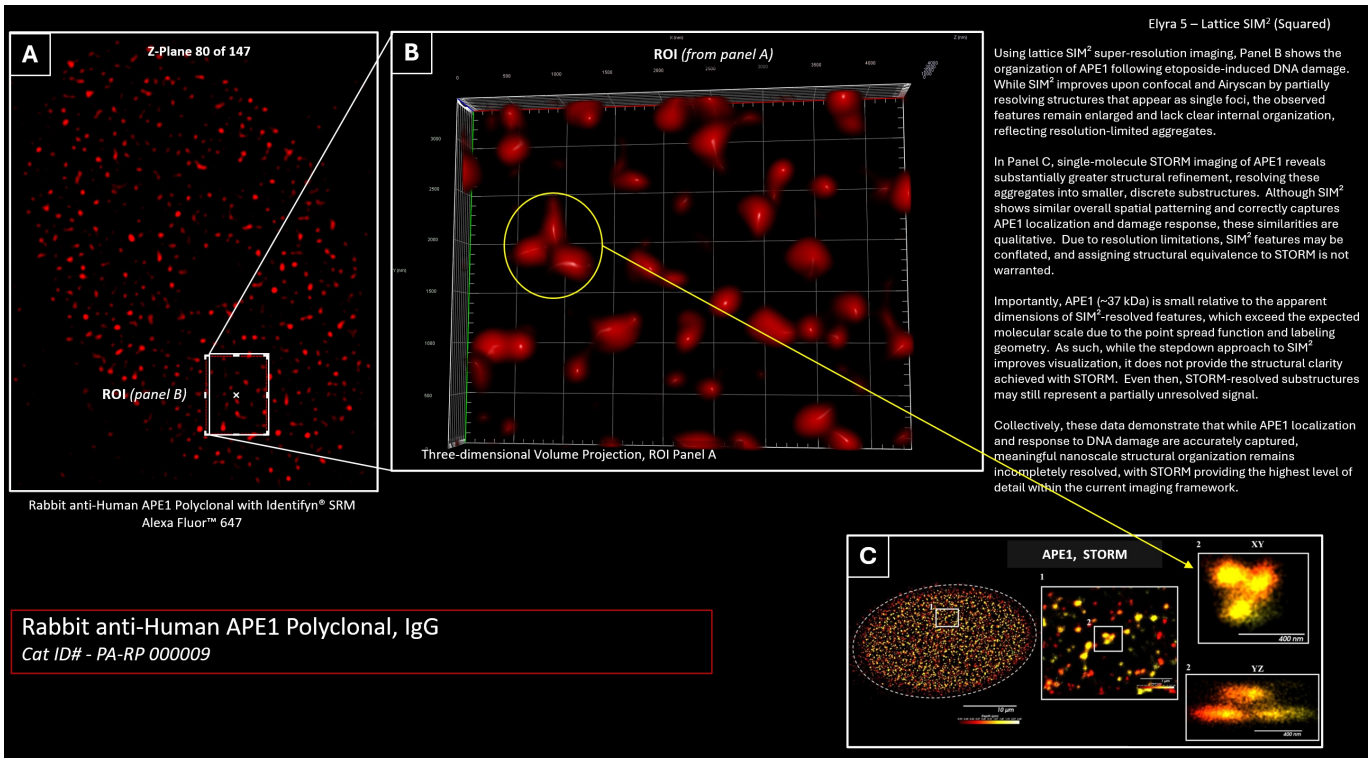
SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, 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	1E0697658E919605F1D90BEA3A08CD9D846DB4986994733E147FF691FABE722F
Method PDF SHA-256	DBB9F7C36C63036E62119185A59242044981174F6F4EE1D34D0BF4A4271C941D

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	52

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 a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens
Z-Stack	147 Slices (4.38 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.02980 µm
Image Size (Pixels)	1583 X 1446 | 207 X 161
Image Size (Scaled)	33.42 µm X 30.53 µm | 4.37 µm X 3.40 µm
Bit Depth	16 Bit
Image Center Position	X: 63.80 mm, Y: 37.58 mm
Stage Position	X: 63.80 mm, Y: 37.58 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 555
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR
Excitation Wavelength	640 | 561
Emission Wavelength	729 | 597
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820
Camera Adapter	1X
Exposure Time	120 ms | 150 ms
Depth of Focus	1.13 µm | 0.92 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 5.0% | 561 nm @ 4.0%
Frame Time	1.33 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000034

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates DNA repair, restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20 °C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific was incubated for 1 hour at room temperature at a dilution of 1:100. 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 a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 80 of 147, Panel A

Z-Stack = 147 Slices (4.38 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image Size (Pixels) = 1583 X 1446
Image Size (Scaled) = 33.42 µm X 30.53 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.80 mm, Y: 37.58 mm
Stage Position = X: 63.80 mm, Y: 37.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3D Volume Projection, Panel B

Z-Stack = 147 Slices (4.38 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image Size (Pixels) = 207 X 161
Image Size (Scaled) = 4.37 µm X 3.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 63.80 mm, Y: 37.58 mm
Stage Position = X: 63.80 mm, Y: 37.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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 = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 5.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

555 - Collected but not Shown in the Data Analysis

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 = 555
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 561
Emission Wavelength = 597
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 0.92 µm
Binning Mode = 2,2
Laser Wavelength = 561 nm @ 4.0%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View- Panel A

Shows Rabbit anti-Human APE1 Polyclonal, IgG, visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

3D Image Processing - Panel B

3D Volume Projection = Precise
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Shown from the same product dataset to demonstrate the resolution limitations of Lattice SIM² while also highlighting its improved localization and structural detail relative to conventional Lattice SIM imaging. The observed signal distribution remains broadly consistent with the corresponding STORM dataset; however, direct structural equivalence should be interpreted with caution, as SIM² remains partially resolution-limited compared with single-molecule localization microscopy.

Summary

Using lattice SIM² super-resolution imaging, Panel B shows the organization of APE1 following etoposide-induced DNA damage. While SIM² improves upon confocal and Airyscan by partially resolving structures that appear as single foci, the observed features remain enlarged and lack clear internal organization, reflecting resolution-limited aggregates.

In Panel C, single-molecule STORM imaging of APE1 reveals substantially greater structural refinement, resolving these aggregates into smaller, discrete substructures. Although SIM² shows similar overall spatial patterning and correctly captures APE1 localization and damage response, these similarities are qualitative. Due to resolution limitations, SIM² features may be conflated, and assigning structural equivalence to STORM is not warranted.

Importantly, APE1 (~37 kDa) is small relative to the apparent dimensions of SIM²-resolved features, which exceed the expected molecular scale due to the point spread function and labeling geometry. As such, while the stepdown approach to SIM² improves visualization, it does not provide the structural clarity achieved with STORM. Even then, STORM-resolved substructures may still represent a partially unresolved signal.

Collectively, these data demonstrate that while APE1 localization and response to DNA damage are accurately captured, meaningful nanoscale structural organization remains incompletely resolved, with STORM providing the highest level of detail within the current imaging framework.

Image Correction

None

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

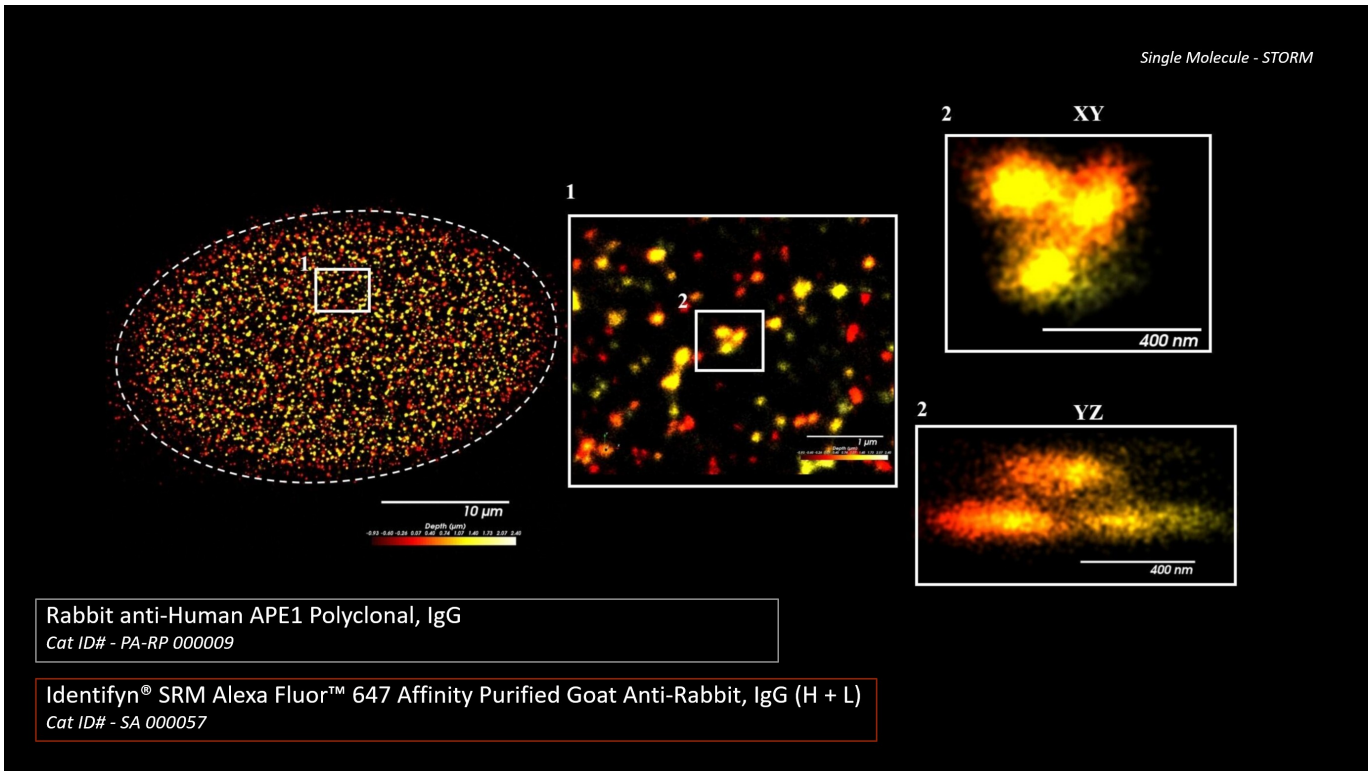
SOURCE PROVENANCE

Catalog	PA-RP-000009
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27 and 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	76126ED3AC7FFDA629F9C1320A43BFAB5094B4DDB561FB3BC066DB237B955D80
Method PDF SHA-256	522A3ECDB503ECAD08FF54F7AE19F7ED8438A8DC16427AC043DB1994DB10A124

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	73

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	635 nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Coarse Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100 ms | 20 ms
Super Resolution Camera Exposure Time	20 ms
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	20 ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200 µm
Capture Mode	Live

FIELD	SOURCE-DOCUMENTED VALUE
Cell(s) or Cellular Structure Localization	The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused. | Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Current	182.6 µm
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50 µm is Selected
Piezo Record	Begin: 182.6; End: 184.2
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	8
Cycles	22
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	50
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
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	Constant
Particle size	25 nm
Color by	Depth
Color Table	Single

FIELD	SOURCE-DOCUMENTED VALUE
Opacity Mode	Depth; Opacity 1: 0.25
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	30
Axial precision	65

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance in repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on the function of APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can occur due to spontaneous base loss or through the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 48 hours, followed by -20 °C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human APE1 Polyclonal 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®'s 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:200. 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: 635 nm Dichroic
Objective: Silicon Selected
Stage Insert: Rectangular Selected
Coarse 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: 100 ms
Super Resolution Camera Exposure Time: 20 ms
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: 20 ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200 µm
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 the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 182.6 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 (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100 ms
Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640 nm" 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: 50 µm 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 focused.

Piezo Record: Begin: 182.6; End: 184.2

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: 20 ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 8
Cycles: 22
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 50

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16 px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1 px
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: Constant
Particle size: 25 nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.25
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 8 Intervals
Pixel Size: 50 nm
Smoothing: 8.00

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

Radial precision: 30
Axial precision: 65

The other parameters were not applied.

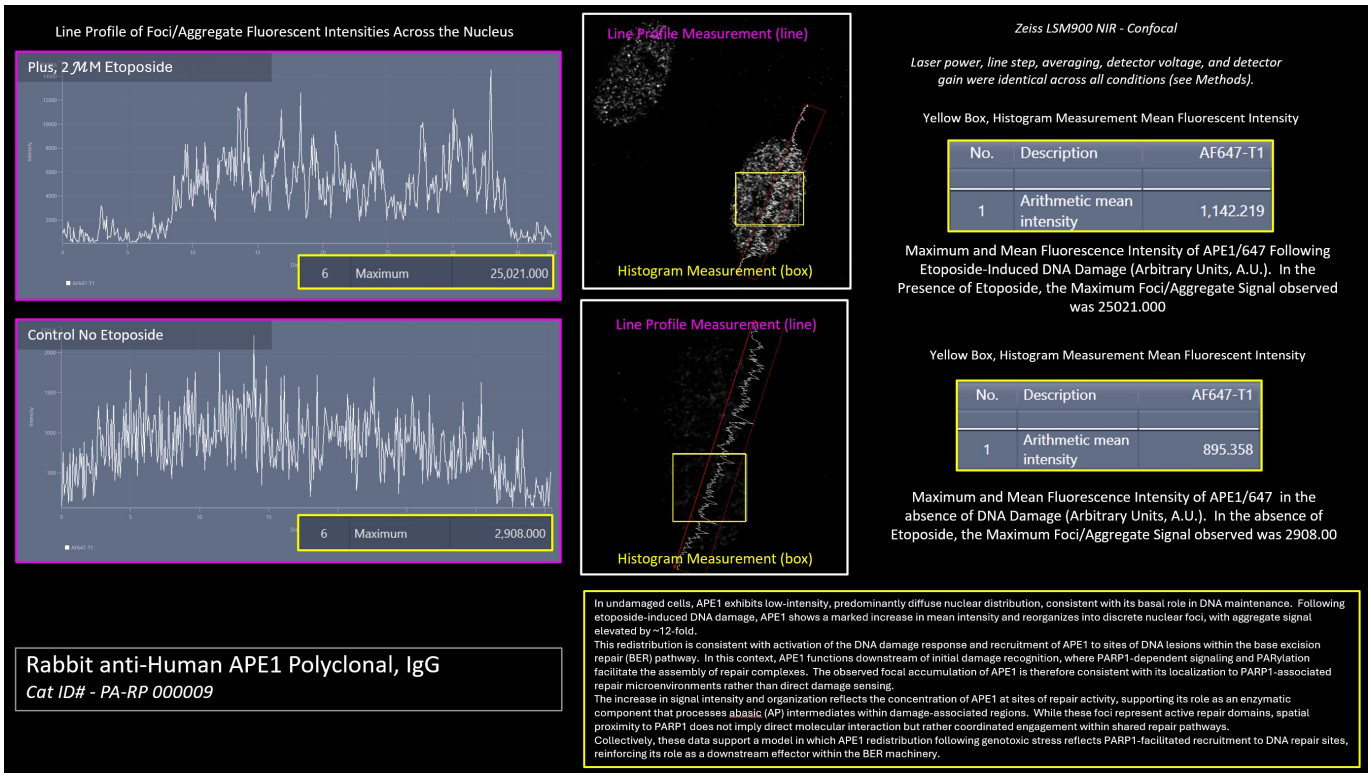
Statistical Analysis - Not Applied

Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RP-000009
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	73
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	3FAFAC1DF160BE44FE4BAD218AEA05EC5BCA7EE82354B9EDB4FF394649DBEE76
Method PDF SHA-256	BA1A448DC766D71DF34EEDFDA48150F8993458F72C1C937CB3DF2F25FFADB82E

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	52

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 LSM 900 Confocal, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	141 Slices (4.2 µm) | 181 Slices (5.4 µm)
Channels	2 | 1
Scaling (Per Pixel)	0.071 µm X 0.071 µm X 0.030 µm
Image Size (Pixels)	898 X 898 | 501 X 524
Image Size (Scaled)	63.38 µm X 63.38 µm | 35.37 µm X 37.00 µm
Bit Depth	16 Bit
Image Center Position	X: 86.46 mm, Y: 46.80 mm | X: 73.71 mm, Y: 50.38 mm
Stage Position	X: 86.64 mm, Y: 46.80 mm | X: 73.71 mm, Y: 50.38 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 56 µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640 nm @ 0.80%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6 | 1.8
Rotation	0°
Sampling	1.00
Pixel Time	0.59 µs | 0.55 µs
Frame Time	2.22 s | 3.26 s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Averaging	4 | 8
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	630 - 700
Imaging Device	GaAsP-Pmt2
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	600 V
Detector Offset	0
Detector Digital Gain	1.0
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 37.60), Y (Min 0.0 and Max 15259) | X and Y axes were set to Auto - X (Min 0.0 and Max 35.5), Y (Min 0.0 and Max 2322)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000000) | X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 200000)
Arithmetic Mean intensity	1142.219 | 895.358

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 APE1 Polyclonal, IgG
Cat ID# - PA-RP 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

APE1 (AP endonuclease 1), also known as APEX1 or Ref-1, is a pivotal enzyme in the base excision repair (BER) pathway. This pathway, which is of paramount importance for repairing single-strand breaks (SSBs) and other forms of DNA damage, relies heavily on APE1.

APE1 in Base Excision Repair (BER)

Base excision repair (BER) is a significant pathway for correcting small base lesions, such as oxidized or deaminated bases, which can result in single-strand breaks if not repaired properly. APE1 is an essential component of the BER pathway and is specifically involved in the following:

AP Site Recognition and Cleavage: APE1's primary function is to recognize and cleave abasic sites, which are locations in DNA where the base has been removed but the phosphate backbone remains intact. These abasic sites, also known as apurinic/apyrimidinic (AP) sites, can arise from spontaneous base loss or from the action of DNA glycosylases.

Endonuclease Activity: After recognizing an AP site, APE1 cleaves the DNA backbone, generating a single-strand break with a 3'-hydroxyl (3'-OH) end and a 5'-deoxyribose phosphate (5'-dRP) end. This cleavage creates a suitable entry point for further repair steps in the BER pathway.

APE1 and Single-Strand Break Repair

Once APE1 creates a single-strand break during base excision repair, the following steps occur to complete the repair process:

Gap Filling and Strand Sealing: DNA polymerase β (Pol β) extends the 3'-OH end by adding the appropriate nucleotide(s) to fill the gap. DNA ligase III, often in complex with XRCC1 (X-ray Repair Cross-Complementing Protein 1), seals the break by forming a phosphodiester bond at the 5'-dRP end.

Interaction with Other Proteins: APE1 interacts with multiple proteins involved in SSB and BER, including XRCC1 and DNA ligase III. These interactions help coordinate the repair process and ensure efficiency.

Additional Functions of APE1

Besides its crucial role in DNA repair, APE1 has other essential functions:

Redox Activity: APE1 acts as a redox factor, regulating the activity of various transcription factors by maintaining their redox state. This role is critical for controlling gene expression related to stress response, growth, and survival.

Therapeutic Implications: Given its central role in DNA repair, APE1 is a potential therapeutic target in cancer therapy. Modulating APE1 activity may influence DNA repair capacity and cellular response to DNA damage.

APE1's role in single-strand break repair is crucial within the base excision repair pathway. By recognizing and processing AP sites, APE1 initiates the repair process, successfully restoring DNA integrity. Its additional functions and therapeutic implications make it a protein of considerable interest in basic research and clinical applications.

Method

Plus Damage -

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 APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20° C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human APE1 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Microscope

Zeiss LSM 900 Confocal, 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) - 2D at Z-Plane 71 of 141, Panel A, from the Confocal Dataset - Plus Damage

Z-Stack = 141 Slices (4.2 μm)

Channels = 2
Scaling (Per Pixel) = 0.071 μm X 0.071 μm X 0.030 μm
Image Size (Pixels) = 898 X 898
Image Size (Scaled) = 63.38 μm X 63.38 μm
Bit Depth = 16 Bit
Image Center Position = X: 86.46 mm, Y: 46.80 mm
Stage Position = X: 86.64 mm, Y: 46.80 mm
ROI Center Offset = X: 0.00 μm, Y: 0.00 μm

Z Stack - (3D) - 2D at Z-Plane 80 of 181 - Control, Minus Damage

Z-Stack = 181 Slices (5.4 µm)

Channels = 1
Scaling (Per Pixel) = 0.071 µm X 0.071 µm X 0.030 µm
Image Size (Pixels) = 501 X 524
Image Size (Scaled) = 35.37 µm X 37.00 µm
Bit Depth = 16 Bit
Image Center Position = X: 73.71 mm, Y: 50.38 mm
Stage Position = X: 73.71 mm, Y: 50.38 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Plus Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640 nm @ 0.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.59 µs
Frame Time = 2.22 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0

647 - Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 56 µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640 nm @ 0.80%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55 µs
Frame Time = 3.26 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 630 - 700
Imaging Device = GaAsP-Pmt2
Detector Type = GaAsP-PMT
Detector Gain = 600 V
Detector Offset = 0
Detector Digital Gain = 1.0

Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 37.60), Y (Min 0.0 and Max 15259)

This panel measures APE1 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately 15K A.U. at its maximum.

Measured at the Z-plane, 71 of 141.

Profile View Display - Minus DNA Damage, Bottom

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 35.5), Y (Min 0.0 and Max 2322)

This panel measures APE1 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately 2K A.U. at its maximum.

Measured at the Z-plane, 80 of 181.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 5000000)

Arithmetic Mean intensity = 1142.219

The histogram was generated from the yellow-boxed region to quantify the overall APE1 response within a defined nuclear area following DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - Minus DNA Damage, Bottom

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0.0 and Max 200000)

Arithmetic Mean intensity = 895.358

The histogram was generated from the yellow-boxed region to quantify the overall APE1 protein within a defined nuclear area in the absence of DNA damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Control Conclusions -

In undamaged cells, APE1 exhibits low-intensity, predominantly diffuse nuclear distribution, consistent with its basal role in DNA maintenance. Following etoposide-induced DNA damage, APE1 shows a marked increase in mean intensity and reorganizes into discrete nuclear foci, with aggregate signal elevated by ~12-fold.

This redistribution is consistent with activation of the DNA damage response and recruitment of APE1 to sites of DNA lesions within the base excision repair (BER) pathway. In this context, APE1 functions downstream of initial damage recognition, where PARP1-dependent signaling and PARylation facilitate the assembly of repair complexes. The observed focal accumulation of APE1 is therefore consistent with its localization to PARP1-associated repair microenvironments rather than direct damage sensing.

The increase in signal intensity and organization reflects the concentration of APE1 at sites of repair activity, supporting its enzymatic role in processing abasic (AP) intermediates within damage-associated regions. While these foci represent active repair domains, spatial proximity to PARP1 does not imply direct molecular interaction but rather coordinated engagement within shared repair pathways.

Collectively, these data support a model in which APE1 redistribution following genotoxic stress reflects PARP1-facilitated recruitment to DNA repair sites, reinforcing its role as a downstream effector within the BER machinery.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RP-000009

SOURCE PROVENANCE

Stage	Control
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, 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	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6A35FD3EE9E3DB3FD68BE4A0414BD12543AD4A1D4EDE690A46E1B287D0BB296C
Method PDF SHA-256	40A86609424338CA9B2187EFEECFA3806844FC97EE7429AC5DB18958834CF03F