

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)

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

7

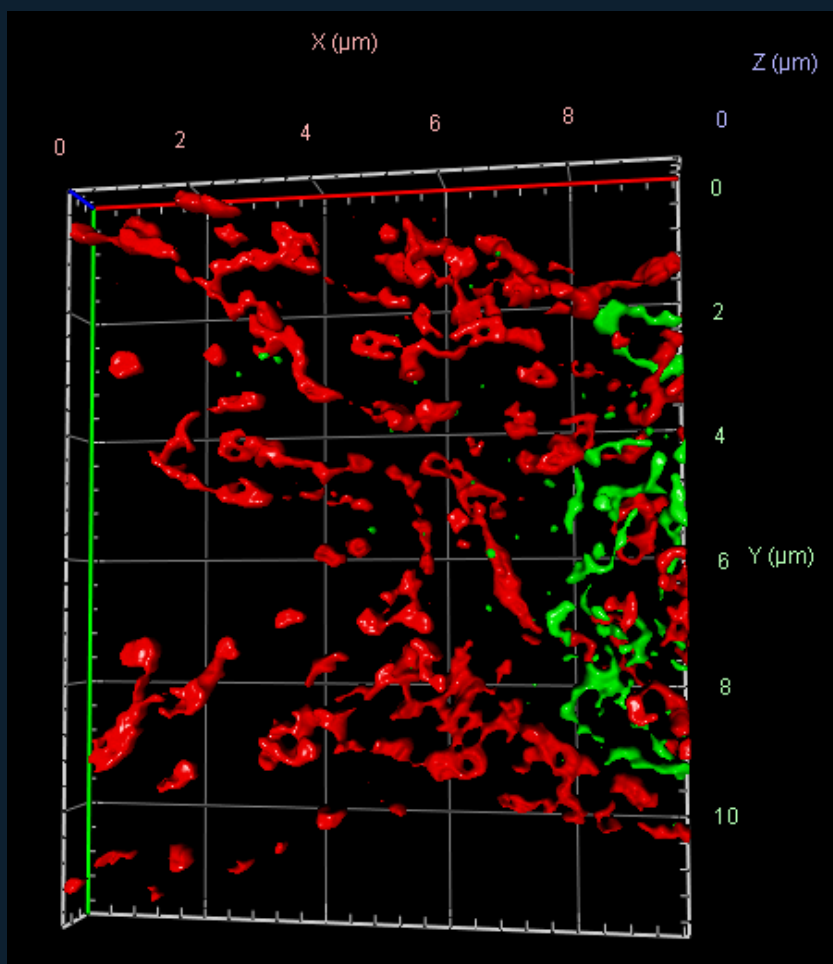
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000053 | 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
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (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-000053 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-000053 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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	2; 45 Texas Red; 38 eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	3; None; 561nm @0.10%; 488nm @0.03%; 405nm @0.5%; 590; 493; 353
Airyscan	405/DAPI; 488; 594	2; None; 561nm @0.5%; 488nm @0.2%; 590; 493; 618; 517
SIM	405/DAPI; 488; 594	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; 561; 405
SIM ²	405/DAPI; 488; 594	2; 561nm @5.0%; 488nm @1.50%; 561; 488; 515; 655; 561.000000 (594)
Control	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 590

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 594.

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: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 1208 X 788; Image Size (Pixels): 1208 X 788; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 50ms; 45ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; X-Cite Xylis Lamp Intensity @6.0%. 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; 594.

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: 98 Slices (9.7µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 614 X 977; Image Size (Pixels): 614 X 977; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 150ms; 180ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @8.0%; X-Cite Xylis Lamp Intensity @15%. 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: 51.

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; 594.

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: Z-Stack: 43 Slices (2.94µm); Channels: 3; Scaling (Per Pixel): 0.152µm X 0.152µm X 0.070µm; Image size (Pixels): 512 X 512; Image Size (Pixels): 512 X 512; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt2; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.85µs; Frame Time: 2.09s. Illumination: Laser Wavelength: 561nm @0.10%; 488nm @0.03%. Optical/scan: Pinhole: 1.00AU / 54µm; 1.00AU / 45µm; Scan Zoom: 1.3; LSM Scan Speed: 10; 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. Structured source metadata values: 52.

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; 594.

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 AiryScan, 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: 66 Slices (3.25µm); Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 50.00nm; Image size (Pixels): 1719 X 1576; Image Size (Pixels): 1719 X 1576; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.73µs; Frame Time: 14.22s. Illumination: Laser Wavelength: 561nm @0.5%; 488nm @0.2%. Optical/scan: Pinhole: 5.00 AU / 268µm; 5.31 AU / 236µm; Scan Zoom: 1.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; 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 9.6 (3D, Auto); Super Resolution 10.0 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 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: 45 Slices (2.20µm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 1751 X 1456; Image Size (Pixels): 1751 X 1456; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2; AxioCam 820#2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @3.00%; 405nm @7.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 53°, 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 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; 594.

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 7. Objective: Plan-Apochromat 63X/1.46 oil Korr M27. Platform: Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 20 Slices (1.14µm); Channels: 2; Scaling (Per Pixel): 15.65nm X 15.65nm X 60.00nm; Image size (Pixels): 618 X 755; Image Size (Pixels): 618 X 755; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 561nm @5.0%; 488nm @1.50%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 4; Effective NA: 1.46. 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: 59.

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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): 1084 X 1070; Image Size (Pixels): 1084 X 1070; 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; 594.

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-000053 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): 35.30nm X 35.30nm X 50.00nm -> 0.03530 μ m X 0.03530 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)=1719 X 1576; Image Size (Scaled)=60.68 μ m X 55.63 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689 μ m X 0.01689 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)=1751 X 1456; Image Size (Scaled)=29.57 μ m X 24.59 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 15.65nm X 15.65nm X 60.00nm -> 0.01565 μ m X 0.01565 μ m X 0.06000 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)=618 X 755; Image Size (Scaled)=9.67 μ m X 11.82 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

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

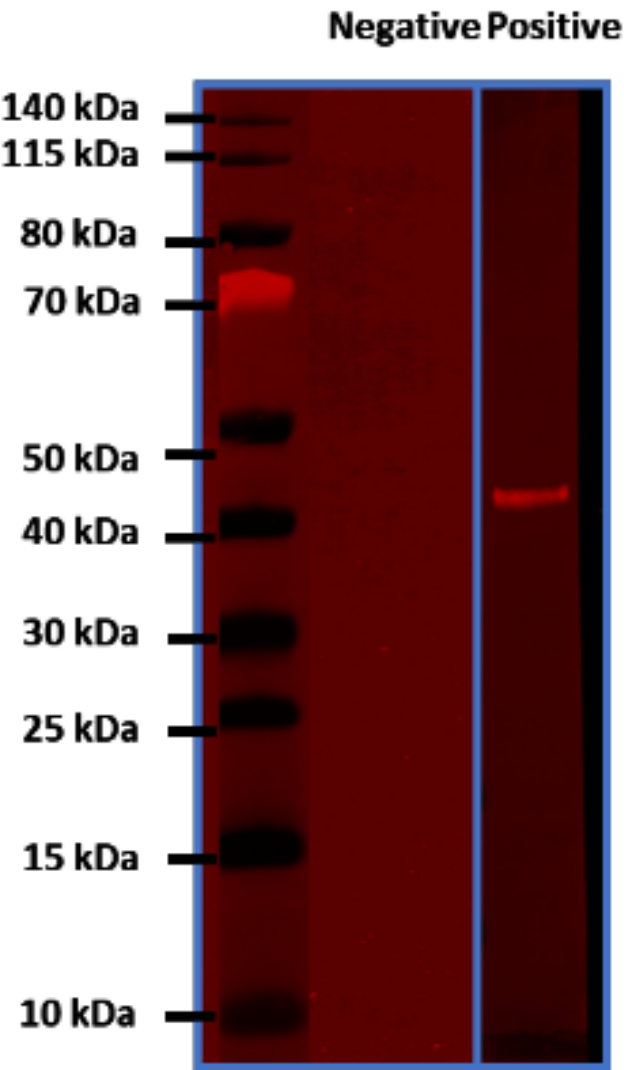
This dossier preserves the complete available evidence progression for SA-000053. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 7	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	594
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	624±40nm
Detector	Avalanche Photodiode
Intensity	4
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™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)
Cat ID# - SA 000053

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™ 594 Affinity Purified Donkey anti-Mouse F(ab)₂ 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 = 532nm
Emission Filter = 624±40nm
Detector = Avalanche Photodiode
Intensity = 4
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.

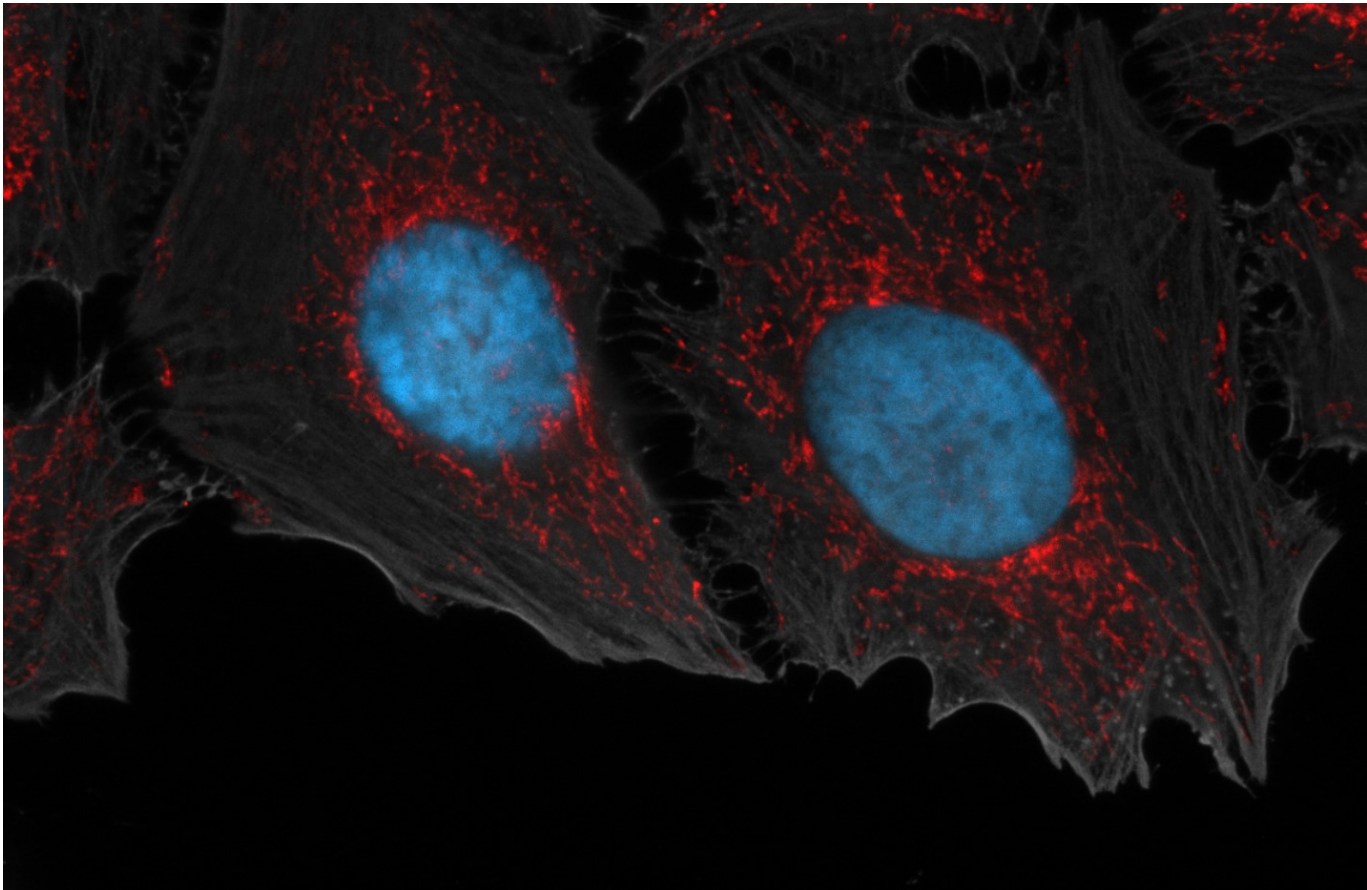
SOURCE PROVENANCE

Catalog	SA-000053
Stage	Western blot
Instrument	Azure Sapphire FL

SOURCE PROVENANCE

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	A57ECD68F5BB141C9D0B916F0FD206AE8B0283D8C0E8391CB9C9FC4FFC4CDD97
Method PDF SHA-256	F345927B64656EE809D3D7EDB89FDF2D88D2F2B1A0C48768813E798479A89334

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
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	2
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	1208 X 788
Image Size (Scaled)	83.40µm X 54.40µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	45 Texas Red 38 eGFP 49 DAPI
Beam Splitter	585 495 395
Filter Excitation Wavelength	540 - 580 450 - 490 335 - 383
Filter Emission Wavelength	593 - 668 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @6.0% X-Cite Xylis Lamp Intensity @12%
Exposure Time	50ms 45ms 20ms
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.96µm 0.80µm 0.72µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

Identifyn® SRM Alexa Fluor™ 488 Affinity Goat Anti-Rabbit IgG (H + L)

Cat ID# - SA 000013

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 first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 1208 X 788

Image Size (Scaled) = 83.40µm X 54.40µm

Bit Depth = 14 Bit

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

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

594 -

Reflector = 45 Texas Red

Beam Splitter = 585

Filter Excitation Wavelength = 540 - 580

Filter Emission Wavelength = 593 - 668

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @10%

Exposure Time = 50ms

Excitation Wavelength = 590

Emission Wavelength = 618

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 0.96µm

Binning Mode = 2,2

488 -

Reflector = 38 eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @6.0%
 Exposure Time = 45ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80µm
 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 @12%
 Exposure Time = 20ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 2,2

Post Processing

None

Image Correction

Identifyn® SRM Alexa Fluor™ 488 was pseudo-colored in Zen Software from green, the default color, to dark grey to provide visual contrast between the 594/488 channels.

Image by J.K. Bennett

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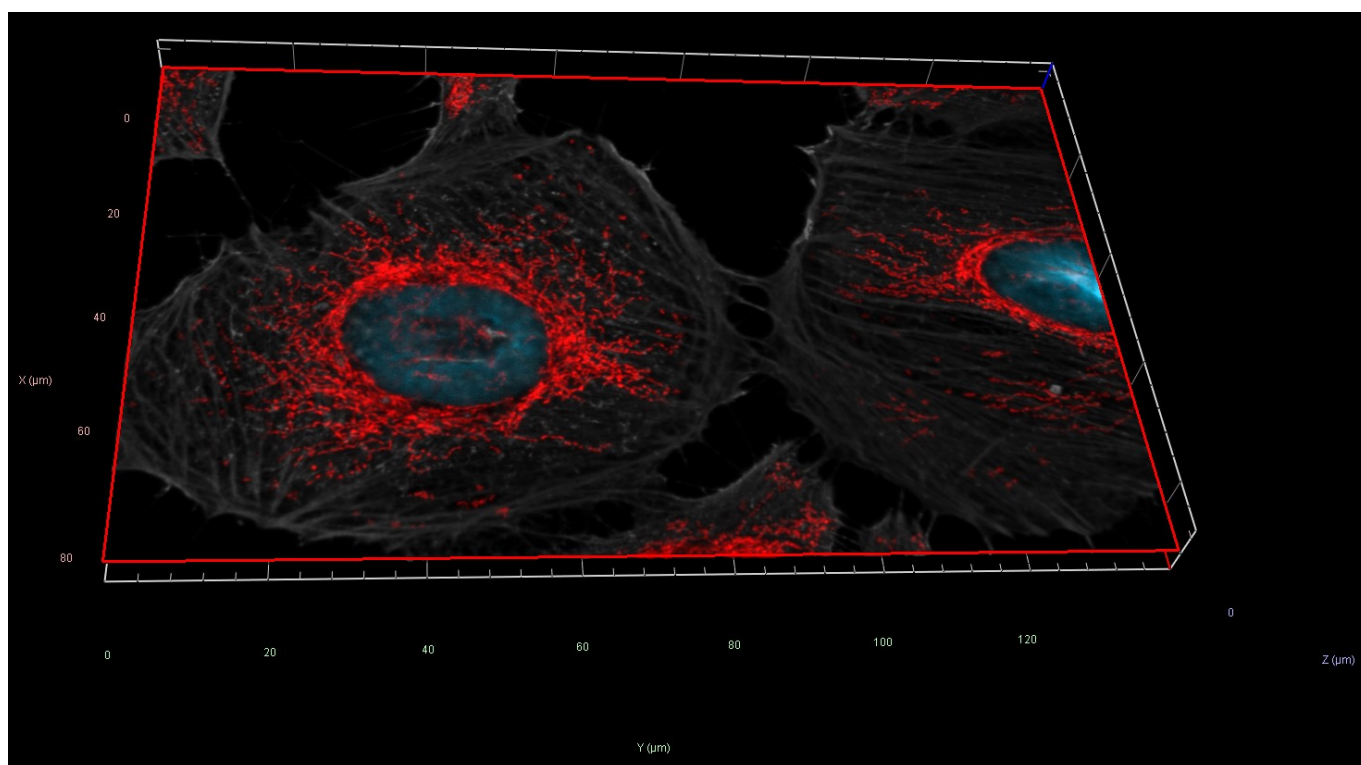
SOURCE PROVENANCE

Catalog	SA-000053
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SOURCE PROVENANCE

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 Xylys 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	B9D6A4EA0D44F07D0F9A6858707B3F741CFEE5A042D69CB173E2C5254EF52B0C
Method PDF SHA-256	569BE52C52E3A22AF6FF085FD069AAE72F618972BC770436AD0D8552A5C583C7

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	51

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	98 Slices (9.7µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	614 X 977
Image Size (Scaled)	87.71µm X 139.57µm
Bit Depth	14 Bit
Image Center Position	X: 91.09mm, Y: 56.35mm
Stage Position	X: 91.09mm, Y: 56.35mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	45 Texas Red 38HE eGFP 49 DAPI
Beam Splitter	585 495 395
Filter Excitation Wavelength	540 - 580 450 - 490 335 - 383
Filter Emission Wavelength	593 - 668 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @8.0% X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms 180ms 18ms
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.20µm 1.00µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
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.

FIELD	SOURCE-DOCUMENTED VALUE
X - Y	Active was selected, Textured Opaque was chosen to remove the obstruction from the image above the cut plane, and a red outline of the clipped perimeter was shown; Clip Back was Selected, and Clip Front was NOT selected.
Position of Clip	-10%
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® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

Identifyn® SRM Alexa Fluor™ 488 Affinity Goat Anti-Rabbit IgG (H + L)

Cat ID# - SA 000013

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 first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 98 Slices (9.7µm)

Channels = 3

Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.100µm

Image Size (Pixels) = 614 X 977

Image Size (Scaled) = 87.71µm X 139.57µm

Bit Depth = 14 Bit

Image Center Position = X: 91.09mm, Y: 56.35mm

Stage Position = X: 91.09mm, Y: 56.35mm

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

594 -

Reflector = 45 Texas Red
 Beam Splitter = 585
 Filter Excitation Wavelength = 540 - 580
 Filter Emission Wavelength = 593 - 668
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @8.0%
 Exposure Time = 150ms
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.20µm
 Binning Mode = 2,2

488 -

Reflector = 38HE 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%
 Exposure Time = 180ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.00µm
 Binning Mode = 2,2

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 @8.0%
 Exposure Time = 18ms
 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 Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing

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 = Active was selected, Textured Opaque was chosen to remove the obstruction from the image above the cut plane, and a red outline of the clipped perimeter was shown; Clip Back was Selected, and Clip Front was NOT selected.

Position of Clip = -10%

Clip X = 0°

Clip Y = 0°

Image Corrections

None

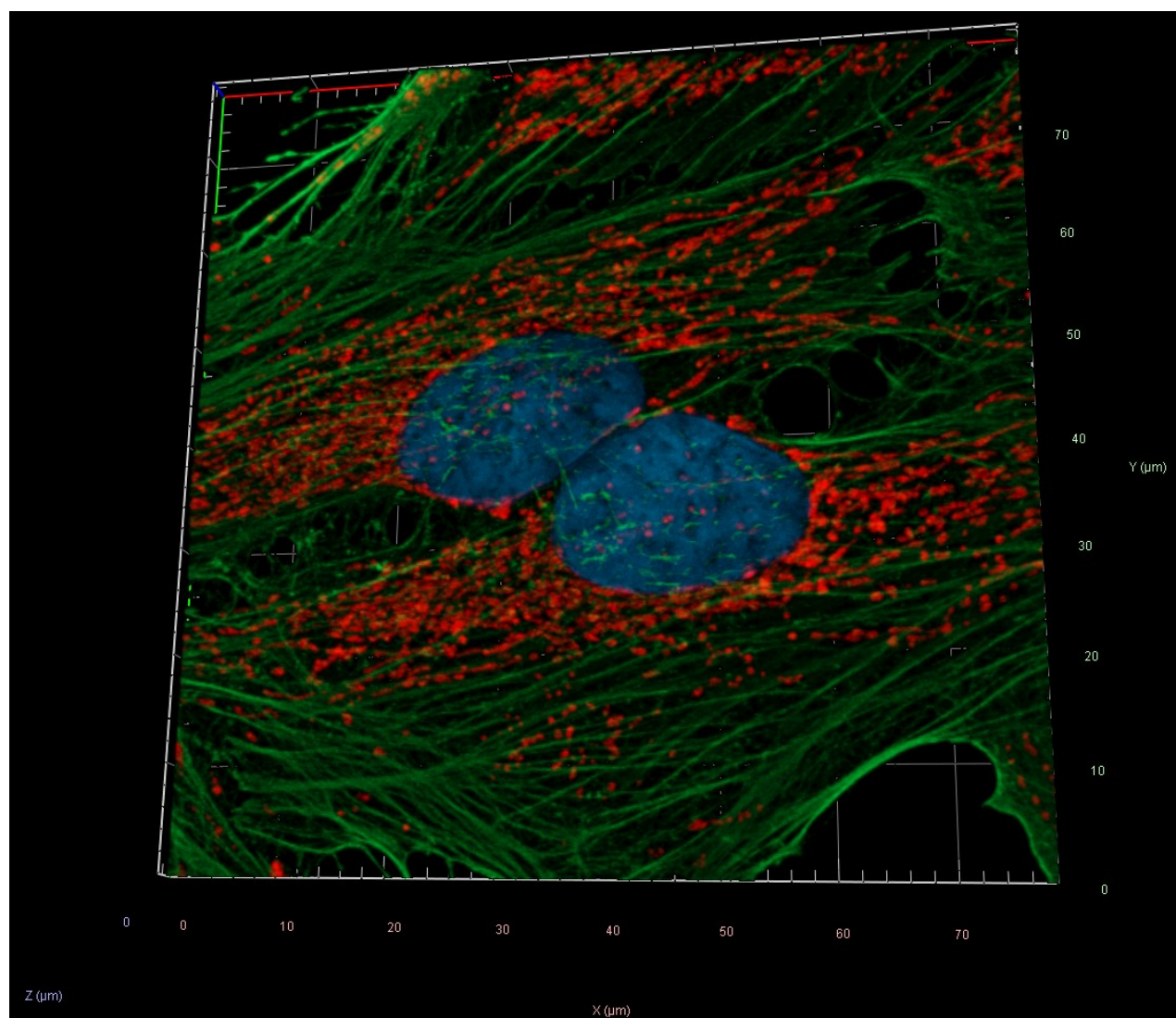
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000053
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	51
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B8D552958505808C08015AE481029017B6A8F6F76848D8C67F884A22358CB03A
Method PDF SHA-256	F8403ADF3AB04D6C236E633070D03B2E5C9664D7EF44957506E9716DE08E0EC8

ORIGINAL IMAGE EVIDENCE / CONFOCAL



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

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
Z-Stack	43 Slices (2.94µm)
Channels	3
Scaling (Per Pixel)	0.152µm X 0.152µm X 0.070µm
Image size (Pixels)	512 X 512
Image Size (Scaled)	78.01µm X 78.01µm
Bit Depth	16 Bit
Image Center Position	X: 70.36mm, Y: 51.03mm
Stage Position	X: 70.36mm, Y: 51.03mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 54µm 1.00AU / 45µm 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.10% 488nm @0.03% 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	0°
Sampling	0.46
Pixel Time	0.85µs
Frame Time	2.09s
LSM Scan Speed	10
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	590 493 353
Emission Wavelength	618 517 465
Effective NA	1.4
Detection Wavelength	590 - 700 496 - 575 410 - 470
Imaging Device	GaAsP-Pmt2 Airyscan GaAsP-PMT1
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	700 V 650 V
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% all channels, Ramp 0% all channels, , Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

Identifyn® SRM Alexa Fluor™ 488 Affinity Goat Anti-Rabbit IgG (H + L)

Cat ID# - SA 000013

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 first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 43 Slices (2.94µm)

Channels = 3

Scaling (Per Pixel) = 0.152µm X 0.152µm X 0.070µm

Image size (Pixels) = 512 X 512

Image Size (Scaled) = 78.01µm X 78.01µm

Bit Depth = 16 Bit

Image Center Position = X: 70.36mm, Y: 51.03mm

Stage Position = X: 70.36mm, Y: 51.03mm

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

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 54µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @0.10%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 0.46
 Pixel Time = 0.85µs
 Frame Time = 2.09s
 LSM Scan Speed = 10
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 590 - 700
 Imaging Device = GaAsP-Pmt2
 Detector Type = GaAsP-PMT
 Detector Gain = 700 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 45µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.03%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 0.46
 Pixel Time = 0.85µs
 Frame Time = 2.09s
 LSM Scan Speed = 10
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 496 - 575
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 700 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 @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 0.46
 Pixel Time = 0.85µs
 Frame Time = 2.09s
 LSM Scan Speed = 10
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsP-PMT1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

3D Image Processing

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

Image Corrections

None

Image by P. Raut

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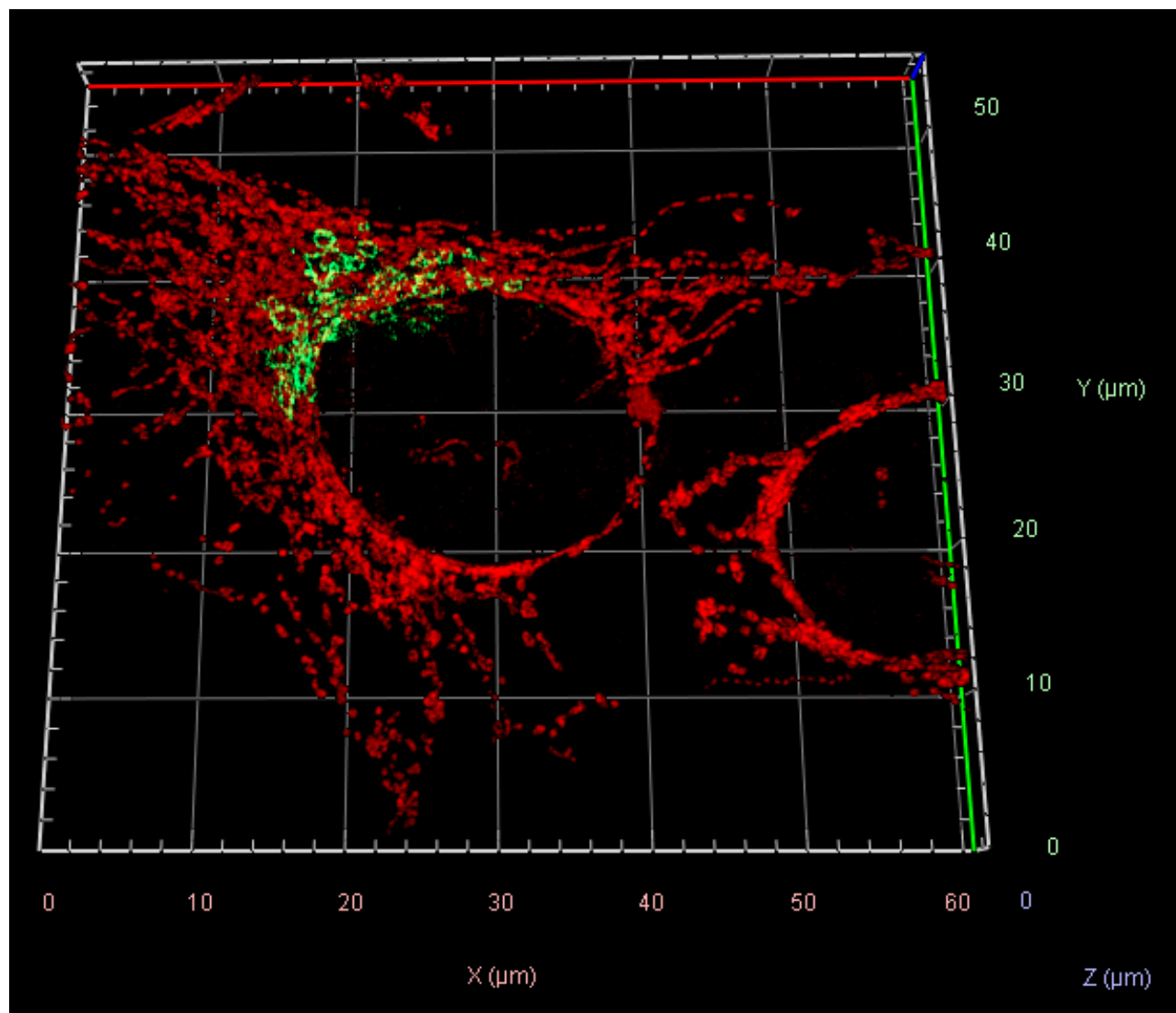
SOURCE PROVENANCE

Catalog	SA-000053
Stage	Confocal

SOURCE PROVENANCE

Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	74916E1396B99CFEF518EECB4D34BEC7CD6D9AAF69D9B58C754AB23FF45A2437
Method PDF SHA-256	C6D3DB1BCF08ED339E6E9B0ADB6B2F110FDA983BE73B90B47DB14B0AE77A2825

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	46

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 AiryScan, 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	66 Slices (3.25µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.05000 µm
Image Size (Pixels)	1719 X 1576
Image Size (Scaled)	60.68µm X 55.63µm
Bit Depth	16 Bit
Image Center Position	X: 79.15mm, Y: 51.73mm
Stage Position	X: 79.15mm, Y: 51.73mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 268µm 5.31 AU / 236µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.5% 488nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	0°
Sampling	2.00
Pixel Time	0.73µs
Frame Time	14.22s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	590 493
Emission Wavelength	618 517
Effective NA	1.4
Detection Wavelength	581-700 495-569
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 9.6 (3D, Auto) Super Resolution 10.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (594), Threshold 1% (488), Ramp 0% (594), Ramp 0% (488), 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® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000018

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 first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 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 AiryScan, 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 = 66 Slices (3.25µm)

Channels = 2

Scaling (Per Pixel) = 35.30nm X 35.30nm X 50.00nm

Image Size (Pixels) = 1719 X 1576

Image Size (Scaled) = 60.68µm X 55.63µm

Bit Depth = 16 Bit

Image Center Position = X: 79.15mm, Y: 51.73mm

Stage Position = X: 79.15mm, Y: 51.73mm

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

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 268µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.73µs
 Frame Time = 14.22s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 581-700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.6 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.31 AU / 236µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.73µs
 Frame Time = 14.22s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 495-569
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 10.0 (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 1% (594), Threshold 1% (488), Ramp 0% (594), Ramp 0% (488), 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 -

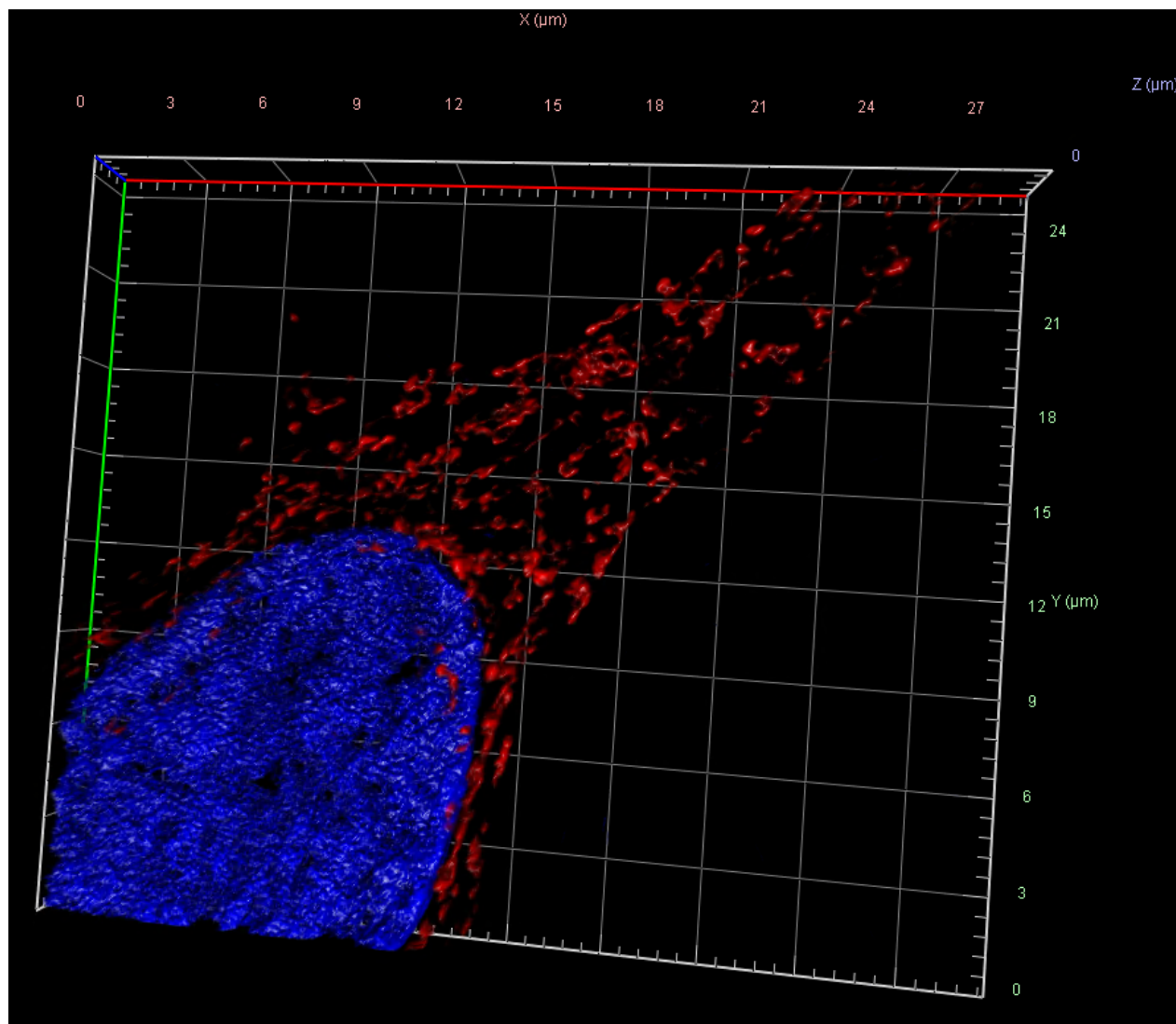
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000053
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, 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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	0D0809CB3A932C7A38F046EE6E9D91BB87E7447E043B4737440A795F5DFAC5A2
Method PDF SHA-256	77CBD527F9DAEC2285DA32EA5B9F0B1FD462EE04239F120870E5F2FC042E1904

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	MCF-7
DETECTED DYES	405/DAPI; 488; 594
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	45 Slices (2.20µm)
Channels	2 1
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	1751 X 1456
Image Size (Scaled)	29.57µm X 24.59µm
Bit Depth	16 Bit
Image Center Position	X: 62.69mm, Y: 35.40mm
Stage Position	X: 62.69mm, Y: 35.40mm
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	561 405
Channel Name	T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
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 Axiocam 820#2
Camera Adapter	1X
Exposure Time	120ms 50ms
Depth of Focus	0.92µm 0.69µm
Binning Mode	2,2
Laser Wavelength	561nm @3.00% 405nm @7.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.7685
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 2% (594), Threshold 2% (DAPI), Ramp 98% (594), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 150%, Azimuth 74°, Elongation -122°, Enabled Tone Mapping
Projection	View Angle 53°, 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 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™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

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, 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® 594 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 = 45 Slices (2.20µm)

Channels = 2

Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm

Image Size (Pixels) = 1751 X 1456

Image Size (Scaled) = 29.57µm X 24.59µm

Bit Depth = 16 Bit

Image Center Position = X: 62.69mm, Y: 35.40mm

Stage Position = X: 62.69mm, Y: 35.40mm

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

594 -

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 = 561
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 561
 Emission Wavelength = 597
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503-546, 576-617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 120ms
 Depth of Focus = 0.92µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @3.00%
 Frame Time = 1.33s
 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 = T2-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 @7.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 2
 Channels = 1
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.7685
 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 Volume Projection = Precise
 Appearance = Threshold 2% (594), Threshold 2% (DAPI), Ramp 98% (594), Ramp 98% (DAPI), Maximum 100% all channels
 Background = Black
 Light = Brightness 150%, Azimuth 74°, Elongation -122°, Enabled Tone Mapping
 Projection = View Angle 53°, 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

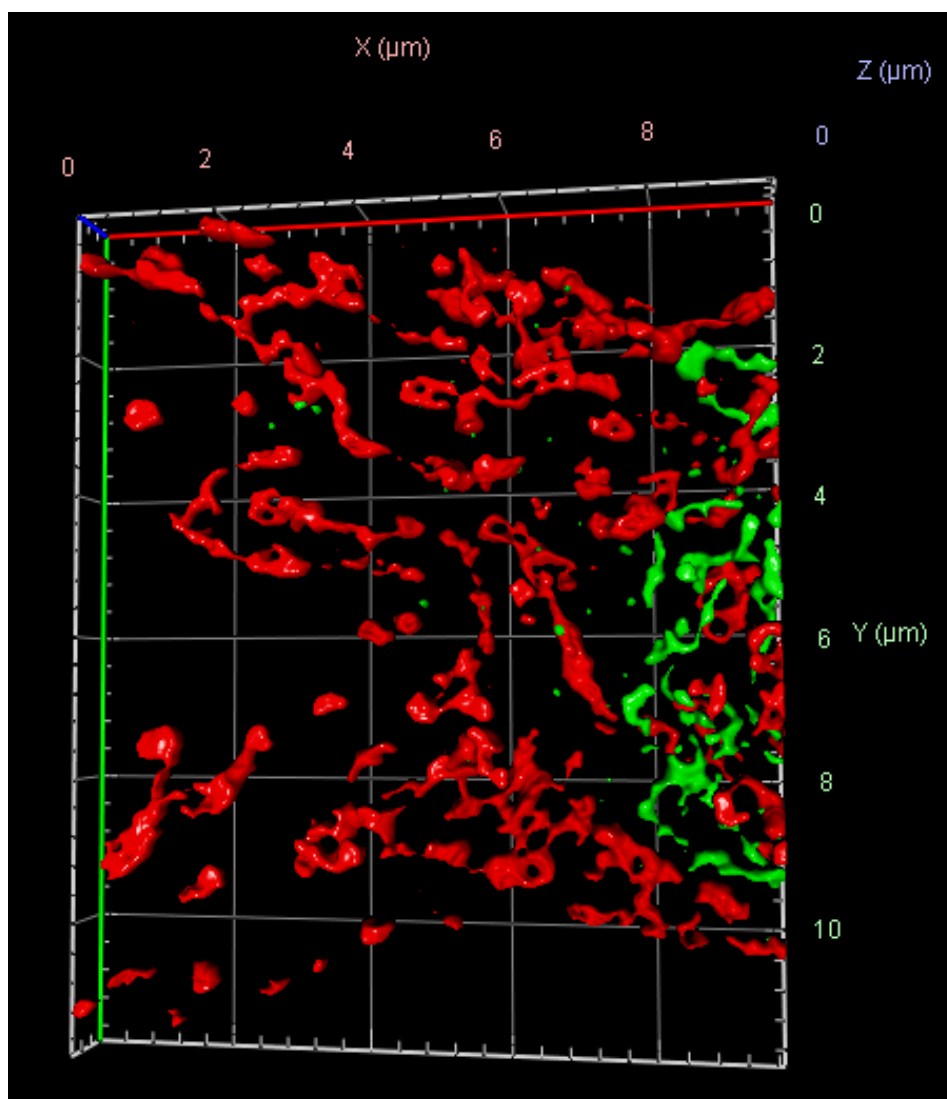
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SOURCE PROVENANCE	
Catalog	SA-000053
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	MCF-7
Image SHA-256	83DB2409626196B34984A1B8BD4F635920E775802D828B4F3A68F406851B3861

SOURCE PROVENANCE

Method PDF SHA-256

3E34349289FAD6825495FDB8BC8FEBE7D0A69C9A5EA90DCDBC4AD2479C4D507F

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	59

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 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.46 oil Korr M27
Filters	-2147483648
Beam Splitter 594	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 560
Beam Splitter 488	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Calibration Marker Positions	Marker 1: 0.00µm, 0.00µm, Marker 2: 0.00µm, 0.00µm, Marker 3 0.00µm, 0.00µm
Z-Stack	20 Slices (1.14µm)
Channels	2
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.06000 µm
Image Size (Pixels)	618 X 755
Image Size (Scaled)	9.67µm X 11.82µm
Bit Depth	16 Bit
Image Center Position	X: 4.68mm, Y: 2.07mm
Stage Position	X: 4.68mm, Y: 2.07mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @5.0% 488nm @1.50%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	4
Excitation Wavelength	561 488
Emission Wavelength	515 655
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms 50ms
Depth of Focus	0.73µm 0.93µm
Binning Mode	1x1
Detector Type	Camera

FIELD	SOURCE-DOCUMENTED VALUE
Depth Offset	0
Detector Digital Gain	0.00
Channel	561.000000 (594) 488.000000
Grating Period (μm)	32.000000 27.500000
Processing	3D
Input SNR	Medium
Iterations	16
Regularization Weight	0.065000
Processing Sampling	4
Output Sampling	4
Filter	Median
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Surface Projection	Precise
Appearance	Threshold 14% (594), Threshold 20% (488), Ambient Light 0% all channels, Specular Light 100% all channels, Shininess 30% all channels
Background	Black
Light	Azimuth 80°, Elongation -130°
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™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000018

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 first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017)), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 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 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:

Filters = -2147483648

Beam Splitter 594 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642

Dual Camera Beam Splitter = SBS LP 560

Beam Splitter 488 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642

Dual Camera Beam Splitter = SBS LP 560

Calibration Marker Positions: Marker 1: 0.00µm, 0.00µm, Marker 2: 0.00µm, 0.00µm, Marker 3 0.00µm, 0.00µm

Z Stack - (3D)

Z-Stack = 20 Slices (1.14µm)

Channels = 2

Scaling (Per Pixel) = 15.65nm X 15.65nm X 60.00nm

Image Size (Pixels) = 618 X 755

Image Size (Scaled) = 9.67µm X 11.82µm

Bit Depth = 16 Bit

Image Center Position = X: 4.68mm, Y: 2.07mm

Stage Position = X: 4.68mm, Y: 2.07mm

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

594 -

Contrast Method = Fluorescence
 Laser Wavelength = 561nm @5.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 4
 Excitation Wavelength = 561
 Emission Wavelength = 515
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 100ms
 Depth of Focus = 0.73µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 561.000000 (594)

Grating Period (µm): 32.000000

SIM2

Processing: 3D
 Input SNR: Medium
 Iterations: 16
 Regularization Weight: 0.065000
 Processing Sampling: 4
 Output Sampling: 4
 Filter: Median
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

488 -

Contrast Method = Fluorescence
 Laser Wavelength = 488nm @1.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 4
 Excitation Wavelength = 488
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 50ms
 Depth of Focus = 0.93µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 488.000000
Grating Period (µm): 27.500000

SIM2

Processing: 3D
 Input SNR: Medium
 Iterations: 16
 Regularization Weight: 0.065000
 Processing Sampling: 4
 Output Sampling: 4
 Filter: Median
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Surface Projection = Precise
 Appearance = Threshold 14% (594), Threshold 20% (488), Ambient Light 0% all channels, Specular Light 100% all channels, Shininess 30% all channels
 Background = Black
 Light = Azimuth 80°, Elongation -130°
 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

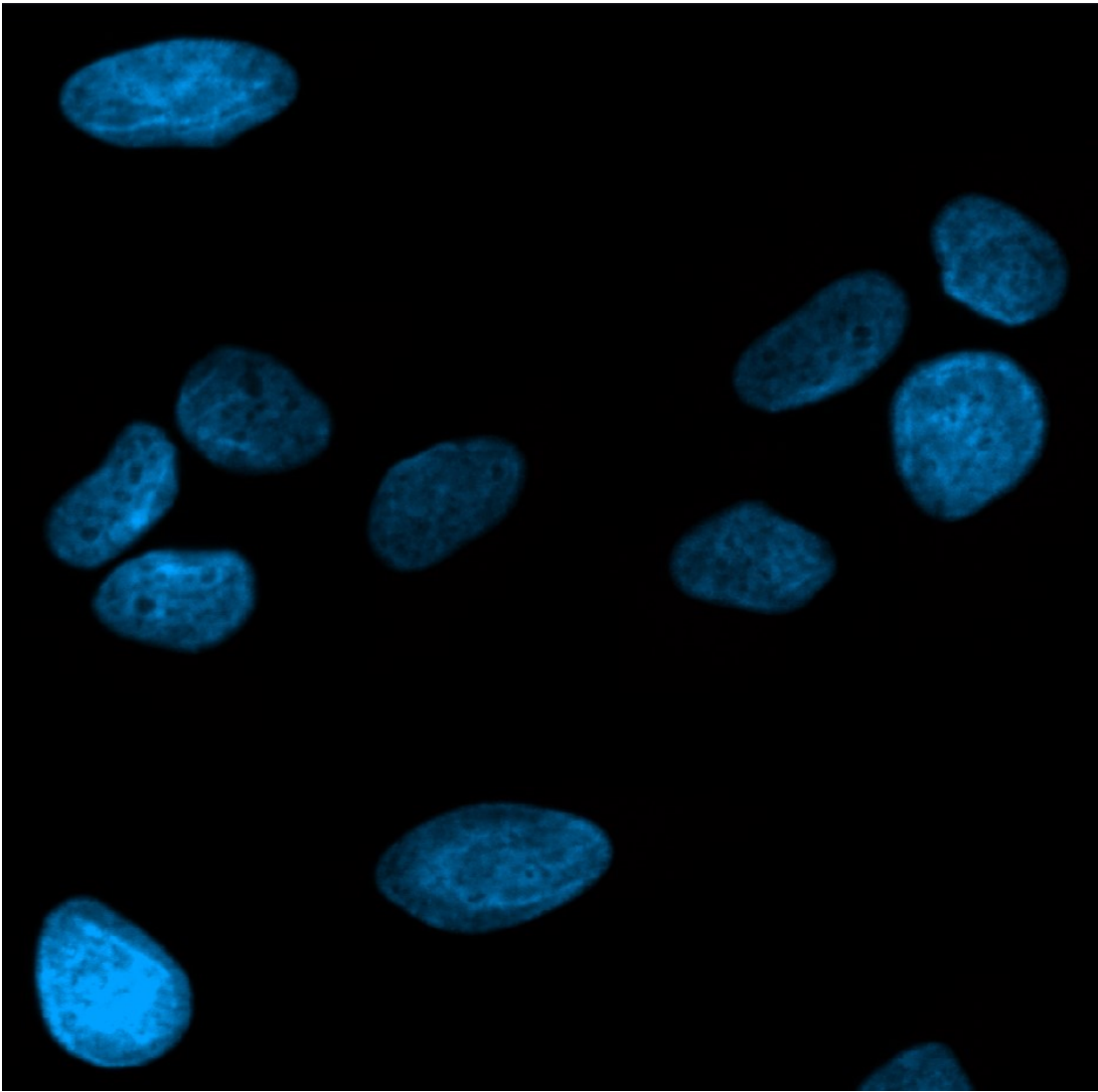
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000053
Stage	SIM²
Instrument	ZEISS Elyra 7
Microscope configuration	Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Structured source metadata values	59
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D671A286D0A77733F007294EE867F0D739C66AFE5794B313B74C3E538F574D4D
Method PDF SHA-256	90F975323EBD1D5DD4767C6A38A37050A0717C2FFBBC9490DA8715E4D8BCE153

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
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)	1084 X 1070
Image Size (Scaled)	154.86µm X 152.86µm
Bit Depth	14 Bit
Image Center Position	X: 50.54mm, Y: 51.35mm
Stage Position	X: 50.54mm, Y: 51.35mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	45 Texas Red 49 DAPI
Beam Splitter	585 395
Filter Excitation Wavelength	540 - 580 335 - 383
Filter Emission Wavelength	593 - 668 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	590 353
Emission Wavelength	618 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.20µm 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™ 594 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000053

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® 594 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) = 1084 X 1070

Image Size (Scaled) = 154.86µm X 152.86µm

Bit Depth = 14 Bit

Image Center Position = X: 50.54mm, Y: 51.35mm

Stage Position = X: 50.54mm, Y: 51.35mm

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

594 -

Reflector = 45 Texas Red

Beam Splitter = 585

Filter Excitation Wavelength = 540 - 580

Filter Emission Wavelength = 593 - 668

Contrast Method = Fluorescence

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

Exposure Time = 200ms

Excitation Wavelength = 590

Emission Wavelength = 618

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.20µm

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-000053
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	017D75AB49ED724A46D47BB35FBAE4F12F0718A081B50EFE331C6B84A5A80631
Method PDF SHA-256	166965C8F59C2E7BF5941A6212401914271A65C4FDCFA7A623E0FF13BED9DECE