

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM²

7

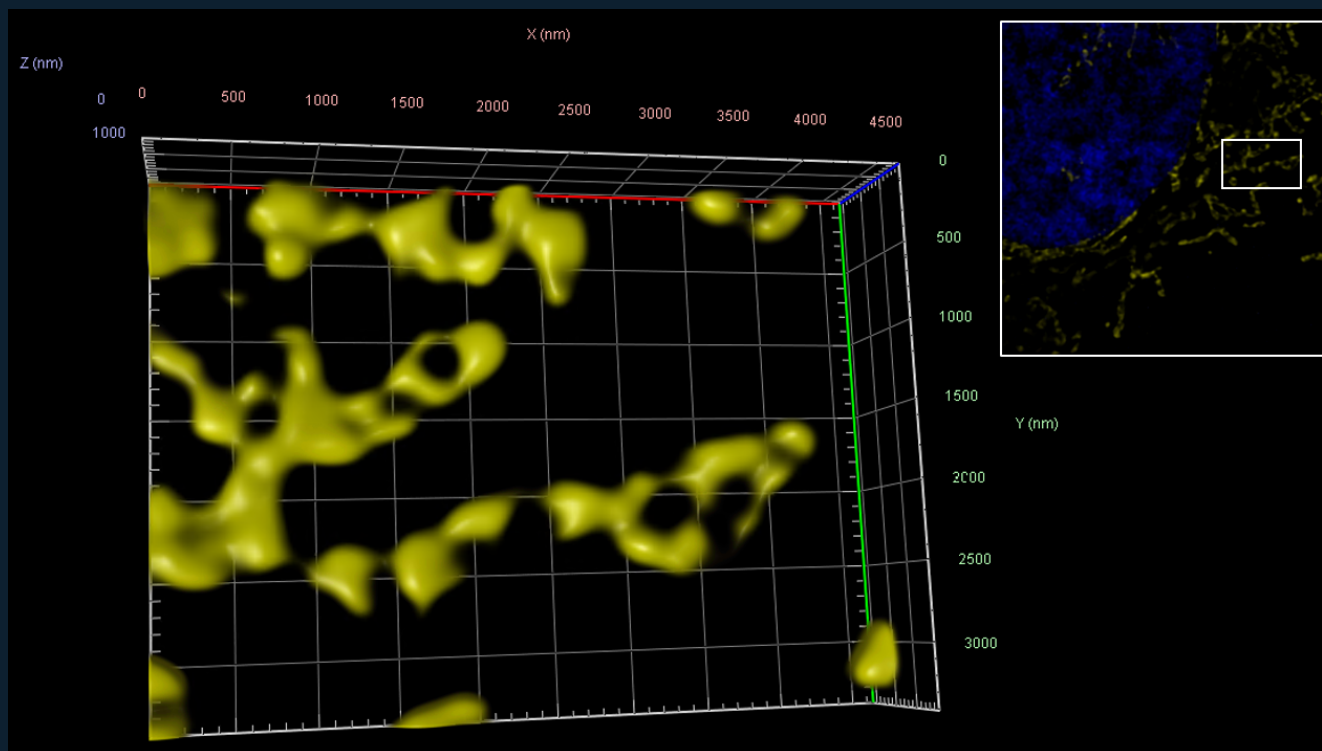
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000026 | 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
- 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 Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse, IgG (H + L), 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 -> Control

BENNETT ASSESSMENT

The preserved SA-000026 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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 / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved SA-000026 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 7 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	532	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555	2; 4AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 528
Widefield SIM — Apotome	405/DAPI; 532; 555	2; 4AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 528
Confocal	405/DAPI; 488; 532	2; None; 561nm @3.5%; 405nm @0.2%; 528; 353; 553; 465
Airyscan	405/DAPI; 488; 532; 647	2; None; 488nm @1.50%; 640nm @0.7%; 528; 653; 553; 668
SIM	405/DAPI; 488; 532	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820#2-SR; T3-Axiocam 820 #2-SR; 488
SIM ²	405/DAPI; 488; 532	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820#2-SR; T3-Axiocam 820 #2-SR; 488
Control	405/DAPI; 532; 555	2; AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 528

MODALITY ASSESSMENT / PART 1 OF 8

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 532.

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 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: EC Plan-Neofluar 20X/0.50 M27. Platform: Zeiss Axio Imager.M1 Widefield, using an EC Plan-Neofluar 20X/0.50 M27, AxioCam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.323µm X 0.323µm; Image size (Pixels): 364 X 360; Image Size (Pixels): 364 X 360; Bit Depth: 12 Bit. Detector/camera: Imaging Device: AxioCam MR R3. Timing: Exposure Time: 300ms; 60ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @35%; X-Cite Xylis Lamp Intensity @5.0%. Optical/scan: Effective NA: 0.5; .5. Structured source metadata values: 31.

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; 532; 555.

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 8

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: 14 Slices (3.90µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.300µm; Image size (Pixels): 761 X 758; Image Size (Pixels): 761 X 758; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 100ms; 10ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @40%; X-Cite Xylis Lamp Intensity @5%. 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: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 532; 555.

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 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.071m X 0.071µm; Image size (Pixels): 1596 X 1596; Image Size (Pixels): 1596 X 1596; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66µs; Frame Time: 31.57s; 431.57s. Illumination: Laser Wavelength: 561nm @3.5%; 405nm @0.2%. Optical/scan: Pinhole: 1.00AU / 48µm; 1.00AU / 37µm; Scan Zoom: 0.90; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 16; Effective NA: 1.4. Structured source metadata values: 40.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 532.

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 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 57 Slices (2.8µm); Channels: 2; Scaling (Per Pixel): 42.53nm X 42.53nm X 50.00nm; Image size (Pixels): 1963 X 1963; Image Size (Pixels): 1963 X 1963; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.53µs; Frame Time: 4.92s. Illumination: Laser Wavelength: 488nm @1.50%; 640nm @0.7%. Optical/scan: Pinhole: 5.00AU / 229µm; 5.00AU / 280µm; Scan Zoom: 1.2; LSM Scan Speed: 7; Scan Direction: Bidirectional; 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; AiryScan Mode: Super Resolution 8.6 (3D, Auto); Super Resolution 9.0 (3D, Auto). Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 39 Slices (1.90µm); Channels: 2; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm; Image size (Pixels): 4110 X 3573; Image Size (Pixels): 4110 X 3573; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820#2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 488nm @7.00%; 405nm @6.0%; Illumination Wavelength: 488; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 35°, Scale Z 32%, 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 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; 532.

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 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 32 Slices (1.55µm); Channels: 2; 1; Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm; Image size (Pixels): 352 X 258; 1508 X 1535; Image Size (Pixels): 352 X 258; 1508 X 1535; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820#2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 488nm @8.00%; 405nm @6.0%; Illumination Wavelength: 488; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 42°, Scale Z 53%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); 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: 62.

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; 532.

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 8

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1600 X 1104; Image Size (Pixels): 1600 X 1104; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

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; 532; 555.

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 SA-000026 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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): 42.53nm X 42.53nm X 50.00nm -> 0.04253 μ m X 0.04253 μ m X 0.05000 μ m. 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)=1963 X 1963; Image Size (Scaled)=83.49 μ m X 83.49 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm -> 0.01320 μ m X 0.01320 μ m X 0.05000 μ m. 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)=4110 X 3573; Image Size (Scaled)=54.23 μ m X 47.15 μ m; 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): 13.20nm X 13.20nm X 50.00nm -> 0.01320 μ m X 0.01320 μ m X 0.05000 μ m. 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)=352 X 258; Image Size (Scaled)=4.64 μ m X 3.40 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.16%.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

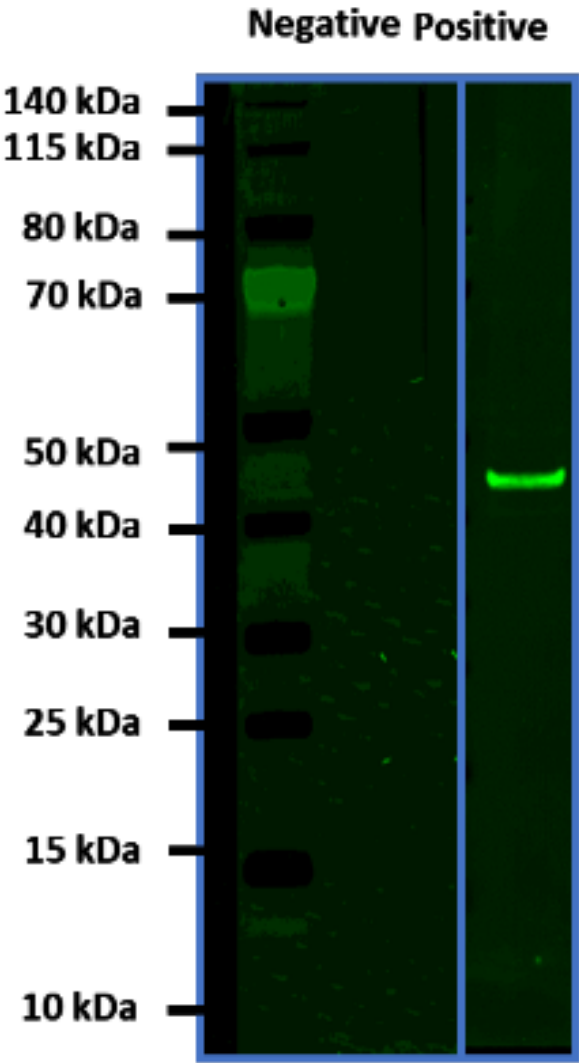
This dossier preserves the complete available evidence progression for SA-000026. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra	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	532
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	532nm
Emission Filter	572±28nm
Detector	Avalanche Photodiode
Intensity	L1
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Identifyn® LLC

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® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

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

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and washed 5X with PBS. The blocked membrane was incubated with Actin Monoclonal Antibody (mAbGEa) (MA1-744) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey anti-Mouse IgG (H+L) was incubated for 1 hour, followed by washing 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 = 532nm

Emission Filter = 572±28nm

Detector = Avalanche Photodiode

Intensity = L1

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

Copyright 2026 GMD12 LLC.

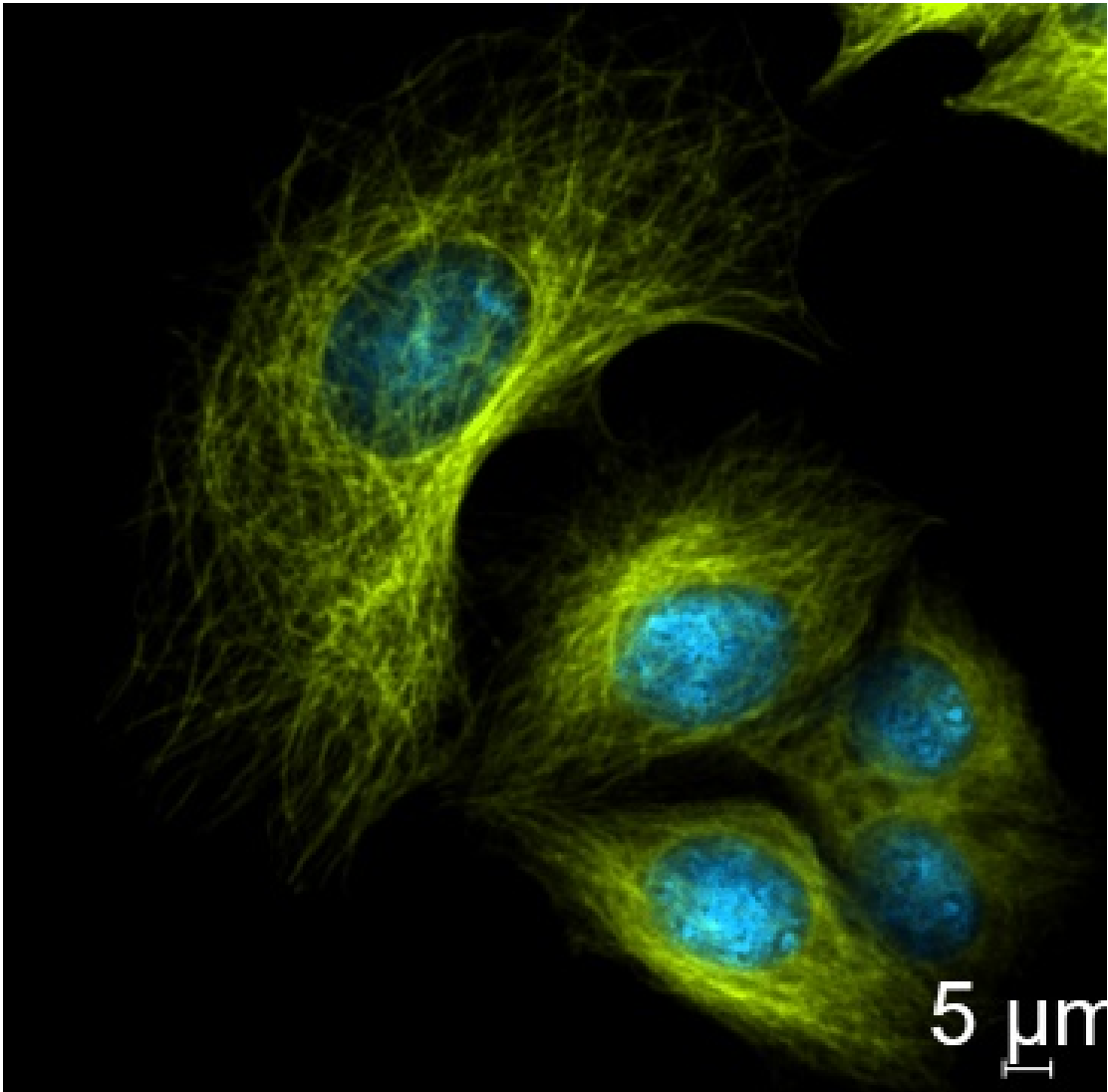
SOURCE PROVENANCE

Catalog	SA-000026
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)

SOURCE PROVENANCE

Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	90390F71528A5411BE2838B8789AAB2C5DABBB4077A02072D0EE4F3784791F9B
Method PDF SHA-256	FA8A0B1A9B413B210A2C6CC603D5089C218C56D352BDD67EEEC29DE166D581DD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555
METADATA VALUES	31

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 an EC Plan-Neofluar 20X/0.50 M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 20X/0.50 M27
Channels	2
Scaling (Per Pixel)	0.323µm X 0.323µm
Image size (Pixels)	364 X 360
Image Size (Scaled)	117.39µm X 116.10µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	4AF532-49014 49 DAPI
Beam Splitter	550 395
Filter Excitation Wavelength	515 - 545 335 - 383
Filter Emission Wavelength	555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @35% X-Cite Xylis Lamp Intensity @5.0%
Exposure Time	300ms 60ms
Excitation Wavelength	528 353
Emission Wavelength	553 465
Effective NA	0.5 .5
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	4.43µm 0.72µm
Binning Mode	1,1

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® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

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#37527X3. The primary antibody, beta-3 Tubulin Monoclonal Antibody (2G10), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Imager.M1 Widefield, using an EC Plan-Neofluar 20X/0.50 M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.323µm X 0.323µm

Image size (Pixels) = 364 X 360

Image Size (Scaled) = 117.39µm X 116.10µm

Bit Depth = 12 Bit

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

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

532 -

Reflector = 4AF532-49014

Beam Splitter = 550

Filter Excitation Wavelength = 515 - 545

Filter Emission Wavelength = 555 - 595

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @35%

Exposure Time = 300ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 0.5

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 4.43µm

Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @5.0%
 Exposure Time = 60ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA =.5
 Imaging Device = Axiocam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 1,1

Post Processing

None

Image Correction

None

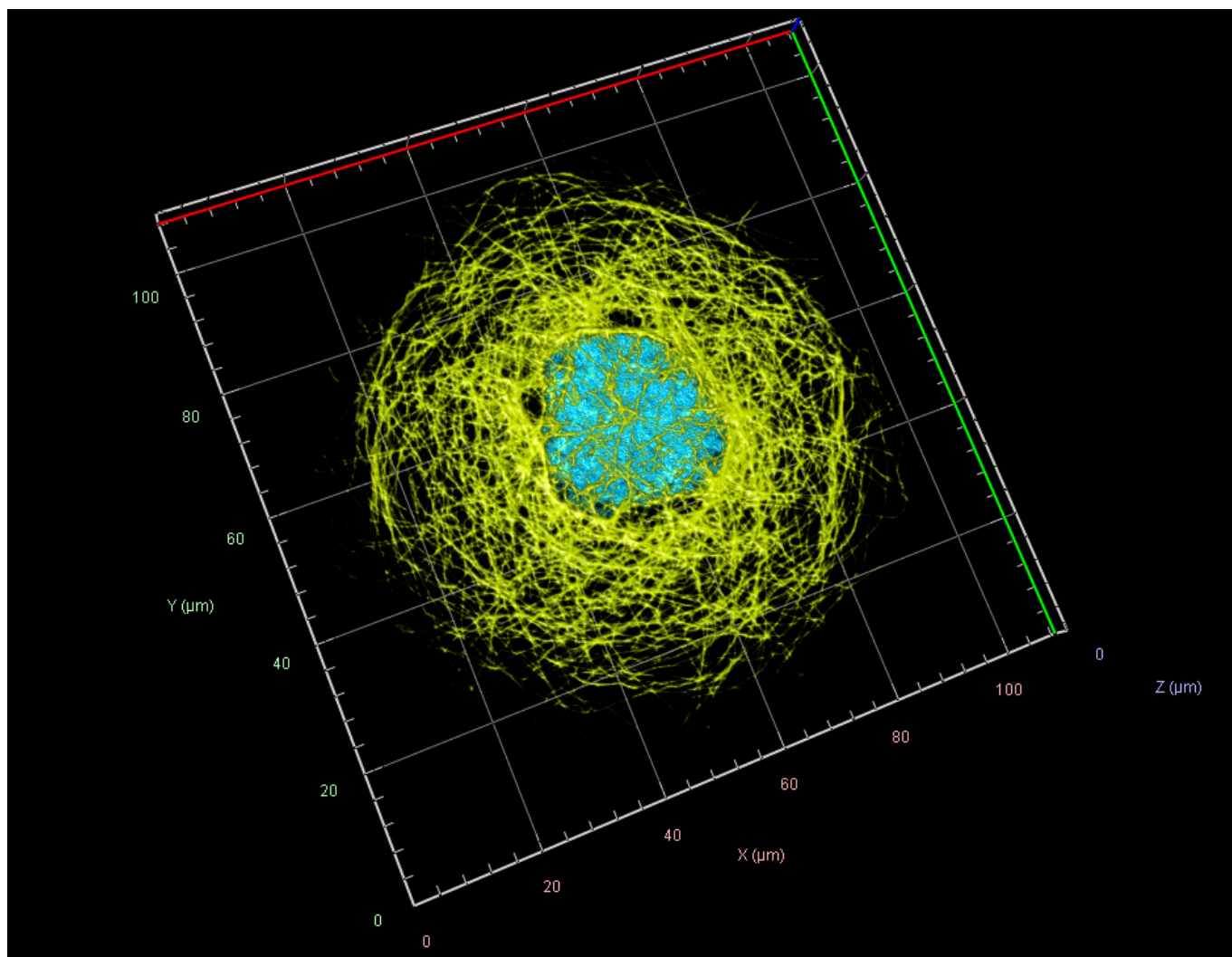
Image by E.F. Simon

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SOURCE PROVENANCE

Catalog	SA-000026
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using an EC Plan-Neofluar 20X/0.50 M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	31
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	84B9ED17046E648FEEC6C3C99788C3FB4C0D33BFB3811985E1153EF242FAE0F5
Method PDF SHA-256	1CEE459A35BE8D4A0A5417D1B14DE3B623255DE9B718521452F45614D36886CD

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	MCF-7
DETECTED DYES	405/DAPI; 532; 555
METADATA VALUES	38

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	14 Slices (3.90µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.300µm
Image Size (Pixels)	761 X 758
Image Size (Scaled)	108.71µm X 108.29µm
Bit Depth	14 Bit
Image Center Position	X: 71.49mm, Y: 71.49mm
Stage Position	X: 71.49mm, Y: 71.49mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	4AF532-49014 49 DAPI
Beam Splitter	550 395
Filter Excitation Wavelength	515 - 545 335 - 383
Filter Emission Wavelength	555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @40% X-Cite Xylis Lamp Intensity @5%
Exposure Time	100ms 10ms
Excitation Wavelength	528 353
Emission Wavelength	553 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.07µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 23% all channels, Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 175%, 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® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

MCF7 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#37527X3. The primary antibody, beta-3 Tubulin Monoclonal Antibody (2G10), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 14 Slices (3.90µm)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.300µm

Image Size (Pixels) = 761 X 758

Image Size (Scaled) = 108.71µm X 108.29µm

Bit Depth = 14 Bit

Image Center Position = X: 71.49mm, Y: 71.49mm

Stage Position = X: 71.49mm, Y: 71.49mm

ROI Center Offset = X: 1.14µm, Y: 0.00µm

532 -

Reflector = 4AF532-49014

Beam Splitter = 550

Filter Excitation Wavelength = 515 - 545

Filter Emission Wavelength = 555 - 595

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @40%

Exposure Time = 100ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.25

Imaging Device = Axiocam 807

Camera Adapter = 1X

Depth of Focus = 1.07µm

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 @5%
Exposure Time = 10ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 23% all channels, Ramp 50% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 175%, 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)

Image Corrections

None

Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

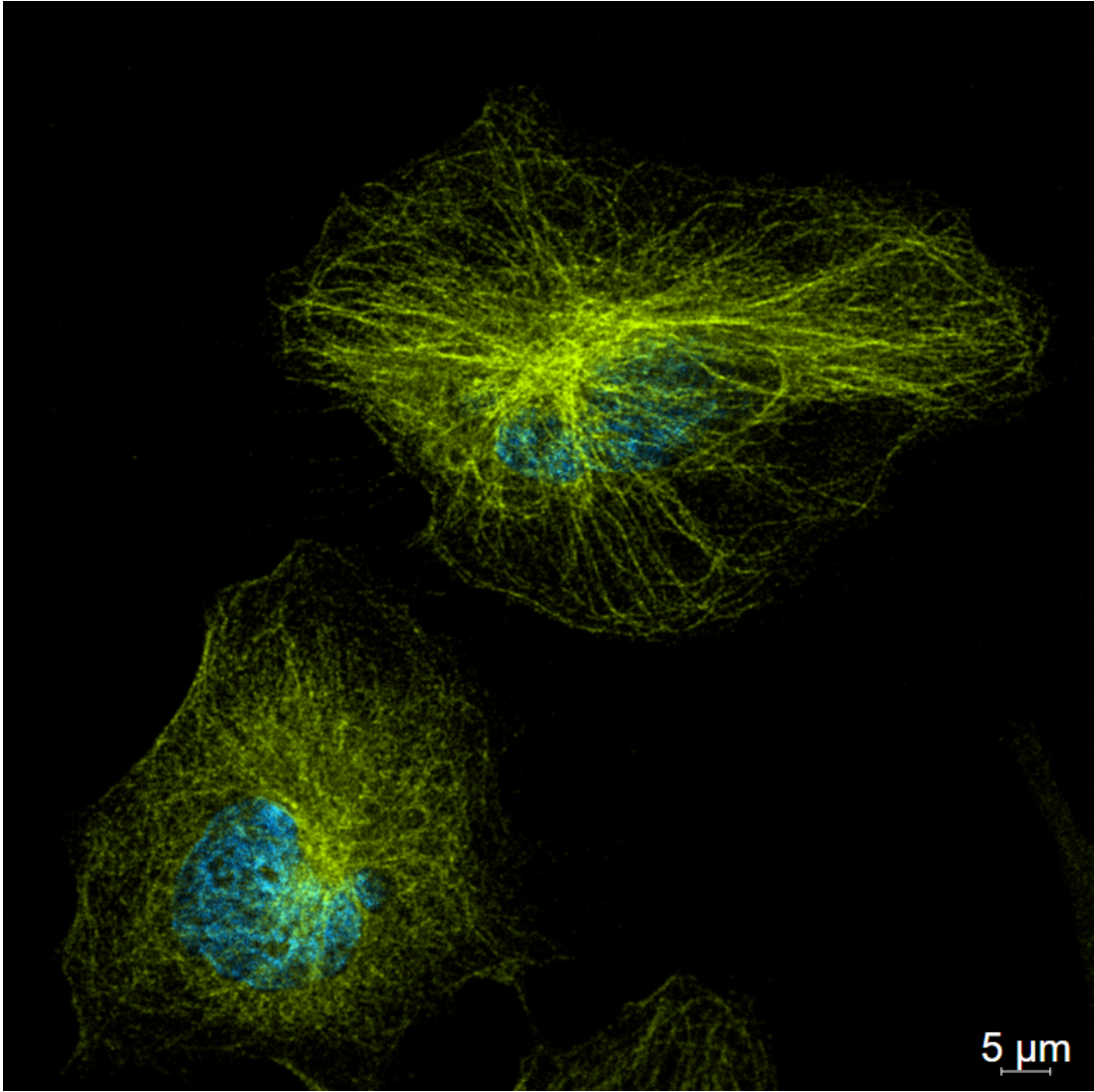
SOURCE PROVENANCE

Catalog	SA-000026
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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	MCF-7
Image SHA-256	6182348D7B60FCEBDC599FFD5C580156D5615559BD896F2B4376892770C9ACC7
Method PDF SHA-256	04197087F6414D87E308A5DDF6B8E0C6102ACD1454748A5B085C980450F622C7

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	40

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Channels	2
Scaling (Per Pixel)	0.071m X 0.071µm
Image size (Pixels)	1596 X 1596
Image Size (Scaled)	112.68µm X 112.68µm
Bit Depth	16 Bit
Image Center Position	X: 54.66mm, Y: 48.69mm
Stage Position	X: 54.66mm, Y: 48.69mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 48µm 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @3.5% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	0.90
Rotation	0°
Sampling	1.00
Pixel Time	0.66µs
Frame Time	31.57s 431.57s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	16
Excitation Wavelength	528 353
Emission Wavelength	553 465
Effective NA	1.4
Detection Wavelength	510 - 590 410 - 470
Imaging Device	Airyscan
Detector Type	GaAsp-PMT
Detector Gain	800 V 650 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0

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® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)
Cat ID# - SA 000026

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#37527X3. The primary antibody, beta-3 Tubulin Monoclonal Antibody (2G10), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.071m X 0.071µm
Image size (Pixels) = 1596 X 1596
Image Size (Scaled) = 112.68µm X 112.68µm
Bit Depth = 16 Bit
Image Center Position = X: 54.66mm, Y: 48.69mm
Stage Position =X: 54.66mm, Y: 48.69mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 48µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @3.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.90
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.66µs
 Frame Time = 31.57s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 16
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Detection Wavelength = 510 - 590
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 37µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.90
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.66µs
 Frame Time = 431.57s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 16
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

Post Processing

N/A

Image Corrections

None

Image by J.K. Bennett

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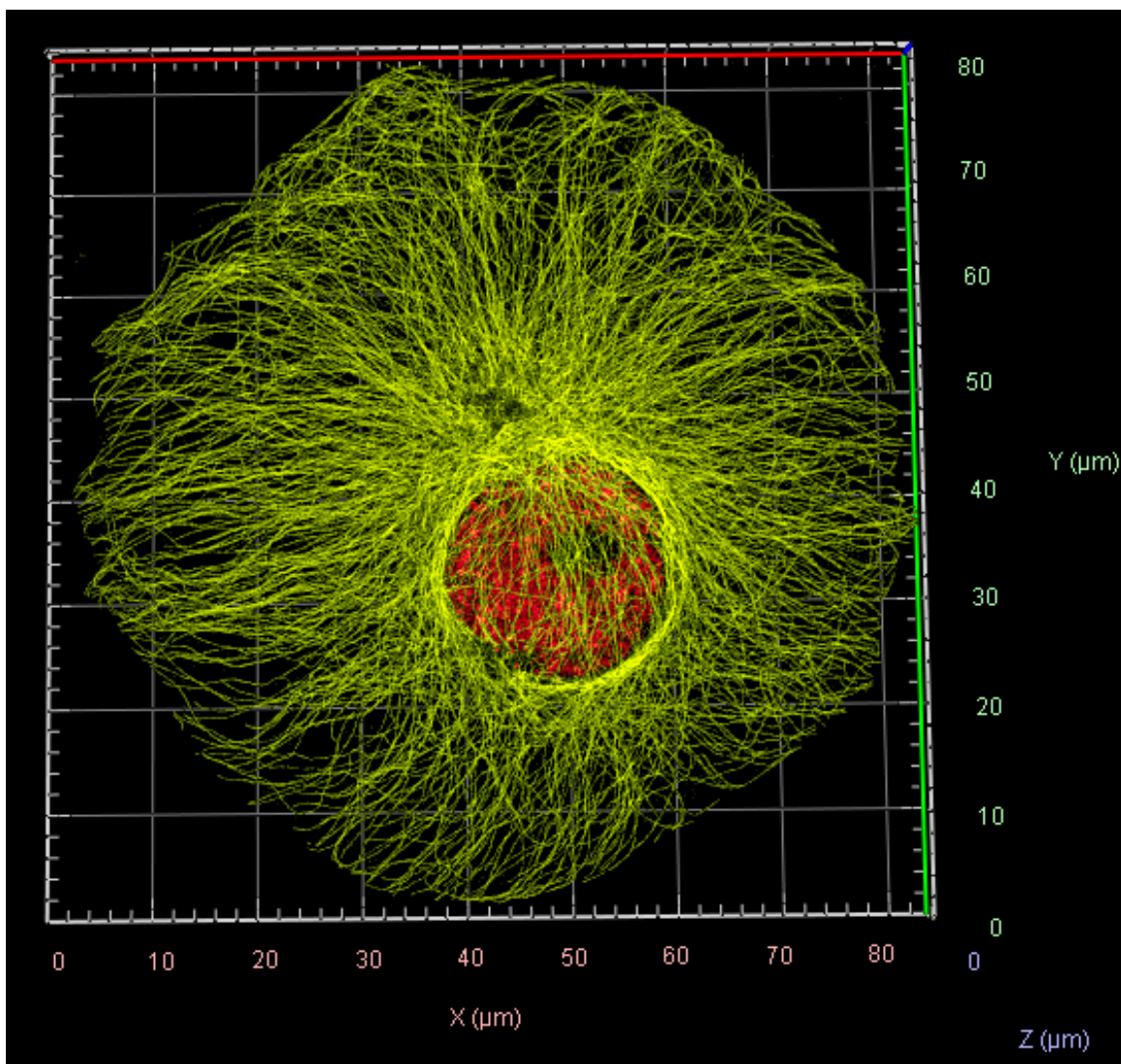
SOURCE PROVENANCE

Catalog	SA-000026
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C7D538DF133A5BFF1A5B370075EECF6E0B574F5F56D3E60B054E21C580FC4EC7
Method PDF SHA-256	EBEC061BB25431A4C1E989DF55D084C72EDB9344FAF5137A9AB5A9467BC8ED54

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	57 Slices (2.8µm)
Channels	2
Scaling (Per Pixel)	0.04253 µm X 0.04253 µm X 0.05000 µm
Image Size (Pixels)	1963 X 1963
Image Size (Scaled)	83.49µm X 83.49µm
Bit Depth	16 Bit
Image Center Position	X: 72.22mm, Y: 46.46mm
Stage Position	X: 72.22mm, Y: 46.46mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 229µm 5.00AU / 280µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @1.50% 640nm @0.7%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.2
Rotation	0°
Sampling	2.00
Pixel Time	0.53µs
Frame Time	4.92s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	528 653
Emission Wavelength	553 668
Effective NA	1.4
Detection Wavelength	515 - 580 635 - 700
Imaging Device	Airyscan
Detector Type	GaAsp-PMT
Detector Gain	850 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.6 (3D, Auto) Super Resolution 9.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 6% (647), Threshold 4% (532), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate
Cat ID# - DC 000020

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)
Cat ID# - SA 000026

Gamma H2AX (γ H2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of DSBs. When DSBs occur in chromatin, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ H2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 μ M 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 γ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 57 Slices (2.8 μ m)

Channels = 2

Scaling (Per Pixel) = 42.53nm X 42.53nm X 50.00nm

Image Size (Pixels) = 1963 X 1963

Image Size (Scaled) = 83.49 μ m X 83.49 μ m

Bit Depth = 16 Bit

Image Center Position = X: 72.22mm, Y: 46.46mm

Stage Position = X: 72.22mm, Y: 46.46mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 229µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @1.50%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.53µs
 Frame Time = 4.92s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 2
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Detection Wavelength = 515 - 580
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.6 (3D, Auto)

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 280µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.7%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.53µs
 Frame Time = 4.92s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 2
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 635 - 700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.0 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 6% (647), Threshold 4% (532), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -**None**

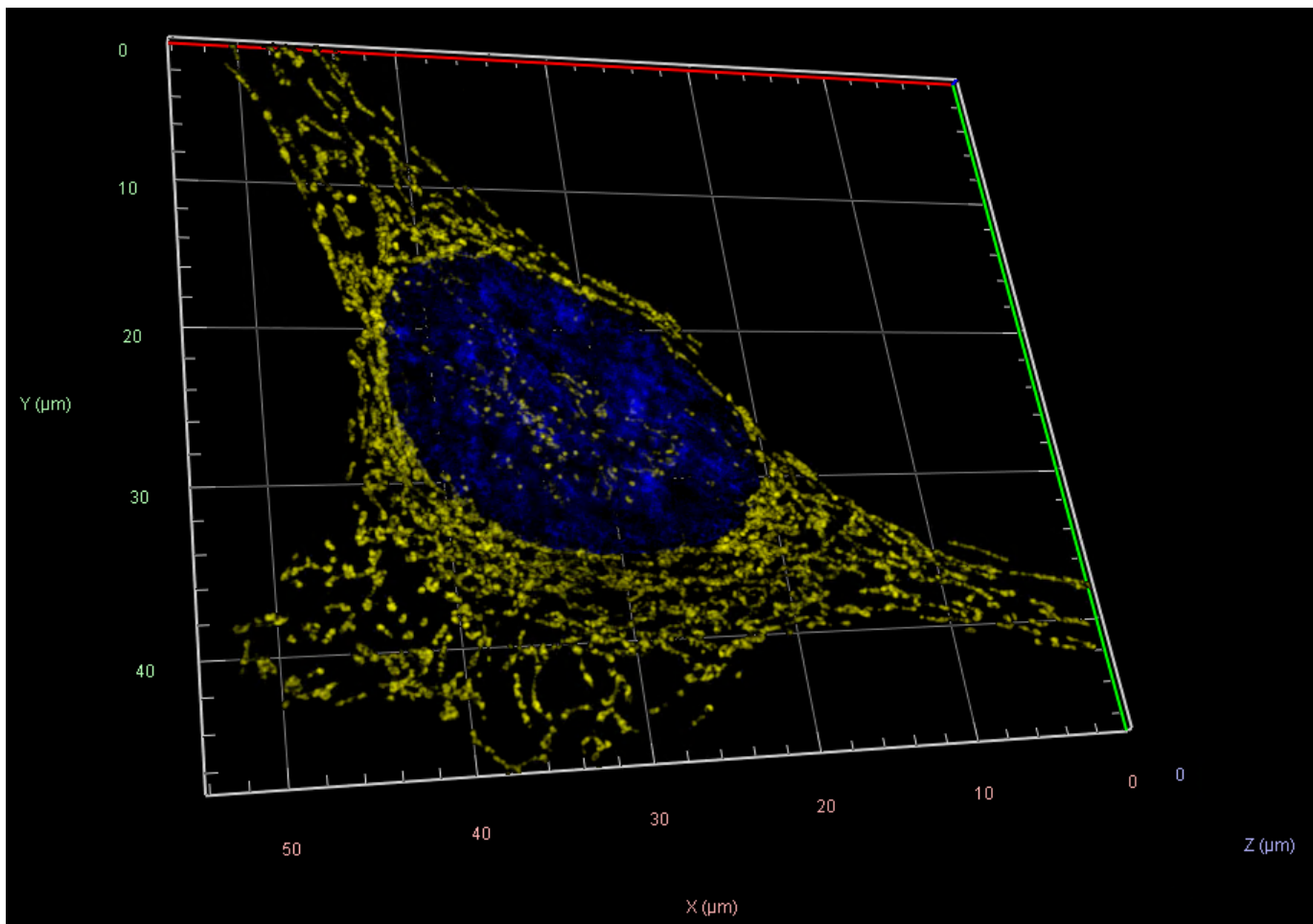
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000026
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	885DD38310572B5045717E2B8E246359C33AD11E7E76FAD064100208CED8E746
Method PDF SHA-256	64CABFB30139842D54E9734388140485ACAC743957387132A52E96C22E00E0DD

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	39 Slices (1.90µm)
Channels	2 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.05000 µm
Image Size (Pixels)	4110 X 3573
Image Size (Scaled)	54.23µm X 47.15µm
Bit Depth	16 Bit
Image Center Position	X: 59.11mm, Y: 42.53mm
Stage Position	X: 59.11mm, Y: 42.53mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 405
Channel Name	T2-Axiocam 820#2-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	488 405
Emission Wavelength	525 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820#2
Camera Adapter	1X
Exposure Time	120ms 50ms
Depth of Focus	0.81µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @7.00% 405nm @6.0%
Frame Time	1.59s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.4463 (532)
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Maximum Projection	Precise
Appearance	Threshold 1% (532), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 80%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 32%, 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 Identifyfyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyfyn Standards for Optical Microscopy Methods™

Identifyfyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

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#37527X3. The primary antibody, Mitochondria Antibody (MA5-12017), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyfyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 39 Slices (1.90µm)

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 50.00nm

Image Size (Pixels) = 4110 X 3573

Image Size (Scaled) = 54.23µm X 47.15µm

Bit Depth = 16 Bit

Image Center Position = X: 59.11mm, Y: 42.53mm

Stage Position = X: 59.11mm, Y: 42.53mm

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

532 -

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#2-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#2
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 488nm @7.00%
 Frame Time = 1.59s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 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 = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D

Result Sampling = 4

Process Sampling = 2

Channels = 1

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.4463 (532)

Input SNR = N/A

Regularization Weight = N/A

Iterations = N/A

Filter = N/A

Fitting = N/A

Normalization = Automatic

Experimental PSF = N/A

3D Image Processing

3D Maximum Projection = Precise

Appearance = Threshold 1% (532), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 80%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 32%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

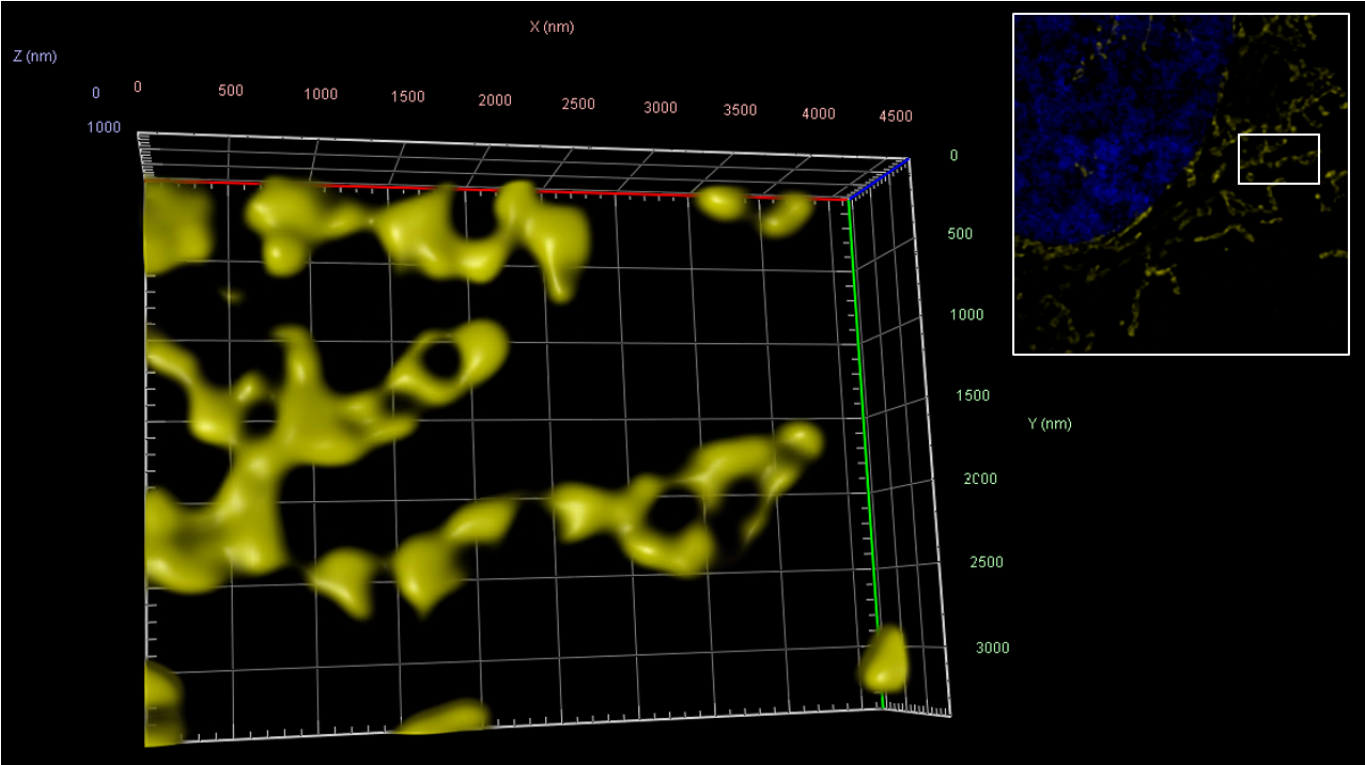
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000026
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5A8EC9D891DFFBF322F4DD9F79FFD3ED985262B9ECDF65C51295A03876265120
Method PDF SHA-256	4484EB608238361656259114E5189B6DF7B7ED471E78C292485C82C7C78C1098

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	62

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	32 Slices (1.55µm)
Channels	2 1
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.05000 µm
Image Size (Pixels)	352 X 258 1508 X 1535
Image Size (Scaled)	4.64µm X 3.40µm 19.90µm X 20.25µm
Bit Depth	16 Bit
Image Center Position	X: 59.61mm, Y: 35.29mm
Stage Position	X: 59.61mm, Y: 35.29mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 405
Channel Name	T2-Axiocam 820#2-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	488 405
Emission Wavelength	525 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820#2
Camera Adapter	1X
Exposure Time	120ms 50ms
Depth of Focus	0.81µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @8.00% 405nm @6.0%
Frame Time	1.59s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.9788
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Volume Projection	Precise
Appearance	Threshold 8% (532), Ramp 92% (DAPI), Maximum 100% Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% call channels, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°Enabled Directional Light and Tone Mapping Brightness 100%,Enabled Tone Mapping
Projection	View Angle 42°, Scale Z 53%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Maximum Projection	Precise

SOURCE METHOD

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

Identifyfyn Standards for Optical Microscopy Methods™

Identifyfyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

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#37527X3. The primary antibody, Mitochondria Antibody (MA5-12017), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyfyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Main Image

Z-Stack = 32 Slices (1.55µm)

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 50.00nm

Image Size (Pixels) = 352 X 258

Image Size (Scaled) = 4.64µm X 3.40µm

Bit Depth = 16 Bit

Image Center Position = X: 59.61mm, Y: 35.29mm

Stage Position = X: 59.61mm, Y: 35.29mm

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

Z Stack - (3D) - Inset Image, Upper Right

Z-Stack = 32 Slices (1.55µm)

Channels = 2

Scaling (Per Pixel) = 13.20nm X 13.20nm X 50.00nm

Image Size (Pixels) = 1508 X 1535

Image Size (Scaled) = 19.90µm X 20.25µm

Bit Depth = 16 Bit

Image Center Position = X: 59.61mm, Y: 35.29mm

Stage Position = X: 59.61mm, Y: 35.29mm

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

532 -

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#2-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#2
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 488nm @8.00%
 Frame Time = 1.59s
 Scan Direction = Unidirectional
 Grating 36.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 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 = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

 Post Processing - SIM2 Processing
 Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 1

Algorithm = SIM2
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 8.9788
 Input SNR = N/A
 Regularization Weight = N/A
 Iterations = N/A
 Filter = N/A
 Fitting = N/A
 Normalization = Automatic
 Experimental PSF = N/A

3D Image Processing - Main Image

3D Volume Projection = Precise
 Appearance = Threshold 8% (532), Ramp 92% (DAPI), Maximum 100%
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°Enabled Directional Light and Tone Mapping
 Projection = View Angle 42°, Scale Z 53%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Inset Image, Upper Right

3D Maximum Projection = Precise
 Appearance = Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% call channels, Maximum 100%
 Background = Black
 Light = Brightness 100%,Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

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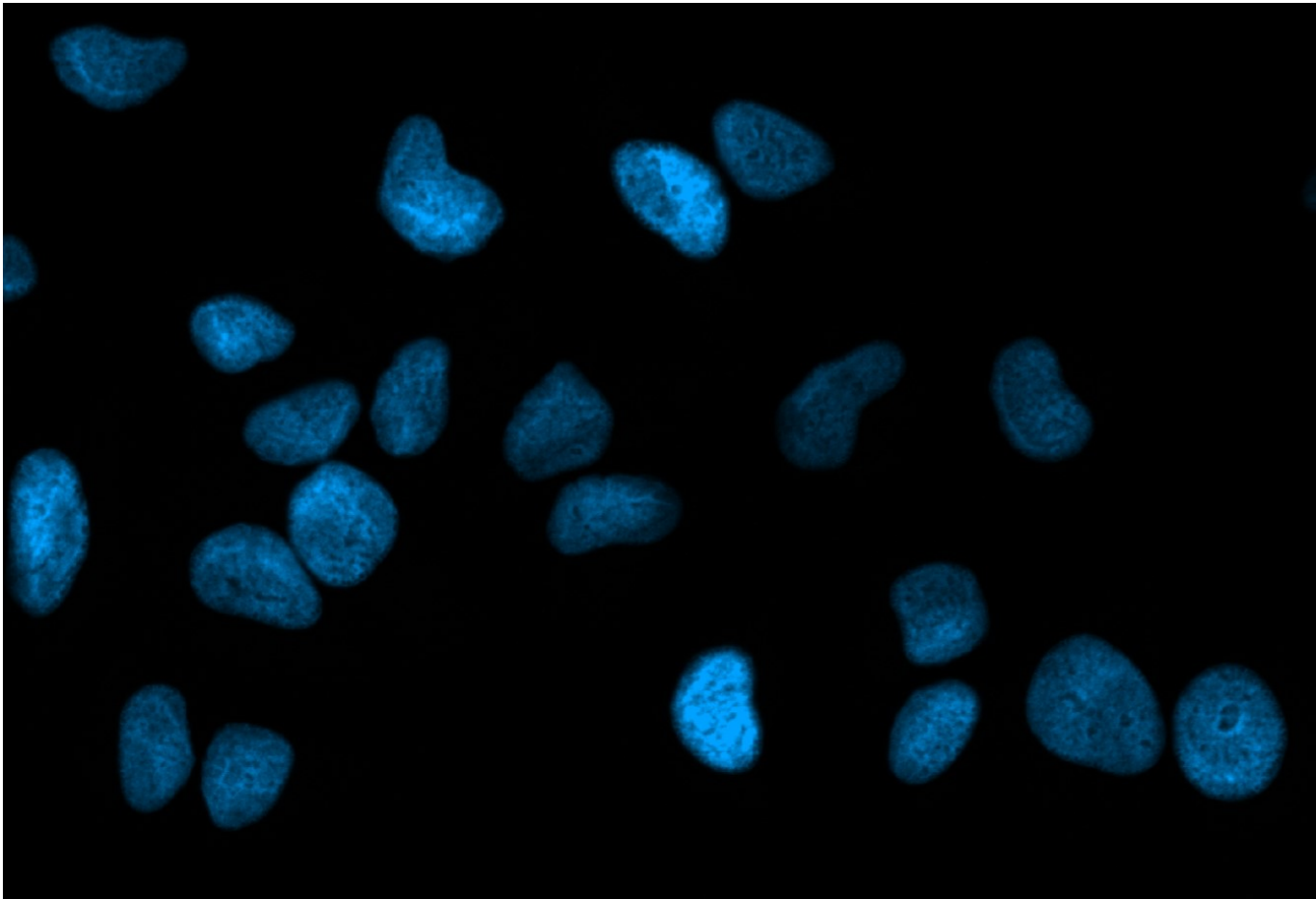
SOURCE PROVENANCE

Catalog	SA-000026
Stage	SIM ²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	AF69A105330CA053395A9032FADFB31BDF87A9C75B9823FD7AE8B1A2B9702CFA
Method PDF SHA-256	F31A2E21FB0FABC28FB8B43E508621026773CDE573CEB9516E0138E27F6B349A

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1600 X 1104
Image Size (Scaled)	228.57µm X 157.71µm
Bit Depth	14 Bit
Image Center Position	X: 52.67mm, Y: 50.57mm
Stage Position	X: 52.67mm, Y: 50.57mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	AF532-49014 49 DAPI
Beam Splitter	550 395
Filter Excitation Wavelength	515 - 545 335 - 383
Filter Emission Wavelength	555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	528 353
Emission Wavelength	553 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.07µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Donkey Anti-Mouse IgG (H + L)

Cat ID# - SA 000026

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#37527X3. In the absence of a primary antibody, the Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) = 1600 X 1104

Image Size (Scaled) = 228.57µm X 157.71µm

Bit Depth = 14 Bit

Image Center Position = X: 52.67mm, Y: 50.57mm

Stage Position = X: 52.67mm, Y: 50.57mm

ROI Center Offset = X: 1.14µm, Y: 0.00µm

532 -

Reflector = AF532-49014

Beam Splitter = 550

Filter Excitation Wavelength = 515 - 545

Filter Emission Wavelength = 555 - 595

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @25%

Exposure Time = 200ms

Excitation Wavelength = 528

Emission Wavelength = 553

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.07µm

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%
 Exposure Time = 30ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.90µm
 Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used.

Image Corrections

None

Control Summary -

In the absence of a primary antibody, Identifyn Standards for Optical Microscopy Methods™ were applied using standard staining methodology and image acquisition settings using the Zeiss Apotome SIM. With no image correction, the image demonstrates that the secondary antibody, minus a primary target, had no off-target binding and no significant presence of free dye within the sample.

The control data suggests the secondary antibody is highly specific and free of unconjugated (free) dye post-conjugation.

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000026
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	32

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7540E2E43C3A876BC29C931DBDF586F28BE31DC9EA6E51492820712F09FBE9ED
Method PDF SHA-256	F9B47FBE6A19E88F58C586BA4684E902BC2230BB7000FD6F940C568D25E7425B