BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

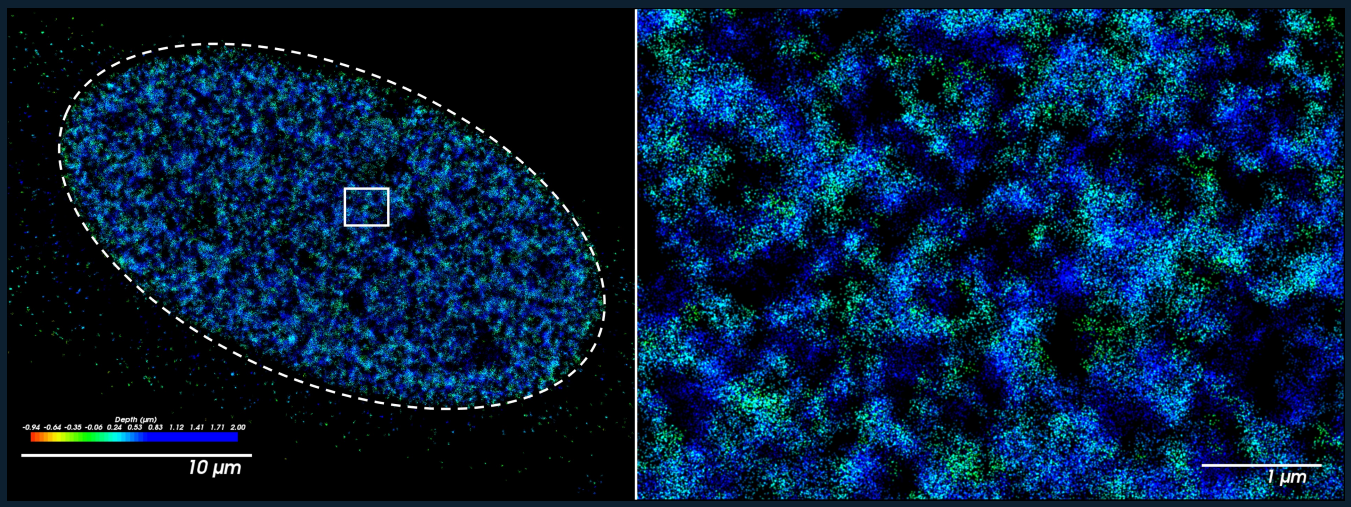
KU70

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human Ku70 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-000006 | 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 Ku70, 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-000006 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-000006 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 50 Cy 5; 32 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 50 Cy 5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	3; None; 639 nm @0.06%; 488 nm @0.04%; 405 nm @0.2%; 653; 493; 353
Airyscan	405/DAPI; 488; 647; 750	3; None; 639 nm @0.05%; 488 nm @1.00%; 405 nm @0.3%; 653; 493; 353
SIM	405/DAPI; 488; 555; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 647	2; 50 Cy 5; 49 DAPI; 625 - 655; 335-383; 665 - 715; 420-470; 653

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s). Structured source metadata values: 11.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 488.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 878 X 929; Image Size (Pixels): 878 X 929; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 100 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 31 Slices (3 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 978 X 971; 289 X 316; Image Size (Pixels): 978 X 971; 289 X 316; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 100 ms; 150 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%; X-Cite Xylis Lamp Intensity @16.00%. 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: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 201 Slices (6 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1066 X 1066; 1038 X 650; Image Size (Pixels): 1066 X 1066; 1038 X 650; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.32 µs; Frame Time: 3.52 s. Illumination: Laser Wavelength: 639 nm @0.06%; 488 nm @0.04%. Optical/scan: Pinhole: 1.00 AU / 65 µm; 1.00 AU / 50 µm; Scan Zoom: 2.1; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 6.9 (3D, Auto); 6.8 (3D, Auto). Structured source metadata values: 69.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 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 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 181 Slices (5.4 µm) - Panels A & B; 181 Slices (5.4 µm) - Panel C; Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 856 X 1191; 422 X 750; Image Size (Pixels): 856 X 1191; 422 X 750; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66 µs; Frame Time: 7.82 s. Illumination: Laser Wavelength: 639 nm @0.05%; 488 nm @1.00%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 250 µm; Scan Zoom: 2.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: 8.7 (3D, Auto); 7.7 (3D, Auto). Structured source metadata values: 71.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 160 Slices (4.77 µm); 78 Slices (2.31 µm); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm; Image size (Pixels): 3560 X 4889; 1825 X 2228; Image Size (Pixels): 3560 X 4889; 1825 X 2228; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. 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); View Angle 15°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 70.

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. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 160 Slices (4.77 µm); 65 Slices (1.92 µm); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm; Image size (Pixels): 3431 X 4860; 1583 X 2118; Image Size (Pixels): 3431 X 4860; 1583 X 2118; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 100 ms; 120 ms; Frame Time: 1.33 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @1.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 67.

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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 79.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 31 z-planes (3 µm) – Uses Z-plane 16 for Analysis.; Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm x 100µm; Image size (Pixels): 978 X 971; 707 X 580; Image Size (Pixels): 978 X 971; 707 X 580; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 100 ms; 12 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10.00%; X-Cite Xylis Lamp Intensity 6.00%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 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-000006 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=422 X 750; Image Size (Scaled)=14.89 µm X 26.47 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm -> 0.01320 µm X 0.01320 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3560 X 4889; Image Size (Scaled)=46.98 µm X 64.51 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm -> 0.01320 µm X 0.01320 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3431 X 4860; Image Size (Scaled)=45.27 µm X 64.13 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

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

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"], "Control": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked Stepdown order. Scientific wording is preserved; missing modalities are not invented.
A preserved control follows all available Stepdown tiers as supporting evidence.

PRODUCT-LEVEL STEPDOWN MAP

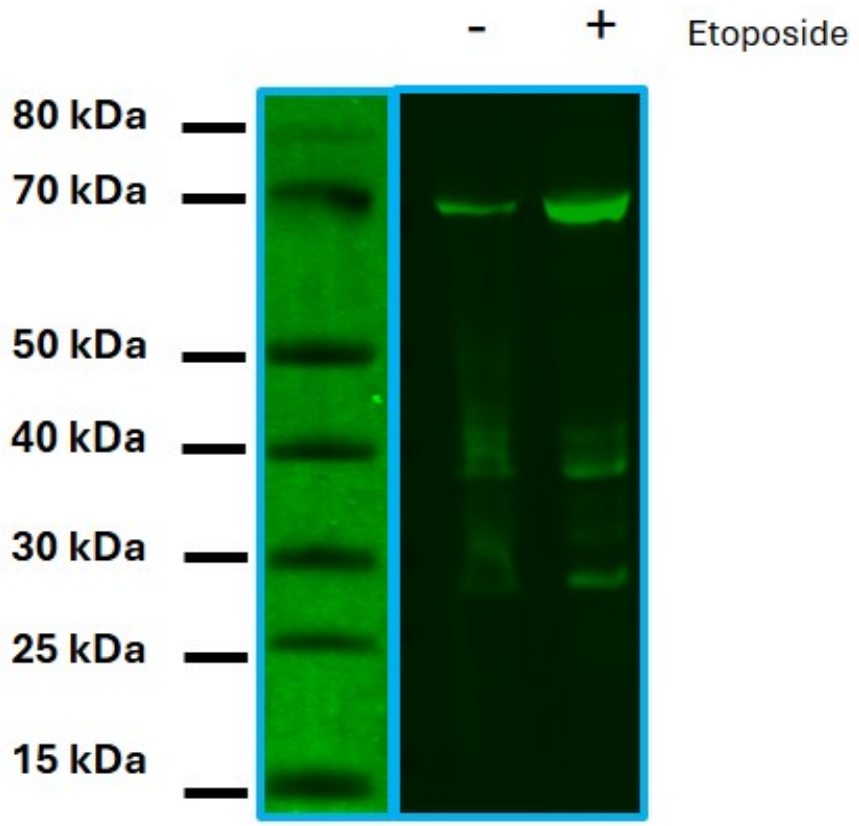
This dossier preserves the complete available evidence progression for PA-RP-000006. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	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	488
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	488nm
Emission Filter	513±17nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Expected Molecular Weight: 70 kDa

HeLa cells were damaged with 200 µM etoposide for 20 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet the cell debris The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and then washed five times with PBS. The blocked membrane was incubated with Rabbit anti-Human Ku70 Polyclonal, IgG, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence
Laser = 488nm
Emission Filter = 513±17nm
Detector = PMT
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

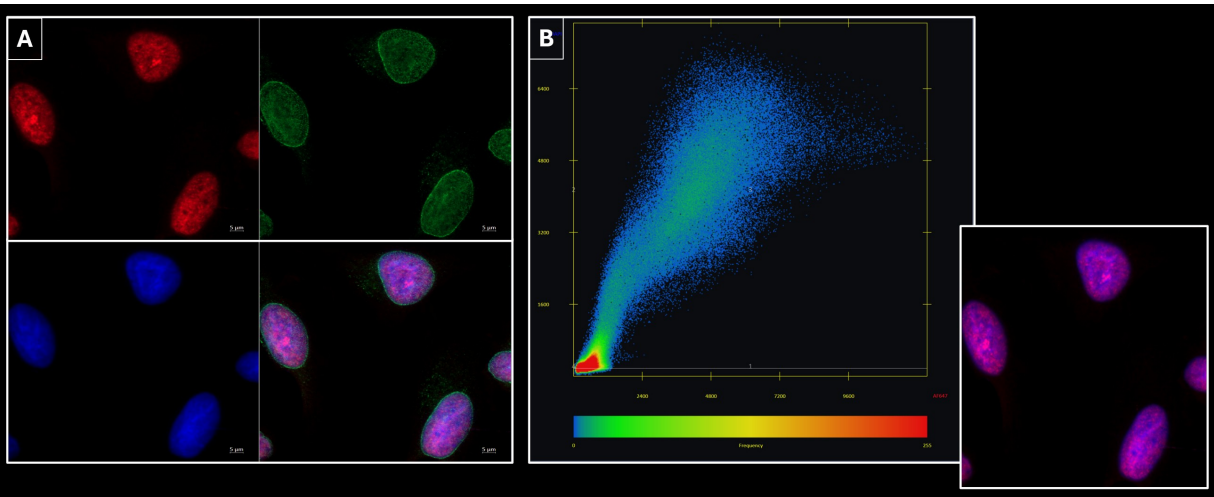
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CD0504F43A947D629F8C7AA7B2FAC329189F4E7A1D3A75C0716714DA33D7B342
Method PDF SHA-256	61A7FFEF1F77196683E7B9A7AA55E4900E4F7F7183544BDE209CECD3B6F64F79

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human Ku70 Polyclonal, IgG antibody. The upper left section features the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The upper right image shows the Nuclear Pore Complex (NPC) with Identifyn™ SRM Alexa Fluor™ 488 (green), while the lower left image is a DAPI nuclear staining (blue). The lower right presents a merged image of the three channels. The merge demonstrates that Ku70 is strongly localized to the nucleus, as indicated by DAPI staining, and the NPC staining, which marks the nuclear periphery, confirms its presence within the nuclear compartment following Etoposide-induced DNA damage.

Panel B - Displays a colocalization view with DAPI (Y-axis) and the Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (X-axis). The colocalization measurement encompasses the entire image. This measurement demonstrates that Ku70 is strongly colocalized with DAPI, suggesting that, as expected for this protein, it is located within the nuclear compartment.

As widefield is the first in our series of the “step down” method, as expected and seen as highly consistent, the staining appears pan-nuclear. As we move through widefield SIM and Confocal, much will stay the same, however, as we cross into super resolution, we will see that Ku70 displays 3-dimensional localization and structural details.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	878 X 929
Image Size (Scaled)	96.16 µm X 101.75 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 | 32 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	100 ms | 150 ms | 25 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0 and Max 0), Y (Min 0 and Max 1)

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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PB. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity

Purified Donkey Anti-Mouse, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 878 X 929
Image Size (Scaled) = 96.16 µm X 101.75 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 @10.00%
Exposure Time = 100 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

488 -

Reflector = 32 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 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 = 25 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The top-left panel features Rabbit anti-Human Ku70 Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L); the top-right panel features nuclear pore complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L); the bottom-left panel features DAPI nuclear staining. The bottom-right panel features the merged image of all channels.

Profile View Display - Panel B

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

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human Ku70 Polyclonal, IgG antibody. The upper left section features the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™SRM Alexa Fluor™ 647 (red). The upper right image shows the Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green), while the lower left image is a DAPI nuclear staining (blue). The lower right presents a merged image of the three channels. The merge demonstrates that Ku70 is strongly localized to the nucleus, as indicated by DAPI staining, and the NPC staining, which marks the nuclear periphery, confirms its presence within the nuclear compartment following Etoposide-induced DNA damage.

Panel B - Displays a colocalization view with DAPI (Y-axis) and the Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (X-axis). The colocalization measurement encompasses the entire image. This measurement demonstrates that Ku70 is strongly colocalized with DAPI, suggesting that, as expected for this protein, it is located within the nuclear compartment.

As widefield is the first in our series of the "step down" method, as expected and seen as highly consistent, the staining appears pan-nuclear. As we move through widefield SIM and Confocal, much will stay the same; however, as we cross into super resolution, we will see that Ku70 displays 3-dimensional localization and structural details.

Image Correction

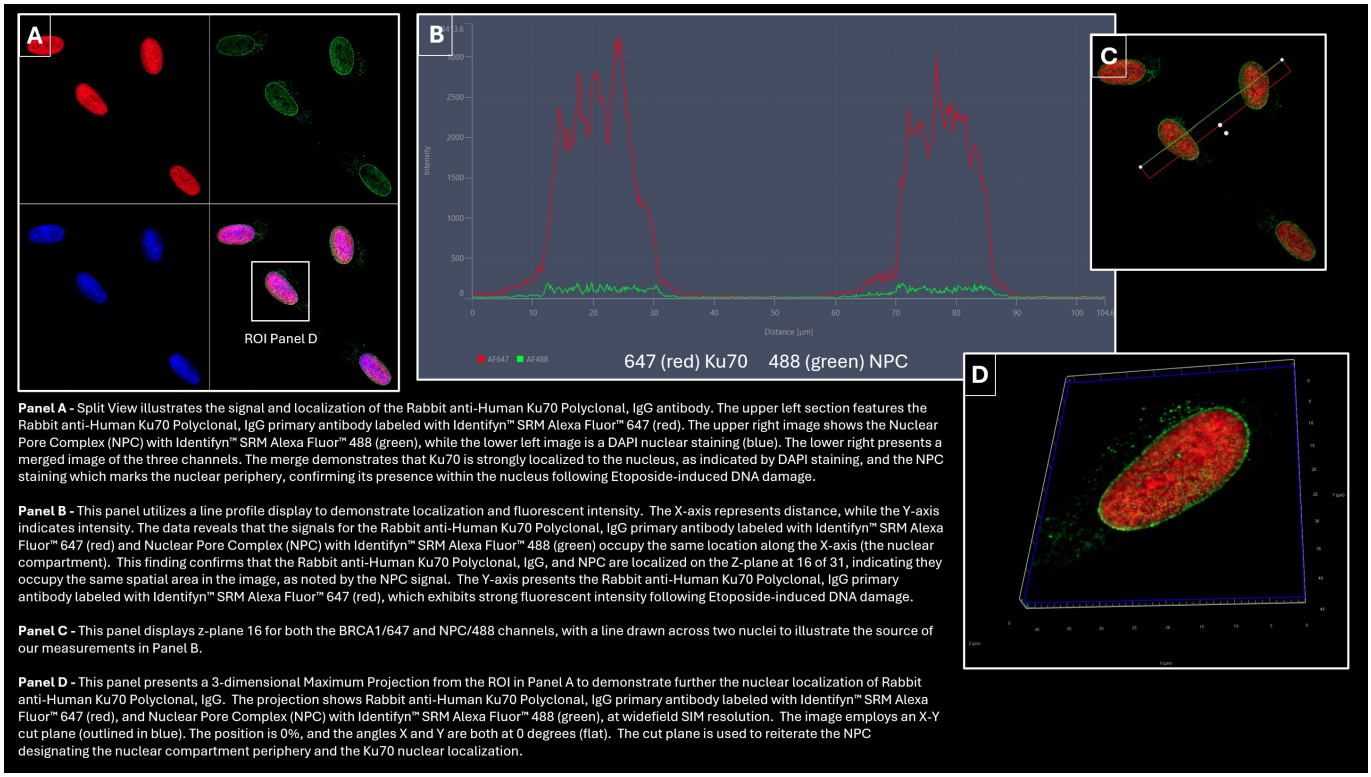
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000006
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	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5206E1E93B9B55C5BD7F2037B74A5F59792CB8F35C98CC9729597D19017683BE
Method PDF SHA-256	D0C8EB1DC984D2A330901D7FDA6430095CFB052C4F63C3008FFA8A59B9FCD9CD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human Ku70 Polyclonal, IgG antibody. The upper left section features the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red). The upper right image shows the Nuclear Pore Complex (NPC) with Identifyn™ SRM Alexa Fluor™ 488 (green), while the lower left image is a DAPI nuclear staining (blue). The lower right presents a merged image of the three channels. The merge demonstrates that Ku70 is strongly localized to the nucleus, as indicated by DAPI staining, and the NPC staining which marks the nuclear periphery, confirming its presence within the nucleus following Etoposide-induced DNA damage.

Panel B - This panel utilizes a line profile display to demonstrate localization and fluorescent intensity. The X-axis represents distance, while the Y-axis indicates intensity. The data reveals that the signals for the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and Nuclear Pore Complex (NPC) with Identifyn™ SRM Alexa Fluor™ 488 (green) occupy the same location along the X-axis (the nuclear compartment). This finding confirms that the Rabbit anti-Human Ku70 Polyclonal, IgG, and NPC are localized on the Z-plane at 16 of 31, indicating they occupy the same spatial area in the image, as noted by the NPC signal. The Y-axis presents the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red), which exhibits strong fluorescent intensity following Etoposide-induced DNA damage.

Panel C - This panel displays z-plane 16 for both the BRCA1/647 and NPC/488 channels, with a line drawn across two nuclei to illustrate the source of our measurements in Panel B.

Panel D - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG. The projection shows Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 647 (red), and Nuclear Pore Complex (NPC) with Identifyn™ SRM Alexa Fluor™ 488 (green), at widefield SIM resolution. The image employs an X-Y cut plane (outlined in blue). The position is 0%, and the angles X and Y are both at 0 degrees (flat). The cut plane is used to reiterate the NPC designating the nuclear compartment periphery and the Ku70 nuclear localization.

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	31 Slices (3 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	978 X 971 | 289 X 316
Image Size (Scaled)	139.71 µm X 138.71 µm | 41.29 µm X 45.14 µm
Bit Depth	14 Bit
Image Center Position	X: 81.11 mm, Y: 50.59 mm
Stage Position	X: 81.11 mm, Y: 50.59 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 HE eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10.00% | X-Cite Xylis Lamp Intensity @16.00% | X-Cite Xylis Lamp Intensity @6.00%
Exposure Time	100 ms | 150 ms | 12 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.00 µm | 0.90 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mod" "Optical Sectioning" - enabled correction selected.
Z-Position	16 of 31
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 104.6), Y (Min 0 and Max 3414)
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

FIELD	SOURCE-DOCUMENTED VALUE
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of special interest.
X-Y	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	0%
Clip X	0°
Clip Y	0°

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity

Purified Donkey Anti-Mouse, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 16 of 31 total - Panels A & C

Z Stack - (3D)

Z-Stack = 31 Slices (3 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 978 X 971
Image Size (Scaled) = 139.71 µm X 138.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11 mm, Y: 50.59 mm
Stage Position = X: 81.11 mm, Y: 50.59 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 31 Slices (3 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 289 X 316
Image Size (Scaled) = 41.29 µm X 45.14 µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11 mm, Y: 50.59 mm
Stage Position = X: 81.11 mm, Y: 50.59 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 @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @16.00%
Exposure Time = 150 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @6.00%
Exposure Time = 12 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mod" "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used.

Split View - Panel A

The top-left panel features Rabbit anti-Human Ku70 Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L); the top-right panel features nuclear pore complexes visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L); the bottom-left panel features DAPI nuclear staining. The bottom-right panel features the merged image of all channels. This image was taken at Z-Position 16 of 31.

Profile View Display - Panel B & C

Z-Position = 16 of 31
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 104.6), Y (Min 0 and Max 3414)

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)

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

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 = 0%
Clip X = 0°
Clip Y = 0°

Summary

Panel A - Split View illustrates the signal and localization of the Rabbit anti-Human Ku70 Polyclonal, IgG antibody. The upper left section features the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The upper right image shows the Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green), while the lower left image is a DAPI nuclear staining (blue). The lower right presents a merged image of the three channels. The merge demonstrates that Ku70 is strongly localized to the nucleus, as indicated by DAPI staining and NPC staining, which marks the nuclear periphery, confirming its presence within the nucleus following Etoposide-induced DNA damage.

Panel B - This panel utilizes a line profile display to demonstrate localization and fluorescent intensity. The X-axis represents distance, while the Y-axis indicates intensity. The data reveal that the signals for the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red) and Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488(green) occupy the same location along the X-axis (the nuclear compartment). This finding confirms that the Rabbit anti-Human Ku70 Polyclonal, IgG, and NPC are localized on the Z-plane at 16 of 31, indicating they occupy the same spatial area in the image, as noted by the NPC signal The Y-axis also presents the Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn™SRM Alexa Fluor™ 647 (red), which exhibits vigorous fluorescent intensity following Etoposide-induced DNA damage.

Panel C - This panel displays z-plane 16 for both the BRCA1/647 and NPC/488 channels, with a line drawn across two nuclei to illustrate the source of our measurements in Panel B.

Panel D - This panel presents a 3-dimensional Maximum Projection from the ROI in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG. The projection shows Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody labeled with Identifyn® SRM Alexa Fluor™ 647 (red), and Nuclear Pore Complex (NPC) with Identifyn® SRM Alexa Fluor™ 488 (green), at widefield SIM resolution. The image employs an X-Y cut plane (outlined in blue. The position is 0%, and the angles X and Y are both at 0 degrees (flat). The cut plane is used to reiterate the NPC designating the nuclear compartment periphery and the Ku70 nuclear localization.

Image Correction

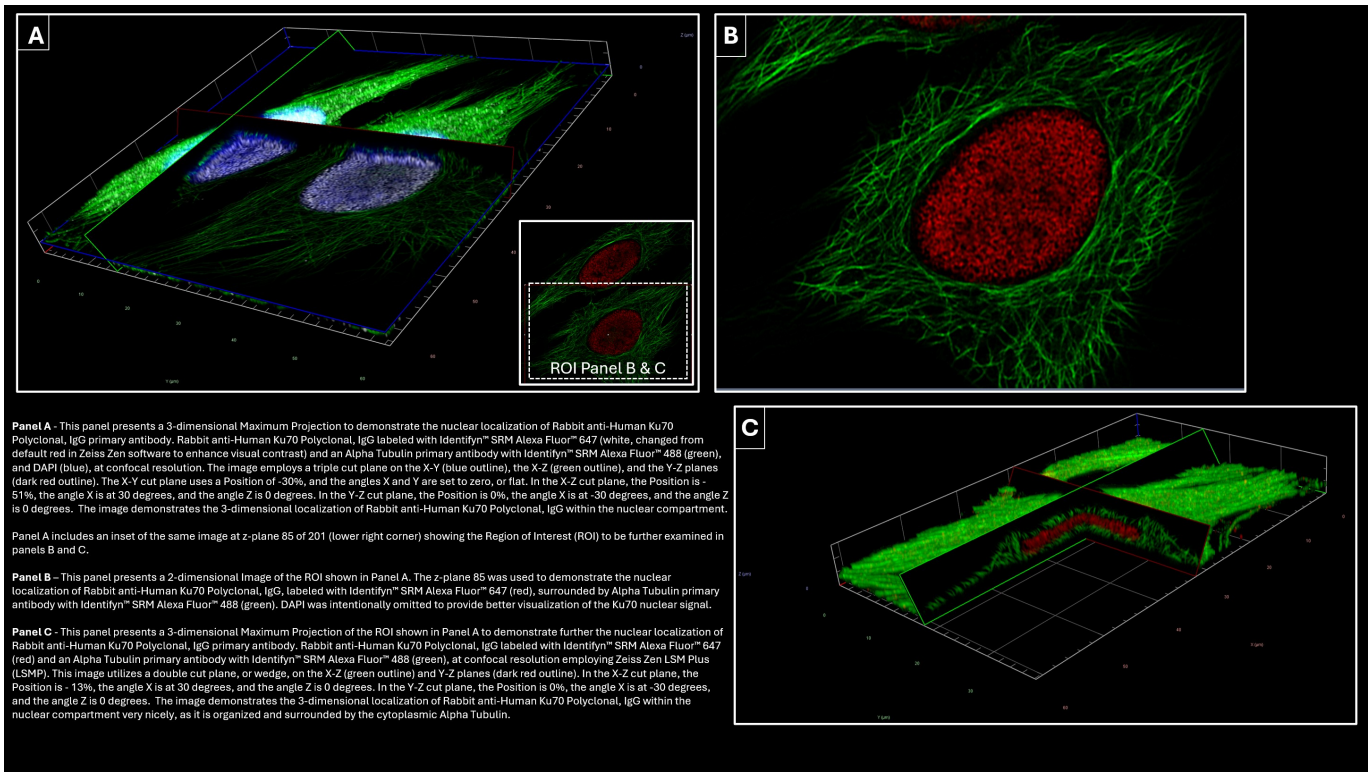
None

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

SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AD67561F027066C23E023FA0C335DC632D4F6E19DEB9F1D6841EA5AC60A54B34
Method PDF SHA-256	C1CB6149FEFB4B730EC2CD1DAA07BBCB875A41FDC93B573B2F8DB2430404BE37

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A - This panel presents a 3-dimensional Maximum Projection to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and an Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (blue), at confocal resolution. The image employs a triple cut plane on the X-Y (blue outline), the X-Z (green outline), and the Y-Z planes (dark red outline). The X-Y cut plane uses a Position of -30%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is -51%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment.

Panel A includes an inset of the same image at z-plane 85 of 201 (lower right corner) showing the Region of Interest (ROI) to be further examined in panels B and C.

Panel B – This panel presents a 2-dimensional Image of the ROI shown in Panel A. The z-plane 85 was used to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG, labeled with Identifyn™ SRM Alexa Fluor™ 647 (red), surrounded by Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green). DAPI was intentionally omitted to provide better visualization of the Ku70 nuclear signal.

Panel C - This panel presents a 3-dimensional Maximum Projection of the ROI shown in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (red) and an Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), at confocal resolution employing Zeiss Zen LSM Plus (LSMP). This image utilizes a double cut plane, or wedge, on the X-Z (green outline) and Y-Z planes (dark red outline). In the X-Z cut plane, the Position is - 13%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment very nicely, as it is organized and surrounded by the cytoplasmic Alpha Tubulin.

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	69

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	201 Slices (6 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1066 X 1066 | 1038 X 650
Image Size (Scaled)	62.72 µm X 62.72 µm | 61.08 µm X 38.25 µm
Bit Depth	16 Bit
Image Center Position	X: 84.41 mm, Y: 51.38 mm
Stage Position	X: 84.41 mm, Y: 51.38 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 65 µm | 1.00 AU / 50 µm | 1.00 AU / 44 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS SP 615 | Mirror
Laser Wavelength	639 nm @0.06% | 488 nm @0.04% | 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.1
Rotation	0°
Sampling	1.20
Pixel Time	0.32 µs
Frame Time	3.52 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	636-732 | 489-550 | 430-499
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	6.9 (3D, Auto) | 6.8 (3D, Auto) | 6.3 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	A clipping plane was introduced to pronounce regions of special interest.
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	-30% | -51% | 0% | -13%
Clip X	0° | 30°
Clip Y	0° | -30°
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 Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a dark 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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified

Donkey Anti-Mouse, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 201 Slices (6 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1066 X 1066
Image Size (Scaled) = 62.72 µm X 62.72 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.41 mm, Y: 51.38 mm
Stage Position = X: 84.41 mm, Y: 51.38 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 85 of 201 total - Panel B

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

Z-Stack = 201 Slices (6 µm)
Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1038 X 650
Image Size (Scaled) = 61.08 µm X 38.25 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.41 mm, Y: 51.38 mm
Stage Position = X: 84.41 mm, Y: 51.38 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 65 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 615
Laser Wavelength = 639 nm @0.06%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636-732
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.9 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 50 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 488 nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 489-550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.8 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 44 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = Mirror
Laser Wavelength = 405 nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.32 µs
Frame Time = 3.52 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430-499
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 6.3 (3D, Auto)

3D Image Processing - Panel A

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

Clipping Image Process = A clipping plane was introduced to pronounce regions of special interest.

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 = -30%
Clip X = 0°
Clip Y = 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 = -51%
Clip X = 30°
Clip Z = 0°

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

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

3D Image Processing - Panel C

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

Clipping Image Process = A clipping plane was introduced to pronounce regions of special interest.

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 = -13%
Clip X = 30°
Clip Z = 0°

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

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

Summary

Panel A - This panel presents a 3-dimensional Maximum Projection to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and an Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue), at confocal resolution. The image employs a triple cut plane on the X-Y (blue outline), the X-Z (green outline), and the Y-Z planes (dark red outline). The X-Y cut plane uses a Position of -30%, and the angles X and Y are set to zero, or flat. In the X-Z cut plane, the Position is - 51%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment.

Panel A includes an inset of the same image at z-plane 85 of 201 (lower right corner) showing the Region of Interest (ROI) to be further examined in panels B and C.

Panel B - This panel presents a 2-dimensional Image of the ROI shown in Panel A. The z-plane 85 was used to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (red), surrounded by Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green). DAPI was intentionally omitted to provide better visualization of the Ku70 nuclear signal.

Panel C - This panel presents a 3-dimensional Maximum Projection of the ROI shown in Panel A to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled withIdentifyn® SRM Alexa Fluor™ 647 (red) and an Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), at confocal resolution employing Zeiss Zen LSM Plus (LSMP). This image utilizes a double cut plane, or wedge, on the X-Z (green outline) and Y-Z planes (dark red outline). The X-Z cut plane, the Position is -13%, the angle X is at 30 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is 0%, the angle X is at -30 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment very nicely, as it is organized and surrounded by the cytoplasmic Alpha Tubulin.

Image Corrections

None

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

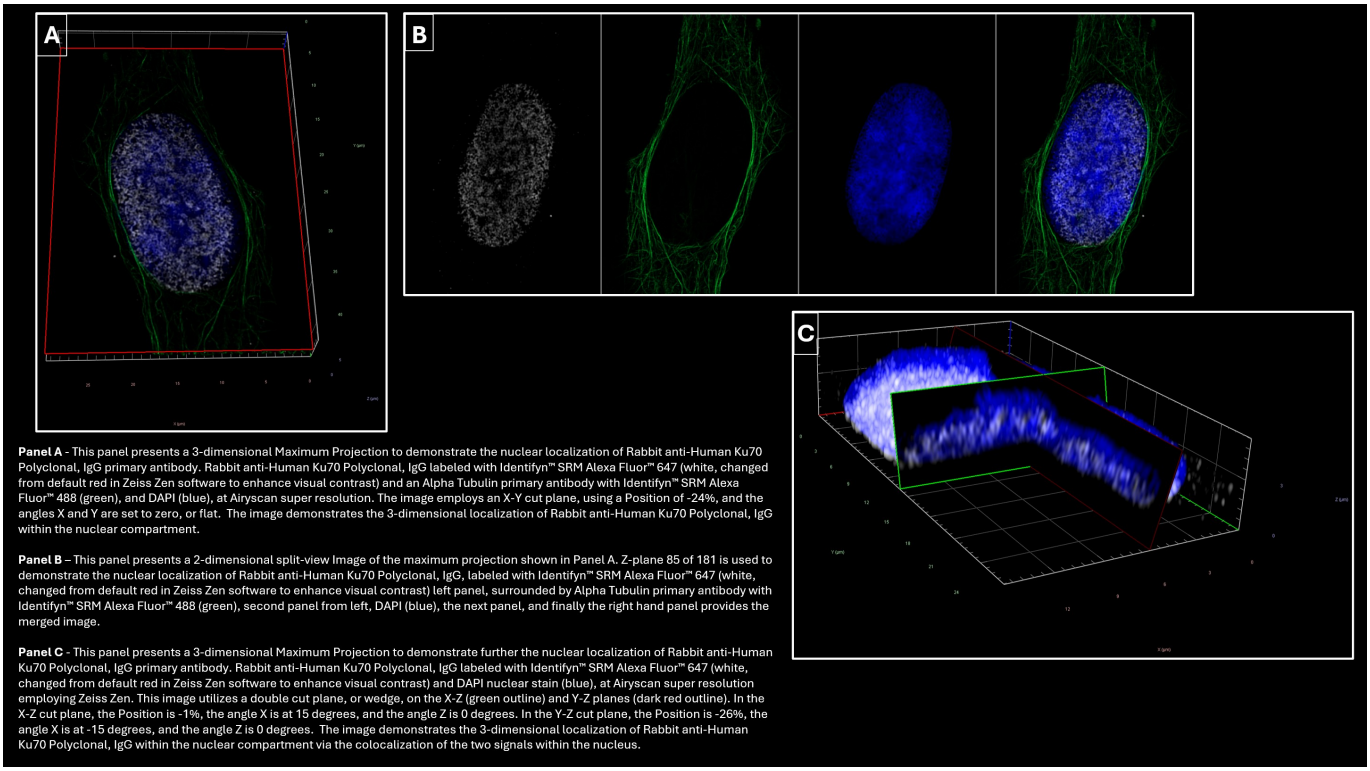
SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	Confocal

SOURCE PROVENANCE

Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	69
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AEBB54E737BDDF0442CF1F428893D4A12BC1AF89DEFE56CB17336D7182ADF483
Method PDF SHA-256	8EBB95845D4E1B7583B15606EAEE24A5A39294A98682FFEC3EB6E4F9ABF5E8F7

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Panel A - This panel presents a 3-dimensional Maximum Projection to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and an Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI (blue), at Airyscan super resolution. The image employs an X-Y cut plane, using a Position of -24%, and the angles X and Y are set to zero, or flat. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment.

Panel B – This panel presents a 2-dimensional split-view Image of the maximum projection shown in Panel A. Z-plane 85 of 181 is used to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG, labeled with Identifyn™ SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) left panel, surrounded by Alpha Tubulin primary antibody with Identifyn™ SRM Alexa Fluor™ 488 (green), second panel from left, DAPI (blue), the next panel, and finally the right hand panel provides the merged image.

Panel C - This panel presents a 3-dimensional Maximum Projection to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn™ SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and DAPI nuclear stain (blue), at Airyscan super resolution employing Zeiss Zen. This image utilizes a double cut plane, or wedge, on the X-Z (green outline) and Y-Z planes (dark red outline). In the X-Z cut plane, the Position is -1%, the angle X is at 15 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is -26%, the angle X is at -15 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment via the colocalization of the two signals within the nucleus.

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 750
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	181 Slices (5.4 µm) - Panels A & B | 181 Slices (5.4 µm) - Panel C
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	856 X 1191 | 422 X 750
Image Size (Scaled)	29.51 µm X 42.04 µm | 14.89 µm X 26.47 µm
Bit Depth	16 Bit
Image Center Position	X: 84.61 mm, Y: 46.58 mm
Stage Position	X: 84.61 mm, Y: 46.58 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 µm | 5.00 AU / 250 µm | 5.00 AU / 235 µm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 640 | SBS LP 550 | SBS SP 505
Laser Wavelength	639 nm @0.05% | 488 nm @1.00% | 405 nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.4
Rotation	0°
Sampling	2.00
Pixel Time	0.66 µs
Frame Time	7.82 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643-735 | 499-548 | 350-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	8.7 (3D, Auto) | 7.7 (3D, Auto)
LMS MBS	MBS 488/639, 405/730
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to define regions of special interest. | Clipping planes are introduced to pronounce regions of special interest.
X-Y	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	-24% | -1% | -26%
Clip X	0° | 15°
Clip Y	0° | -15°
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 Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a dark 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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified

Donkey Anti-Mouse, F(ab')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D) - Z-plane 81 of 181 total - Panel B

Z-Stack = 181 Slices (5.4 µm) - Panels A & B

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 856 X 1191
Image Size (Scaled) = 29.51 µm X 42.04 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.61 mm, Y: 46.58 mm
Stage Position = X: 84.61 mm, Y: 46.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z-Stack = 181 Slices (5.4 µm) - Panel C

Channels = 3
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 422 X 750
Image Size (Scaled) = 14.89 µm X 26.47 µm
Bit Depth = 16 Bit
Image Center Position = X: 84.61 mm, Y: 46.58 mm
Stage Position = X: 84.61 mm, Y: 46.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 640
Laser Wavelength = 639 nm @0.05%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 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 = 643-735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.7 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LSM MBS = MBS 488/639, 405/730
LSM SBS = SBS LP 550
Laser Wavelength = 488 nm @1.00%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499-548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 8.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 235 µm
LMS MBS = MBS 488/639, 405/730
LSM SBS = SBS SP 505
Laser Wavelength = 405 nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.66 µs
Frame Time = 7.82 s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 350-499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = 7.7 (3D, Auto)

3D Image Processing - Panel A

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

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

X-Y = 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 = -24%
Clip X = 0°
Clip Y = 0°

Split View - Panel A

The first panel on the left features Rabbit anti-Human Ku70 Polyclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L), the second panel features alpha tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L), the third panel features DAPi nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 81 of 181.

3D Image Processing - Panel C

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

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

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 = -1%
Clip X = 15°
Clip Z = 0°

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

Position of Clip = -26%
Clip Y = -15°
Clip Z = 0°

Summary

Panel A - This panel presents a 3-dimensional Maximum Projection to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and an Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI (blue), at Airyscan super resolution The image employs an X-Y cut plane, using a Position of -24%, and the angles X and Y are set to zero, or flat. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment.

Panel B - This panel presents a 2-dimensional split-view Image of the maximum projection shown in Panel A. Z-plane 85 of 181 is used to demonstrate the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) left panel, surrounded by Alpha Tubulin primary antibody with Identifyn® SRM Alexa Fluor™ 488 (green), second panel from left, DAPI (blue), the next panel, and finally the right hand panel provides the merged image.

Panel C - This panel presents a 3-dimensional Maximum Projection to demonstrate further the nuclear localization of Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody. Rabbit anti-Human Ku70 Polyclonal, IgG labeled with Identifyn® SRM Alexa Fluor™ 647 (white, changed from default red in Zeiss Zen software to enhance visual contrast) and DAPI nuclear stain (blue), at Airyscan super resolution employing Zeiss Zen. This image utilizes a double cut plane, or wedge, on the X-Z (green outline) and Y-Z planes (dark red outline). In the X-Z cut plane, the Position is -1%, the angle X is at 15 degrees, and the angle Z is 0 degrees. In the Y-Z cut plane, the Position is -26%, the angle X is at -15 degrees, and the angle Z is 0 degrees. The image demonstrates the 3-dimensional localization of Rabbit anti-Human Ku70 Polyclonal, IgG within the nuclear compartment via the colocalization of the two signals within the nucleus.

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

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

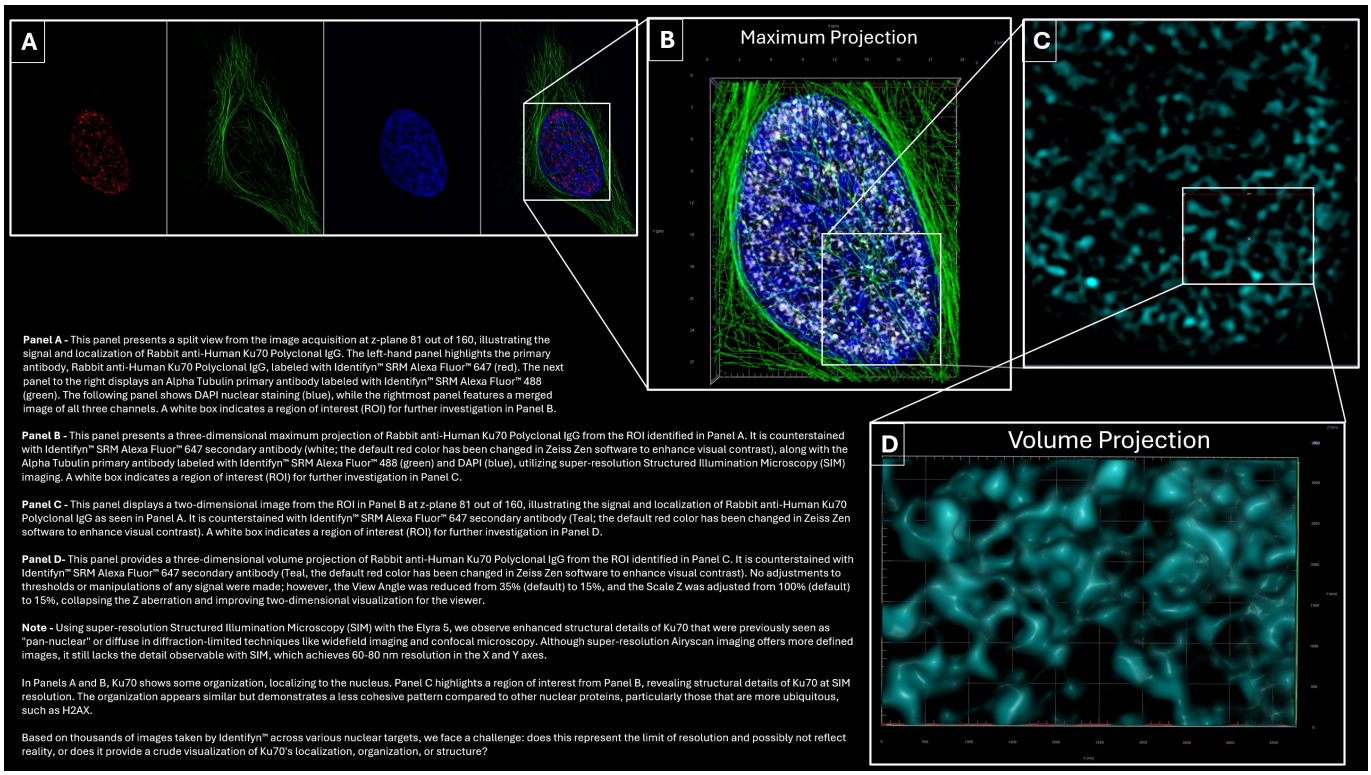
SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	81C678C831B8FFDDCCECAA2EFAEE2D9BF05CCB1A246F1EF30820EE923D1D869D
Method PDF SHA-256	44BDEEC7A49D9B85CA7AA7AFC65126A95AEFCA3DFBF49DE8859D8565E5BE3A1F

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 647
METADATA VALUES	70

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	160 Slices (4.77 µm) | 78 Slices (2.31 µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	3560 X 4889 | 1825 X 2228 | 933 X 960 | 361 X 249
Image Size (Scaled)	46.98 µm X 64.51 µm | 24.08 µm X 29.40 µm | 12.31 µm X 12.61 µm | 4.76 µm X 3.29 µm
Bit Depth	16 Bit
Image Center Position	X: 55.92 mm, Y: 39.25 mm
Stage Position	X: 55.92 mm, Y: 39.25 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100 ms | 120 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.50% | 405 nm @3.00%
Frame Time	1.33 s | 1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	27.5 µm grating | 36.5 µm grating
Result Sampling	4
Process Sampling	N/A

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.4422 (647), 10.1352 (488), 8.6429 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels | Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping Enabled | 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) | View Angle 15°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified

Donkey Anti-Mouse, F('b')□ (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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 81 of 160 total - Panel A

Z Stack - (3D)

Z-Stack = 160 Slices (4.77 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 3560 X 4889
Image Size (Scaled) = 46.98 µm X 64.51 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 160 Slices (4.77 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 1825 X 2228
Image Size (Scaled) = 24.08 µm X 29.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 81 of 160 total - Panel C

Z Stack - (3D)

Z-Stack = 160 Slices (4.77 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 933 X 960
Image Size (Scaled) = 12.31 µm X 12.61 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel D

Z-Stack = 78 Slices (2.31 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 361 X 249
Image Size (Scaled) = 4.76 µm X 3.29 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @3.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 27.5 µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.4422 (647), 10.1352 (488), 8.6429 (DAPI)
Fitting = Fast Fit
Normalization = Auto

Split View - Panel A

The first panel on the left features Rabbit anti-Human BRCA1 Recombinant Monoclonal, IgG visualized with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L), the second panel features alpha tubulin visualized with Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L), the third panel features DAPI nuclear staining, and the last panel on the right features the merged image of all channels. This image was taken at Z-Position 81 of 160.

3D Image Processing - Panel B

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

3D Image Processing - Panel D

3D Volume Projection = Precise
Appearance = Threshold 4% all channels, Ramp 96% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 15°, Scale Z 15%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel presents a split view from the image acquisition at z-plane 81 out of 160, illustrating the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG. The left-hand panel highlights the primary antibody, Rabbit anti-Human Ku70 Polyclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (red). The next panel to the right displays an Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). The following panel shows DAPI nuclear staining (blue), while the rightmost panel features a merged image of all three channels. A white box indicates a region of interest (ROI) for further investigation in Panel B.

Panel B - This panel presents a three-dimensional maximum projection of Rabbit anti-Human Ku70 Polyclonal IgG from the ROI identified in Panel A It is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (white; the default red color has been changed in Zeiss Zen software to enhance visual contrast), along with the Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green) and DAPI (blue), utilizing

super-resolution Structured Illumination Microscopy (SIM) imaging. A white box indicates a region of interest (ROI) for further investigation in Panel C.

Panel C - This panel displays a two-dimensional image from the ROI in Panel B at z-plane 81 out of 160, illustrating the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG as seen in Panel A. It is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (Teal; the default red color has been changed in Zeiss Zen software to enhance visual contrast). A white box indicates a region of interest (ROI) for further investigation in Panel D.

Panel D- This panel provides a three-dimensional volume projection of Rabbit anti-Human Ku70 Polyclonal IgG from the ROI identified in Panel C. It is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (Teal, the default red color has been changed in Zeiss Zen software to enhance visual contrast). No adjustments to thresholds or manipulations of any signal were made; however, the View Angle was reduced from 35% (default) to 15%, and the Scale Z was adjusted from 100% (default) to 15%, collapsing the Z aberration and improving two-dimensional visualization for the viewer.

Note - Using super-resolution Structured Illumination Microscopy (SIM) with the Elyra 5, we observe enhanced structural details of Ku70 that were previously seen as "pan-nuclear" or diffuse in diffraction-limited techniques like widefield imaging and confocal microscopy. Although super-resolution Airyscan imaging offers more defined images, it still lacks the detail observable with SIM, which achieves 60-80 nm resolution in the X and Y axes.

In Panels A and B, Ku70 shows some organization, localizing to the nucleus. Panel C highlights a region of interest from Panel B, revealing structural details of Ku70 at SIM resolution. The organization appears similar but demonstrates a less cohesive pattern compared to other nuclear proteins, particularly those that are more ubiquitous, such as H2AX.

Based on thousands of images taken by Identifyn® across various nuclear targets, we face a challenge: does this represent the limit of resolution and possibly not reflect reality, or does it provide a crude visualization of Ku70's localization, organization, or structure?

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to teal to provide visual contrast.

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

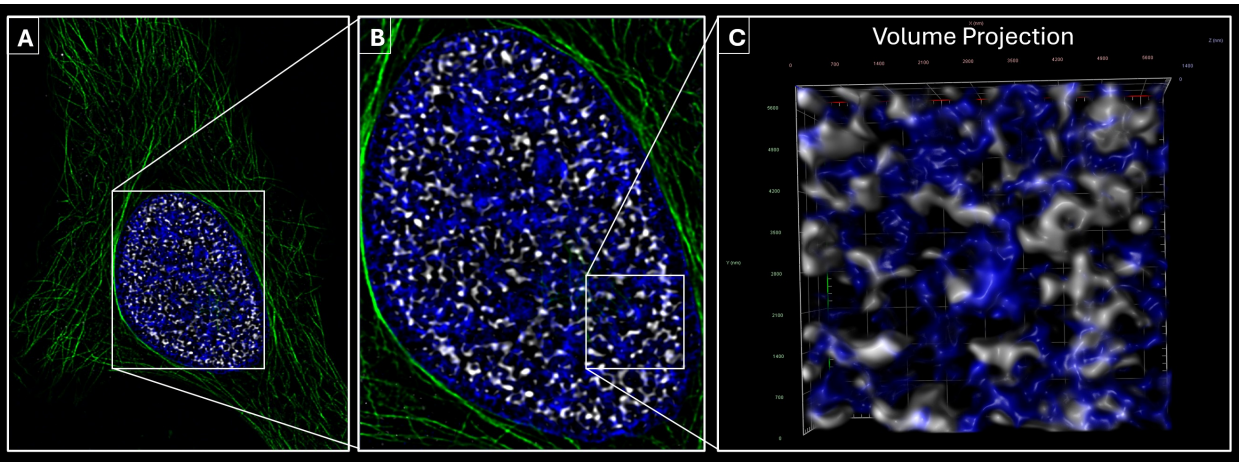
SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	70
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AA70886B8F6369B3C9DF5353F14CBAF12E17DB63DEFBD83DA5DE3A12BAA57CD4
Method PDF SHA-256	B744ECD78D3FFE2EAC977D12995DDF0C637B12F1880058A6192A0B512523C990

ORIGINAL IMAGE EVIDENCE / SIM²



Panel A - This panel presents a 2-dimensional view from the image acquisition at z-plane 54 out of 160, (same Image as used in the SIM analysis), illustrating the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG, labeled with Identifyn™ SRM Alexa Fluor™ 647 (white; the default red color has been changed in Zeiss Zen software to enhance visual contrast), Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue). A white box indicates a region of interest (ROI) for further investigation in Panel B.

Panel B - This panel presents a 2-dimensional view from the ROI in Panel A at the same z-plane, 54. The image illustrates the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG, labeled with Identifyn™ SRM Alexa Fluor™ 647 (white; the default red color has been changed in Zeiss Zen software to enhance visual contrast), Alpha Tubulin primary antibody labeled with Identifyn™ SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue). **Here we see similar structural details to those captured in the SIM resolution**. A white box indicates a region of interest (ROI) for further investigation in Panel C.

Panel C- This panel provides a three-dimensional volume projection of Rabbit anti-Human Ku70 Polyclonal IgG from the ROI identified in Panel B. Ku70 is counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (white, the default red color has been changed in Zeiss Zen software to enhance visual contrast), and DAPI (blue). **No adjustments to thresholds or manipulations of any kind were made; the image represents the image acquisition, in a Volume Projection, at default values, and represents nothing more than what the cameras saw.**

NOTE - Using **super-resolution Structured Illumination Microscopy Squared** with the Elyra 5, **we observe enhanced structural details of Ku70 that were previously seen as "pan-nuclear"** or diffuse under diffraction-limited techniques such as widefield imaging and confocal microscopy. Notably, the **structures appear more defined in SIM Squared compared to standard SIM, as the axial resolution improves from approximately 80 nm to 40 nm. However, the interpretation of these details can be subjective, and we encourage you to draw your own conclusions**

What is clear is that at the SIM Squared resolution, we not only see organized structural details for Ku70, but also for DAPI. While DAPI is often considered merely a counterstain for the nucleus, this level of super-resolution reveals that the previously diffuse blue nucleus now shows distinct structures. These structures exhibit similar patterns and interactions with Ku70 and other nuclear proteins that we regularly image. **Is it possible that DAPI may be a more significant tool in super-resolution than previously recognized?**

EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	67

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	160 Slices (4.77 µm) | 65 Slices (1.92 µm)
Channels	3
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.03000 µm
Image Size (Pixels)	3431 X 4860 | 1583 X 2118 | 454 X 455
Image Size (Scaled)	45.27 µm X 64.13 µm | 20.89 µm X 27.95 µm | 5.99 µm X 6.00 µm
Bit Depth	16 Bit
Image Center Position	X: 55.92 mm, Y: 39.25 mm
Stage Position	X: 55.92 mm, Y: 39.25 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488 | 405
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525 | 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100 ms | 120 ms
Depth of Focus	1.13 µm | 0.81 µm | 0.69 µm
Binning Mode	2,2
Laser Wavelength	640 nm @1.00% | 488 nm @1.50% | 405 nm @3.00%
Frame Time	1.33 s | 1.59 s | 676.00 ms
Scan Direction	Unidirectional
Grating	27.5 µm grating | 36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM²
SIM² Settings	Standard
Input SNR	Low
Regularization	None
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 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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibodies

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

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000042

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F('b')□ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified

Donkey Anti-Mouse, F('b')□ (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 Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Single Plane (2D) - Z-plane 54 of 160 total - Panel A

Z Stack - (3D)

Z-Stack = 160 Slices (4.77 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 3431 X 4860
Image Size (Scaled) = 45.27 µm X 64.13 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Z-plane 54 of 160 total - Panel B

Z Stack - (3D)

Z-Stack = 160 Slices (4.77 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 1583 X 2118
Image Size (Scaled) = 20.89 µm X 27.95 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 65 Slices (1.92 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm
Image Size (Pixels) = 454 X 455
Image Size (Scaled) = 5.99 µm X 6.00 µm
Bit Depth = 16 Bit
Image Center Position = X: 55.92 mm, Y: 39.25 mm
Stage Position = X: 55.92 mm, Y: 39.25 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 = 100 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @1.00%
Frame Time = 1.33 s
Scan Direction = Unidirectional
Grating = 27.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @1.50%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820 #2
Camera Adapter = 1X
Exposure Time = 100 ms
Depth of Focus = 0.69 µm
Binning Mode = 2,2
Laser Wavelength = 405 nm @3.00%
Frame Time = 676.00 ms
Scan Direction = Unidirectional
Grating = 27.5 µm grating

Post Processing - SIM² Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 3
Algorithm = SIM²
SIM² Settings = Standard
Input SNR = Low
Regularization = None
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

3D Image Processing - Panel C

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)

Summary

Here, we present the same image as in the Ku70 super-resolution SIM dataset to facilitate a comparison of halving the

resolution and revealing additional structural details.

Panel A - This panel presents a 2-dimensional view from the image acquisition at z-plane 54 out of 160, illustrating the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (white; the default red color has been changed in Zeiss Zen software to enhancevisual contrast), Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green), and DAPI nuclear staining (blue). A white box indicates a region of interest (ROI) for further investigation in Panel B.

Panel B - This panel presents a 2-dimensional view from the ROI in Panel A at the same z-plane, 54 The image illustrates the signal and localization of Rabbit anti-Human Ku70 Polyclonal IgG, labeled with Identifyn® SRM Alexa Fluor™ 647 (white; the default red color has been changed in Zeiss Zen software to enhance visual contrast), Alpha Tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488 (green). DAPI nuclear staining (blue). Here we see similar structural details to those captured in the SIM resolution. A white box indicates a region of interest (ROI) for further investigation in Panel C.

Panel C- This panel provides a three-dimensional volume projection of Rabbit anti-Human Ku70 Polyclonal IgG from the ROI identified in Panel B. Ku70 is counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (white; the default red color has been changed in Zeiss Zen software to enhance visual contrast), and DAPI (blue). No adjustments to thresholds or manipulations of any kind were made; the image represents the image acquisition, in a Volume Projection, at default values, and represents nothing more than what the cameras saw.

NOTE - Using super-resolution Structured Illumination Microscopy Squared with the Elyra 5, we observe enhanced structural details of Ku70 that were previously seen as "pan-nuclear" or diffuse under diffraction-limited techniques such as widefield imaging and confocal microscopy. Notably, the structures appear more defined in SIM Squared compared to standard SIM, as the axial resolution improves from approximately 80 nm to 40 nm. However, the interpretation of these details can be subjective, and we encourage you to draw your conclusions.

What is clear is that at the SIM Squared resolution, we not only see organized structural details for Ku70, but also for DAPI. While DAPI is often considered merely a counterstain for the nucleus, this level of super-resolution reveals that the previously diffuse blue nucleus now shows distinct structures. These structures exhibit similar patterns and interactions with Ku70 and other nuclear proteins that we regularly image.

Is it possible that DAPI may be a more significant tool in super-resolution than previously recognized?

Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(a'')□ (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast.

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

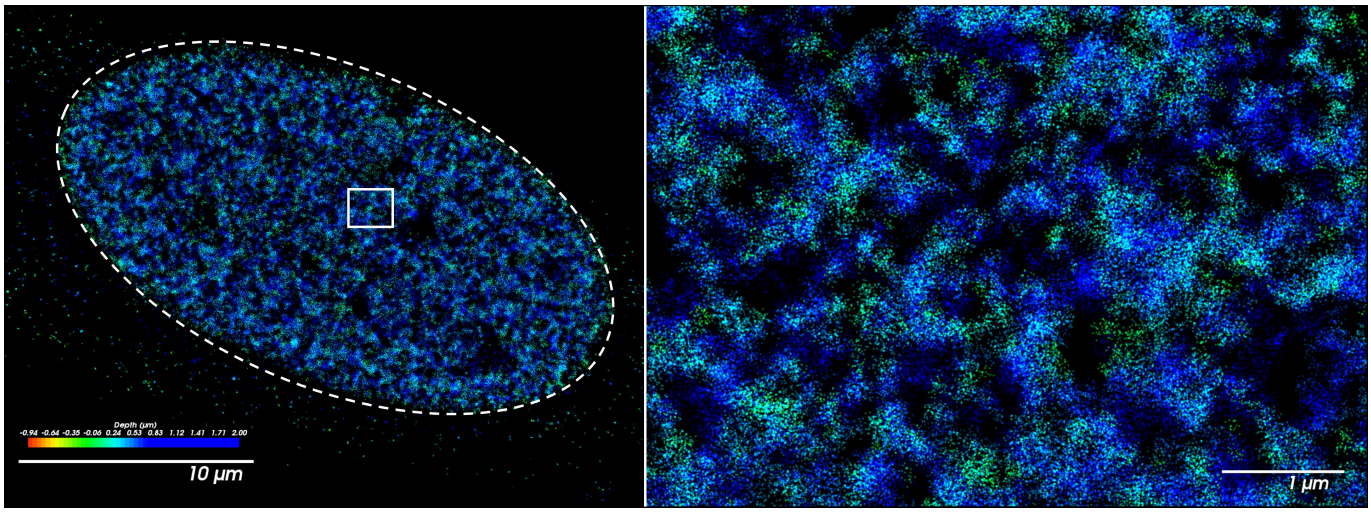
SOURCE PROVENANCE

Catalog	PA-RP-000006

SOURCE PROVENANCE

Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	67
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	39AFA866B51F406C405B053304A395D4F5B8BB564B55F2D2081840FDFC987F48
Method PDF SHA-256	5455B030A39065DBF2C9CB482946C38F839B9C7376DB4501E3F6EC43A38B9E8F

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	79

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms | 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	177 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 174; End: 175.2
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	6 using Steps: 0.2µm (200nm)
Cycles	25
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	90%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 15
Sizing Mode	Constant
Particle size	20nm
Color by	Constant
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.4
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	25
Axial precision	65
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1um (900nm)
Maximum Particle Count to Form Cluster	10
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibody

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

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human Ku70 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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:250 Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope Configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of Sample

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

Piezo Current: 177 µm

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

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

Advanced Tools Option

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

Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

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

View Mode

SuperRes: 50µm is Selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 174; End: 175.2

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: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 6 using Steps: 0.2µm (200nm)
Cycles: 25
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 90%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 15

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Constant
Particle size: 20nm
Color by: Constant
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.4
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 10 Intervals
Pixel Size: 50nm
Smoothing: 8.00

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

PSF Z offset: -600 to +600
Radial precision: 25
Axial precision: 65

The other parameters were not applied.

Statistical analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1um (900nm)
Maximum Particle Count to Form Cluster: 10
Computer Hulls: Not Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

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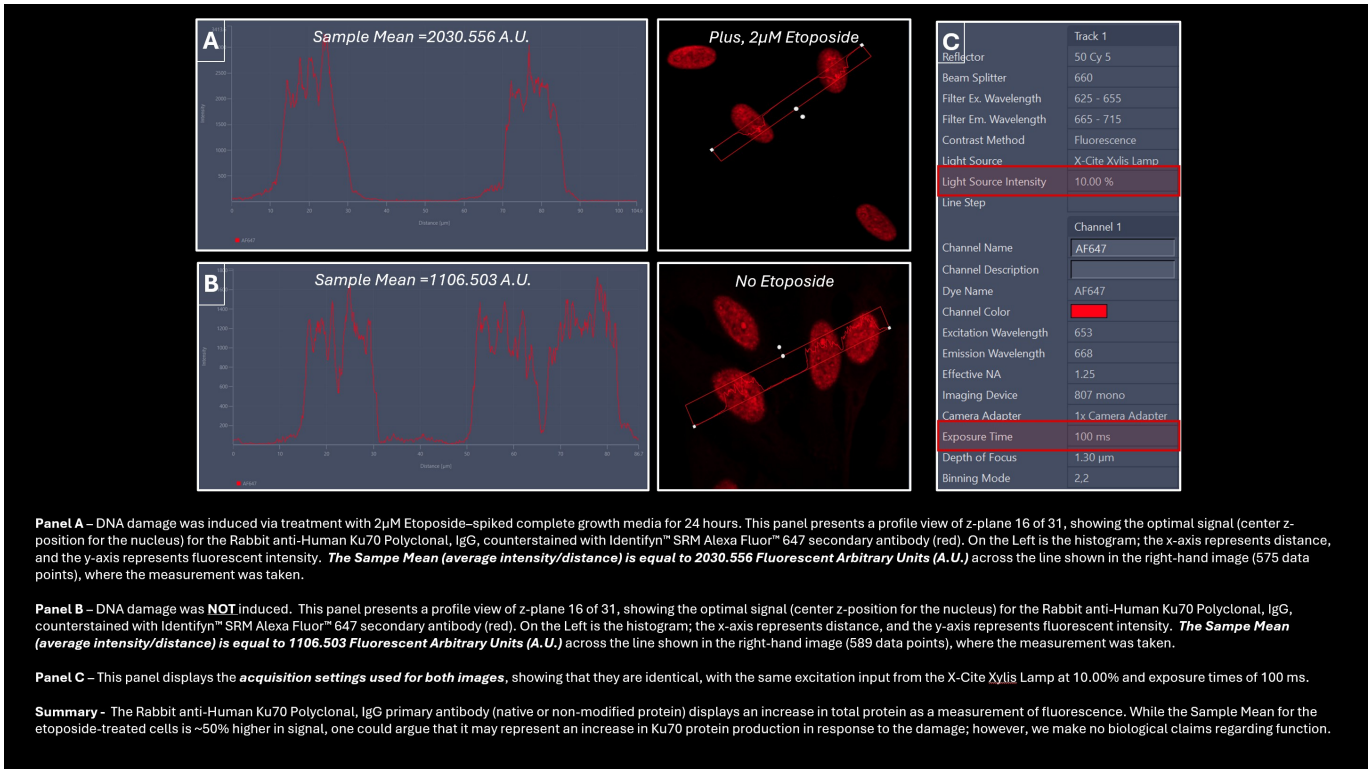
Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RP-000006
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	79
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1D5E34F0195EEE0731255D92F2A8557A820AD6D8AEA285CC59992E47AEC93F42
Method PDF SHA-256	3E47B238E2FD54812D0AE510E1DFF64EAE0FA20C64EFFD282D5B44C0E9910D7B

ORIGINAL IMAGE EVIDENCE / CONTROL



Panel A – DNA damage was induced via treatment with 2μM Etoposide–spiked complete growth media for 24 hours. This panel presents a profile view of z-plane 16 of 31, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human Ku70 Polyclonal, IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sampe Mean (average intensity/distance) is equal to 2030.556 Fluorescent Arbitrary Units (A.U.)*** across the line shown in the right-hand image (575 data points), where the measurement was taken.

Panel B – DNA damage was **NOT** induced. This panel presents a profile view of z-plane 16 of 31, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human Ku70 Polyclonal, IgG, counterstained with Identifyn™ SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. ***The Sampe Mean (average intensity/distance) is equal to 1106.503 Fluorescent Arbitrary Units (A.U.)*** across the line shown in the right-hand image (589 data points), where the measurement was taken.

Panel C – This panel displays the ***acquisition settings used for both images***, showing that they are identical, with the same excitation input from the X-Cite Xylis Lamp at 10.00% and exposure times of 100 ms.

Summary - The Rabbit anti-Human Ku70 Polyclonal, IgG primary antibody (native or non-modified protein) displays an increase in total protein as a measurement of fluorescence. While the Sample Mean for the etoposide-treated cells is ~50% higher in signal, one could argue that it may represent an increase in Ku70 protein production in response to the damage; however, we make no biological claims regarding function.

EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	31 z-planes (3 µm) - Uses Z-plane 16 for Analysis.
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm x 100µm
Image size (Pixels)	978 X 971 | 707 X 580
Image Size (Scaled)	139.71µm X 138.71µm | 101.00µm X 82.86µm
Bit Depth	14 Bit
Image Center Position	X: 81.11µm, Y: 50.59µm | X: 74.56µm, Y: 49.88µm
Stage Position	X: 81.11µm, Y: 50.59µm | X: 74.56µm, Y: 49.88µm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
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 @10.00% | X-Cite Xylis Lamp Intensity 6.00%
Exposure Time	100 ms | 12 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 0.90 µm
Binning Mode	2,2
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 95.9), Y (Min 0 and Max 4948) | Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 86.7), Y (Min 1 and Max 1816)

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 Ku70 Polyclonal, IgG
Cat ID# - PA-RP 000006

Identifyn® Secondary Antibody

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

Ku70 is a crucial protein involved in the Non-Homologous End Joining (NHEJ) pathway, one of DNA's central mechanisms for repairing double-strand breaks (DSBs).

Recognition and Binding to DNA Breaks: Ku70 and Ku80 form the Ku heterodimer. This heterodimer has a high affinity for DNA ends and is responsible for recognizing and binding to DSBs. Its structure allows it to slide onto the DNA strand and form a ring around the break, stabilizing it.

Recruitment of Additional NHEJ Proteins: After binding to the DNA break, the Ku heterodimer acts as a scaffold, recruiting and assembling other proteins essential for the NHEJ pathway. This includes DNA-PKcs (DNA-dependent protein kinase catalytic subunit), which are crucial in further processing the break.

Protection of DNA Ends: By binding to the ends of the DNA at the site of the break, Ku70/80 helps protect the DNA from further degradation or unwanted processing, ensuring that the ends are preserved for the repair process.

Alignment of DNA Ends: Ku70/80 helps align the broken DNA ends, facilitating the ligation step in NHEJ. This alignment is crucial for accurate repair, even though NHEJ does not require extensive sequence homology.

Participation in End Processing and Ligation: Once other NHEJ factors have been recruited, Ku70/80 can assist in processing DNA ends if necessary. Ultimately, it helps bring the DNA ends together for ligation by the ligase complex (such as DNA Ligase IV, XRCC4, and XLF), completing the repair process.

In summary, Ku70, as part of the Ku heterodimer, plays a central role in the NHEJ pathway by recognizing and binding to DNA breaks, recruiting and organizing other repair proteins, protecting DNA ends, and facilitating the alignment and ligation necessary to repair double-strand breaks.

Method

Plus Damage - Panel A

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 Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

No Damage - Panel B

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527. The primary antibody, Rabbit anti-Human Ku70 Polyclonal, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z-Stack - (3D) - Panel A

Z-Stack = 31 z-planes (3 µm) - Uses Z-plane 16 for Analysis.

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm
Image size (Pixels) = 978 X 971
Image Size (Scaled) = 139.71µm X 138.71µm
Bit Depth = 14 Bit
Image Center Position = X: 81.11µm, Y: 50.59µm
Stage Position = X: 81.11µm, Y: 50.59µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

Z-Stack - (3D) - Panel B

Z-Stack = 31 z-planes (3 µm) - Uses Z-plane 16 for Analysis.

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm x 100µm
Image size (Pixels) = 707 X 580
Image Size (Scaled) = 101.00µm X 82.86µm
Bit Depth = 14 Bit
Image Center Position = X: 74.56µm, Y: 49.88µm
Stage Position = X: 74.56µm, Y: 49.88µm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

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 @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335-383
Filter Emission Wavelength = 420-470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity 6.00%
Exposure Time = 12 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90 µm
Binning Mode = 2,2

Profile View Display - Panel A

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 95.9), Y (Min 0 and Max 4948)

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 86.7), Y (Min 1 and Max 1816)

Summary

Panel A - DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours. This panel presents a profile view of z-plane 16 of 31, showing the optimal signal (center z-position for the nucleus) for the Rabbit anti-Human Ku70 Polyclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 secondary antibody (red). On the Left is the histogram; the x-axis represents distance, and the y-axis represents fluorescent intensity. The Sample Mean (average intensity/distance) is equal to 2030.556 Fluorescent Arbitrary Units (A.U.) across the line shown in the right-hand image (575 data points), where the measurement was taken.

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Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RP-000006
Stage	Control
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B1FFC5AA93E6703C3A17D8DD4EFF24A38C688981B481503C721B3D023BA749C5
Method PDF SHA-256	AD32CC4A8155CD2B5FC7064846CB2E212BAC22EF561D120F65DCF0EEAB83132C