

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

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

7

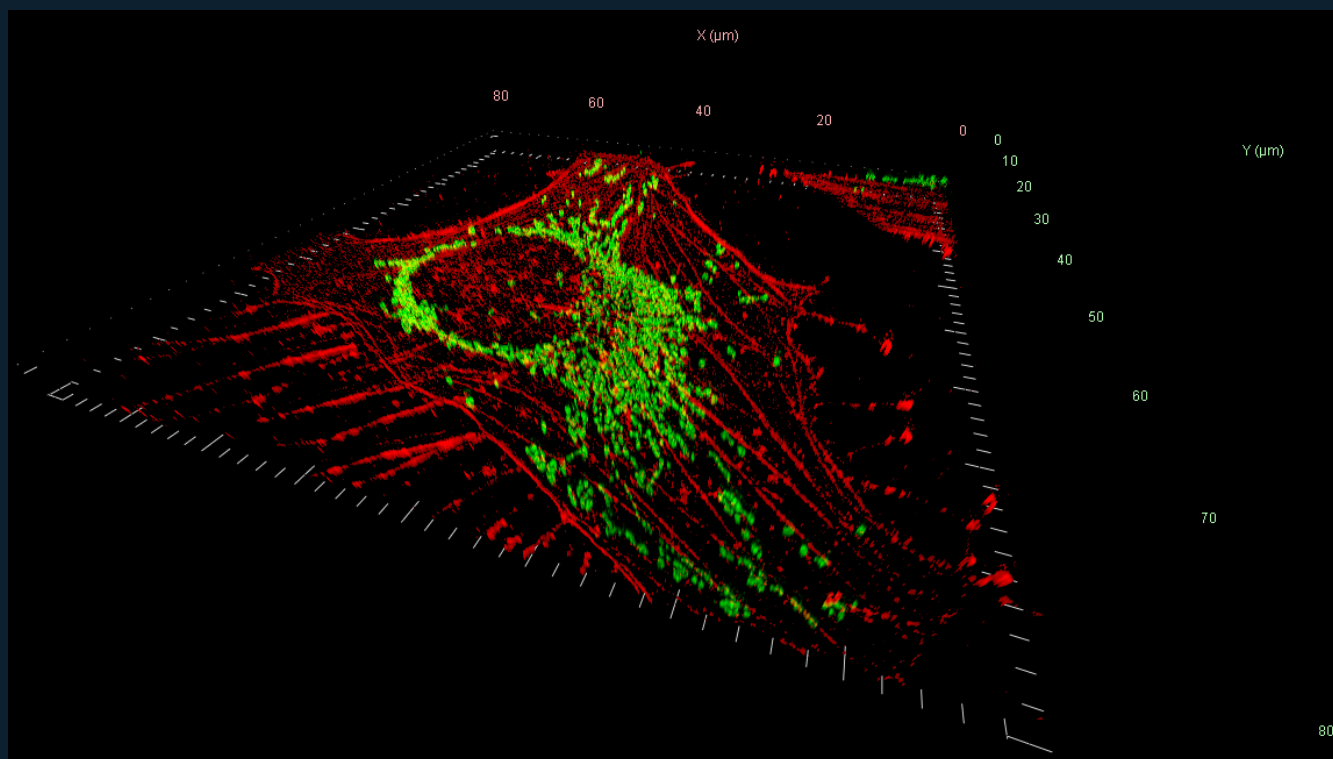
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000054 | 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-Rabbit, 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-000054 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-000054 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 HE eGFP; 540 - 580; 450 - 490; 593 - 668; 500 - 550; 590
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 45 Texas Red; 38 HE eGFP; 49 DAPI; 540 - 580; 450 - 490; 335 - 383; 593 - 668
Confocal	405/DAPI; 488; 594	2; None; 561nm @0.02%; 405nm @0.2%; 590; 353; 618; 465
Airyscan	405/DAPI; 488; 594	2; None; 561nm @0.3%; 488nm @0.7%; 590; 493; 618; 517
SIM	405/DAPI; 488; 594	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T3-Axiocam 820 #2-SR; 561; 405
SIM ²	405/DAPI; 488; 594	2; 561nm @4.0%; 488nm @2.60%; 561; 488; 515; 655; 561.000000
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 MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 770 X 931; Image Size (Pixels): 770 X 931; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 100ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @ 40%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

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: 94 Slices (9.30µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.1423µm X 0.100µm; Image size (Pixels): 1024 X 1024; Image Size (Pixels): 1024 X 1024; 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 141%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 46.

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 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 76 Slices (4.50µm) - 3-Dimensional Model Maximum Projection; Channels: 2; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.060µm; Image size (Pixels): 1323 X 1323; Image Size (Pixels): 1323 X 1323; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29µs; Frame Time: 4.97s. Illumination: Laser Wavelength: 561nm @0.02%; 405nm @0.2%. Optical/scan: Pinhole: 1.00AU / 58µm; 1.00AU / 42µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 148%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 8.0 (3D, Auto); Filter: 6.7 (3D, Auto). Structured source metadata values: 54.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 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: 30 Slices (1.45µm); Channels: 2; Scaling (Per Pixel): 42.52nm X 42.52nm X 50.00nm; Image size (Pixels): 2957 X 2957; Image Size (Pixels): 2957 X 2957; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.71µs; Frame Time: 14.74s. Illumination: Laser Wavelength: 561nm @0.3%; 488nm @0.7%. Optical/scan: Pinhole: 5.00 AU / 268µm; 5.31 AU / 236µm; Scan Zoom: 0.80; 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 9.0 (3D, Auto); Super Resolution 9.8 (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: 73 Slices (3.60µm); Channels: 2; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 982 X 1354; Image Size (Pixels): 982 X 1354; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @2.0%; 405nm @7.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 148%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

VISIBLE OBSERVATION

A preserved presentation image is available for 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: 39Slices (2.66µm); Channels: 2; Scaling (Per Pixel): 15.65nm X 15.65nm X 70.00nm; Image size (Pixels): 5120 X 5120; Image Size (Pixels): 5120 X 5120; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 60ms; 65ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 561nm @4.0%; 488nm @2.60%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Projection: View Angle 90°, Scale Z 130%, 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 Xylys Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1374 X 941; Image Size (Pixels): 1374 X 941; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylys Lamp Intensity @25%; X-Cite Xylys 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-000054 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): 42.52nm X 42.52nm X 50.00nm -> 0.04252 μ m X 0.04252 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)=2957 X 2957; Image Size (Scaled)=125.75 μ m X 125.75 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)=982 X 1354; Image Size (Scaled)=16.59 μ m X 22.87 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 15.65nm X 15.65nm X 70.00nm -> 0.01565 μ m X 0.01565 μ m X 0.07000 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)=5120 X 5120; Image Size (Scaled)=80.14 μ m X 80.14 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

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

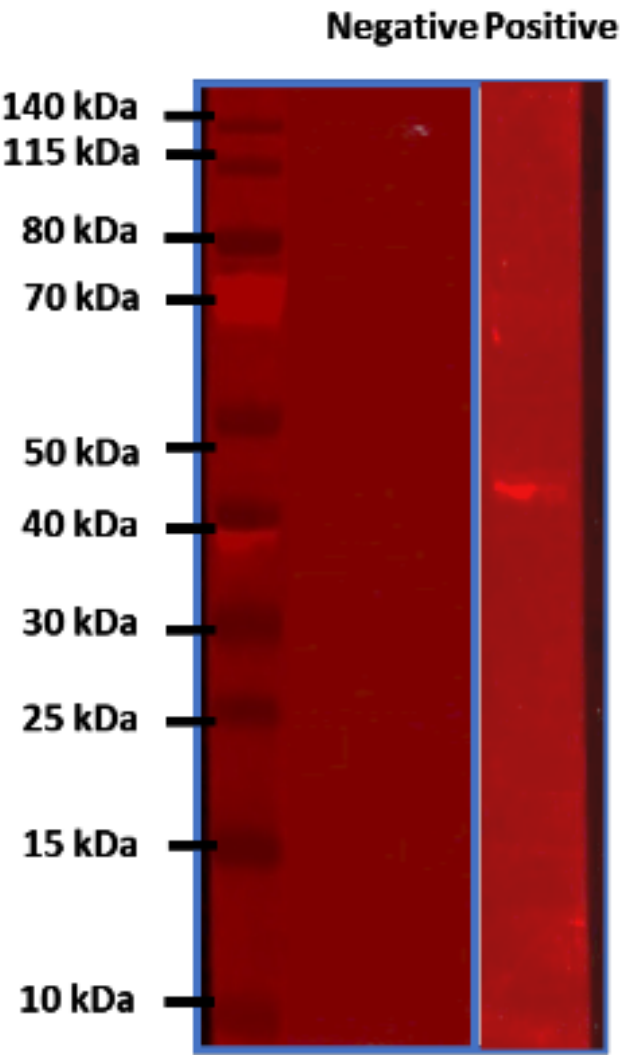
This dossier preserves the complete available evidence progression for SA-000054. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 980	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	8
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-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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 Recombinant Monoclonal Antibody (JF47-01) (MA5-32530) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey anti-Rabbit 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 = 8

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

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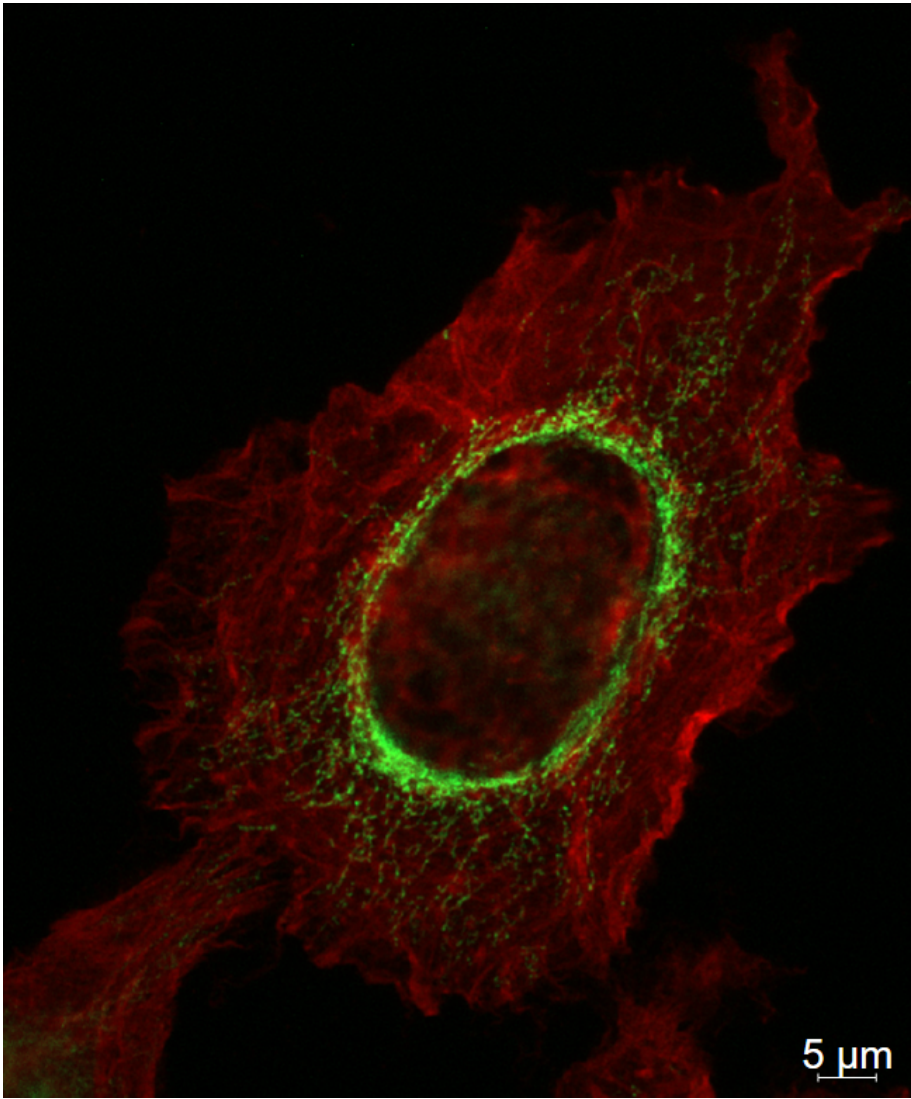
SOURCE PROVENANCE

Catalog	SA-000054
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	A82C5C49522CF39CF7E3E454A2E4E8E8A1F06C55778BCC9311DDC0CCC0F4C337
Method PDF SHA-256	D2E5F3537492B4A4B305005256C2E179F05AC114AE7B1D9354CC44F548EC2150

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	30

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 MR R3, 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)	0.102µm X 0.102µm
Image size (Pixels)	770 X 931
Image Size (Scaled)	78.83µm X 95.32µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	45 Texas Red 38 HE eGFP
Beam Splitter	585 495
Filter Excitation Wavelength	540 - 580 450 - 490
Filter Emission Wavelength	593 - 668 500 - 550
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @ 40%
Exposure Time	100ms 200ms
Excitation Wavelength	590 493
Emission Wavelength	618 517
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.96µm 0.780µm
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™ 594 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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

Cat ID# - SA 000021

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® 488 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® 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 Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil 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) = 0.102µm X 0.102µm

Image size (Pixels) = 770 X 931

Image Size (Scaled) = 78.83µm X 95.32µm

Bit Depth = 12 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 @20%

Exposure Time = 100ms

Excitation Wavelength = 590

Emission Wavelength = 618

Effective NA = 1.4

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 0.96µm

Binning Mode = 1,1

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @ 40%
 Exposure Time = 200ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = AxioCam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.780µm
 Binning Mode = 1,1

Post Processing

None

Image Correction

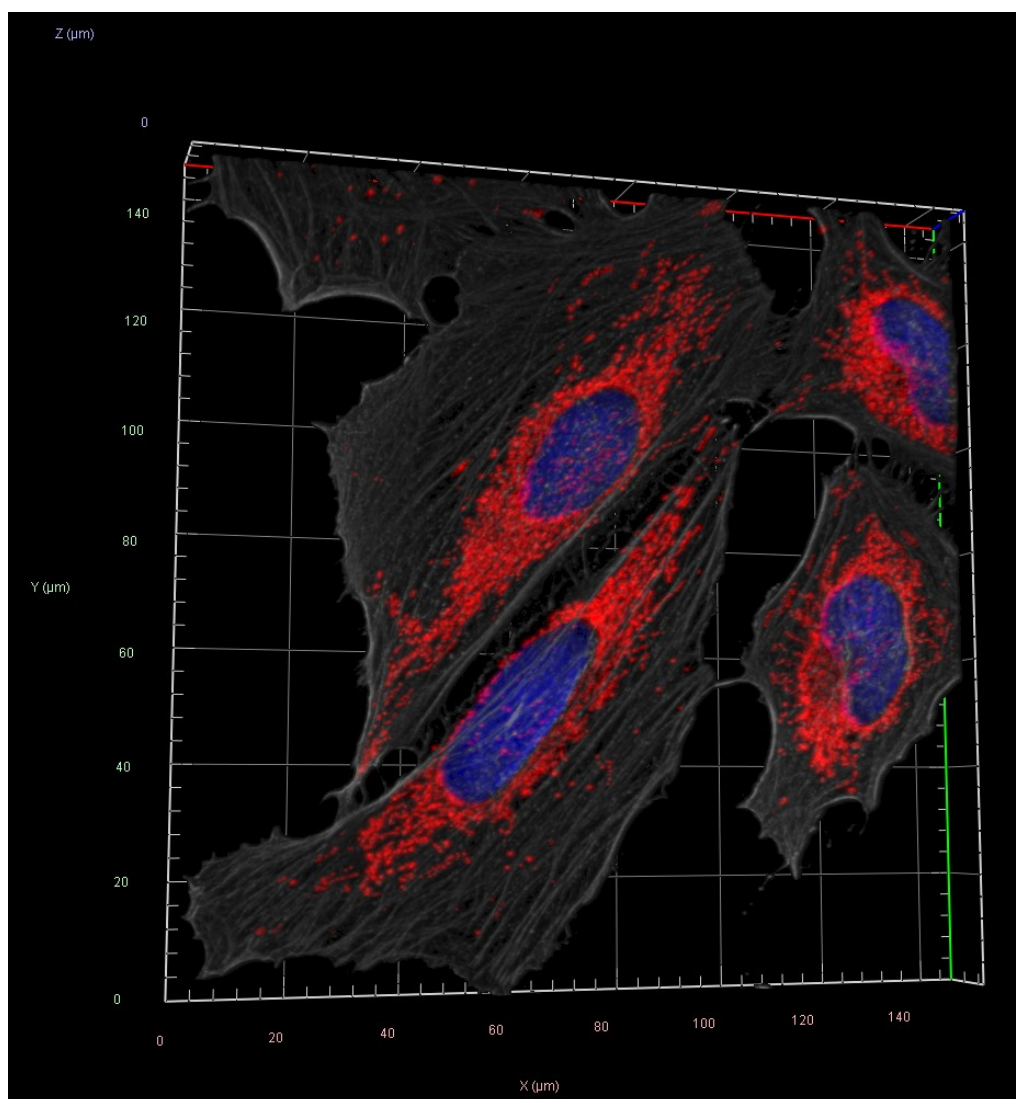
None

Image by E.F. Simon

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SOURCE PROVENANCE	
Catalog	SA-000054
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1A40DE0238344A22C8A5D605B848426A9FC2DCF67B0BEFE99FF94D7B4B8A5DC9
Method PDF SHA-256	4F3DBA15A73E4AF8BF950215D48C956BDF4361677FF05B67BBAD5BDFAF488E1

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	46

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	94 Slices (9.30µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.1423µm X 0.100µm
Image Size (Pixels)	1024 X 1024
Image Size (Scaled)	146.29µm X 146.29µm
Bit Depth	14 Bit
Image Center Position	X: 92.84mm, Y: 51.41µm
Stage Position	X: 92.84mm, Y: 51.41µm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	45 Texas Red 38 HE eGFP 49 DAPI
Beam Splitter	585 594 395
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, Ramp0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping was NOT Enabled
Projection	View Angle 35°, Scale Z 141%, 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-Rabbit F(ab')₂ (H + L)
Cat ID# - SA 000054

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Rabbit Anti-Mouse F(ab')₂ (H + L)
Cat ID# - SA 000021

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 = 94 Slices (9.30µm)

Channels = 3

Scaling (Per Pixel) = 0.143µm X 0.1423µm X 0.100µm

Image Size (Pixels) = 1024 X 1024

Image Size (Scaled) = 146.29µm X 146.29µm

Bit Depth = 14 Bit

Image Center Position = X: 92.84mm, Y: 51.41µm

Stage Position = X: 92.84mm, Y: 51.41µm

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 @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 = 38 HE eGFP
 Beam Splitter = 594
 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%, Tone Mapping was NOT Enabled

Projection = View Angle 35°, Scale Z 141%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

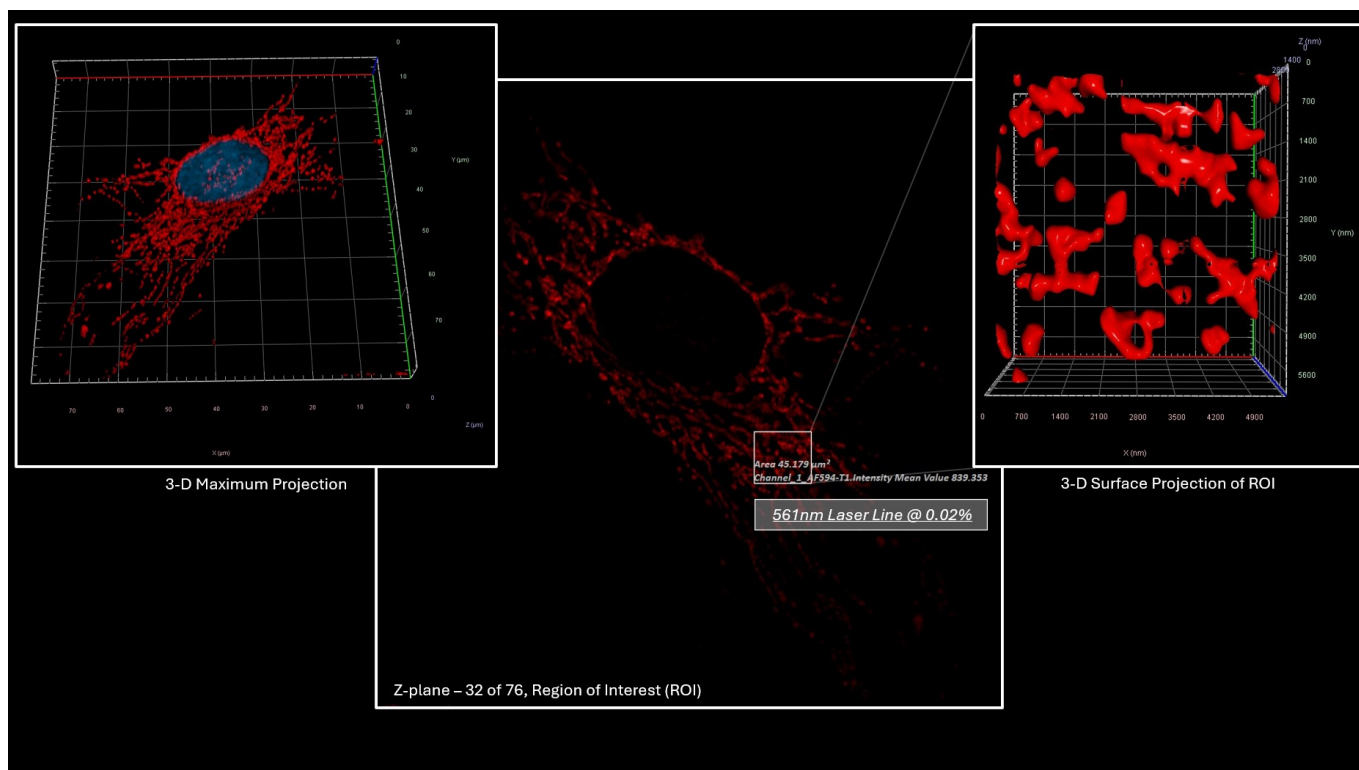
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000054
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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	716B68205BF150BCC103EBB6E887B992237BA39EDCC4FF36195E33953F0C4946
Method PDF SHA-256	93916F9992D10B6969FB6870E63156722A7FE9AE22667A43894AA0C96A0AC16E

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	54

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	76 Slices (4.50µm) - 3-Dimensional Model Maximum Projection
Channels	2
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.060µm
Image Size (Pixels)	1323 X 1323
Image Size (Scaled)	77.82µm X 77.82µm
Bit Depth	16 Bit
Image Center Position	X: 69.79mm, Y: 45.87mm
Stage Position	: 69.79mm, Y: 45.87mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 58µm 1.00AU / 42µm
LMS MBS	Plate MBS 488/561, 405/730
LMS SBS	SBS SP 550 Mirror
Laser Wavelength	561nm @0.02% 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.20
Pixel Time	0.29µs
Frame Time	4.97s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	590 353
Emission Wavelength	618 465
Effective NA	1.4
Detection Wavelength	585 - 618 410 - 470
Imaging Device	GaAsP-Pmt3
Detector Type	GaAsP-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.0 (3D, Auto) Filter: 6.7 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% (594), Threshold 2% (DAPI), Ramp0% (594), Ramp 0% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 60%, Enabled Tone Mapping Azimuth 81°, Elongation -42°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 148%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected) View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise
Surface	Threshold 20%%, Ambient Light 0%, Specular Light 76%, Shininess 45%
Z-Plane	32 of 76 collected
Intensity Mean Value	839.353 for the ROI
Laser Input	561nm @ 0.02

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-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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 Rabbit Recombinant Antibody (MTC02,2860R), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 76 Slices (4.50µm) - 3-Dimensional Model Maximum Projection

Channels = 2

Scaling (Per Pixel) = 0.059µm X 0.059µm X 0.060µm

Image Size (Pixels) = 1323 X 1323

Image Size (Scaled) = 77.82µm X 77.82µm

Bit Depth = 16 Bit

Image Center Position = X: 69.79mm, Y: 45.87mm

Stage Position =: 69.79mm, Y: 45.87mm

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

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 58µm
 LMS MBS = Plate MBS 488/561, 405/730
 LMS SBS = SBS SP 550
 Laser Wavelength = 561nm @0.02%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.7
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.29µs
 Frame Time = 4.97s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 585 - 618
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 8.0 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 42µm
 LMS MBS = Plate MBS 488/561, 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.7
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.29µs
 Frame Time = 4.97s
 LSM Scan Speed = 11
 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-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 6.7 (3D, Auto)

3D Image Processing - Maximum Projection, Left Panel

3D Maximum Projection = Precise
 Appearance = Threshold 2% (594), Threshold 2% (DAPI), Ramp0% (594), Ramp 0% (DAPI), Maximum 100% all channels
 Background = Black
 Light = Brightness 60%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 148%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Surface Projection, Right Panel

3D Surface Projection = Precise
 Surface = Threshold 20%, Ambient Light 0%, Specular Light 76%, Shininess 45%
 Background = Black
 Light = Azimuth 81°, Elongation -42°, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 127%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

2-Dimensional - Middle Panel, ROI

Z-Plane = 32 of 76 collected

Intensity Mean Value = 839.353 for the ROI

Laser Input = 561nm @ 0.02

Image Corrections

None

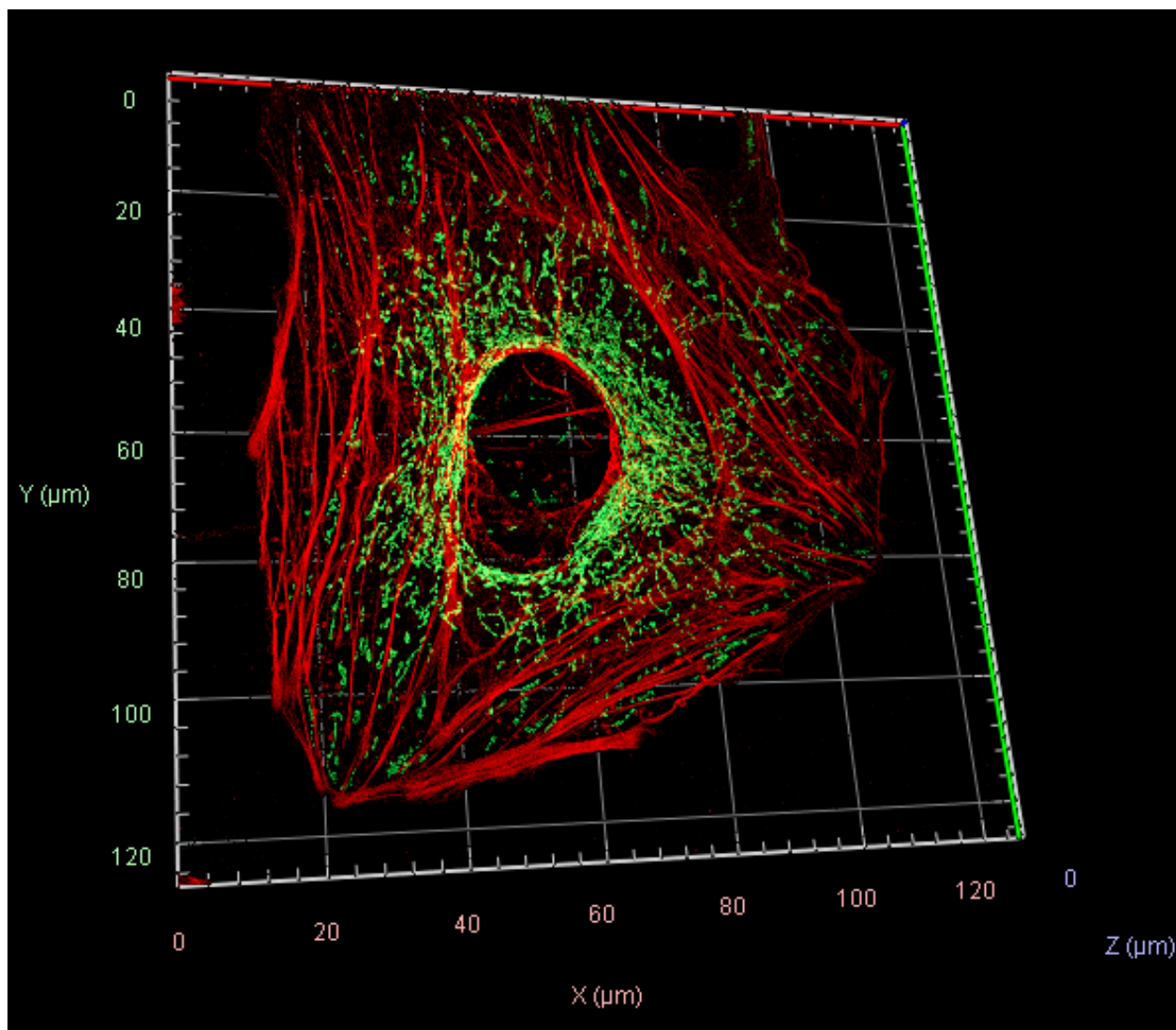
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000054
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DC43DD149CCE92C27548531BEC893C6376D98FDF81845F07C79E509817CDC85E
Method PDF SHA-256	832CB29428B1D458C430A69E266EB368BFCA93B26653F69585AEC438C9C584CC

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	30 Slices (1.45µm)
Channels	2
Scaling (Per Pixel)	0.04252 µm X 0.04252 µm X 0.05000 µm
Image Size (Pixels)	2957 X 2957
Image Size (Scaled)	125.75µm X 125.75µm
Bit Depth	16 Bit
Image Center Position	X: 60.70mm, Y: 47.14mm
Stage Position	X: 60.70mm, Y: 47.14mm
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 @0.3% 488nm @0.7%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	0.80
Rotation	66.98°
Sampling	2.00
Pixel Time	0.71µs
Frame Time	14.74s
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 9.0 (3D, Auto) Super Resolution 9.8 (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-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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

Cat ID# - SA 000021

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® 488 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® 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 = 30 Slices (1.45µm)

Channels = 2

Scaling (Per Pixel) = 42.52nm X 42.52nm X 50.00nm

Image Size (Pixels) = 2957 X 2957

Image Size (Scaled) = 125.75µm X 125.75µm

Bit Depth = 16 Bit

Image Center Position = X: 60.70mm, Y: 47.14mm

Stage Position = X: 60.70mm, Y: 47.14mm

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 @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.80
 Rotation = 66.98°
 Sampling = 2.00
 Pixel Time = 0.71µs
 Frame Time = 14.74s
 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 9.0 (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 @0.7%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.80
 Rotation = 66.98°
 Sampling = 2.00
 Pixel Time = 0.71µs
 Frame Time = 14.74s
 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 9.8 (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)

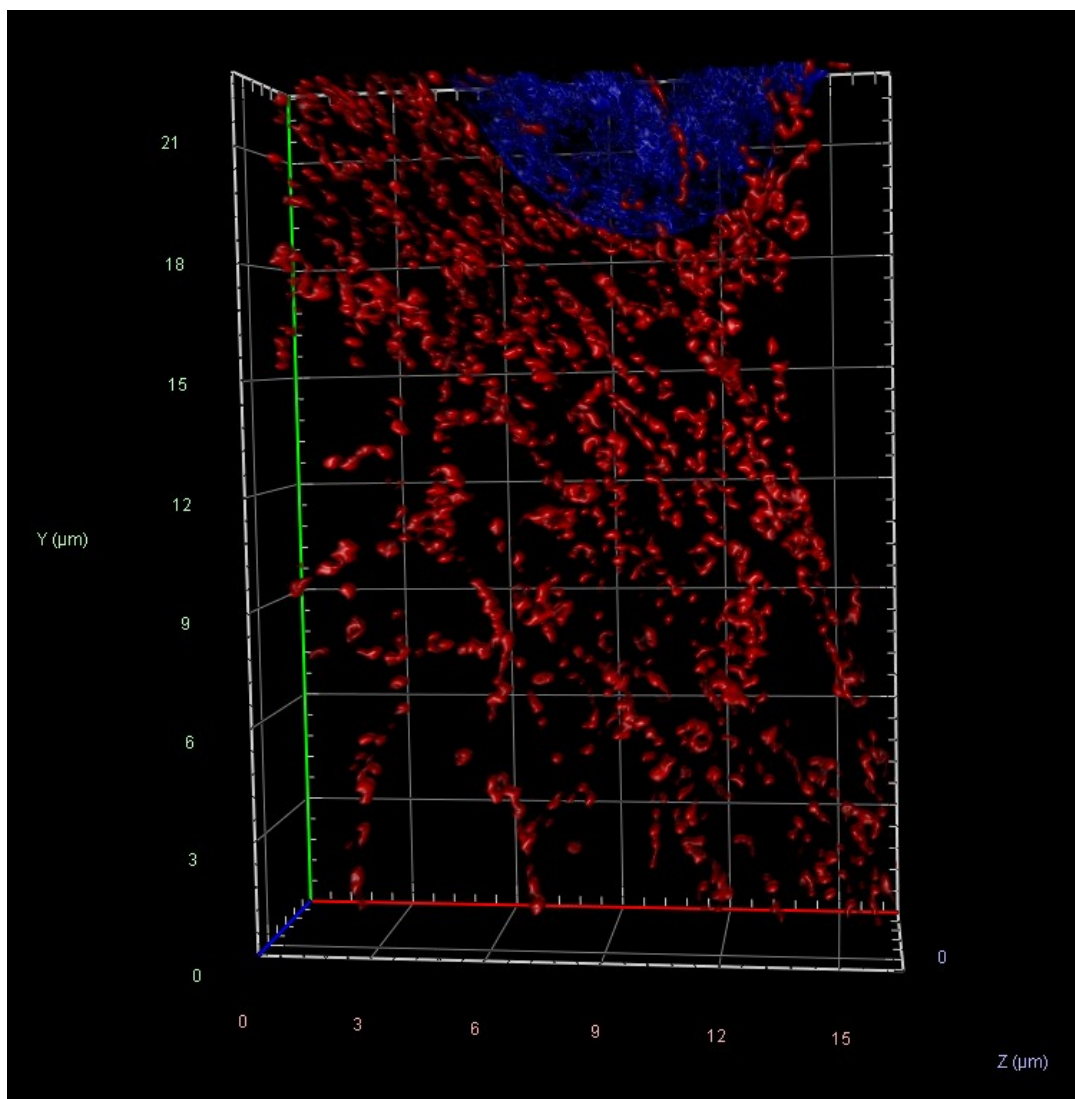
Image Corrections -

Image by P. Raut

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SOURCE PROVENANCE	
Catalog	SA-000054
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	DCA0F2B7DDCC5AD987C2AC7CDB669D253CBFF244BA60B75A6D494C6226542057
Method PDF SHA-256	FDA64B7A24BD49D05C1AA6CBB4DD7A4872B648A969500D125169D26241FD97C0

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	50

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	73 Slices (3.60µm)
Channels	2
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	982 X 1354
Image Size (Scaled)	16.59µm X 22.87µm
Bit Depth	16 Bit
Image Center Position	X: 52.58mm, Y: 41.85mm
Stage Position	X: 52.58mm, Y: 41.85mm
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	T1-Axiocam 820 #2-SR T3-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
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms 50ms
Depth of Focus	0.92µm 0.69µm
Binning Mode	2,2
Laser Wavelength	561nm @2.0% 405nm @7.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.5285 (594), 8.8461 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Volume Projection	Precise
Appearance	Threshold 4% all channels, Ramp96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 114%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 148%, 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-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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 Rabbit Recombinant Antibody (MTC02,2860R), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. 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 = 73 Slices (3.60µm)

Channels = 2

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

Image Size (Pixels) = 982 X 1354

Image Size (Scaled) = 16.59µm X 22.87µm

Bit Depth = 16 Bit

Image Center Position = X: 52.58mm, Y: 41.85mm

Stage Position = X: 52.58mm, Y: 41.85mm

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 = T1-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 @2.0%
 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 = 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 @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 = N/A

Channels = 2

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 10.5285 (594), 8.8461 (DAPI)

Fitting = Fast Fit

Histogram = Scale to Min/Max

Baseline = Cut

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 4% all channels, Ramp96% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 114%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 148%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera

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

Image Corrections

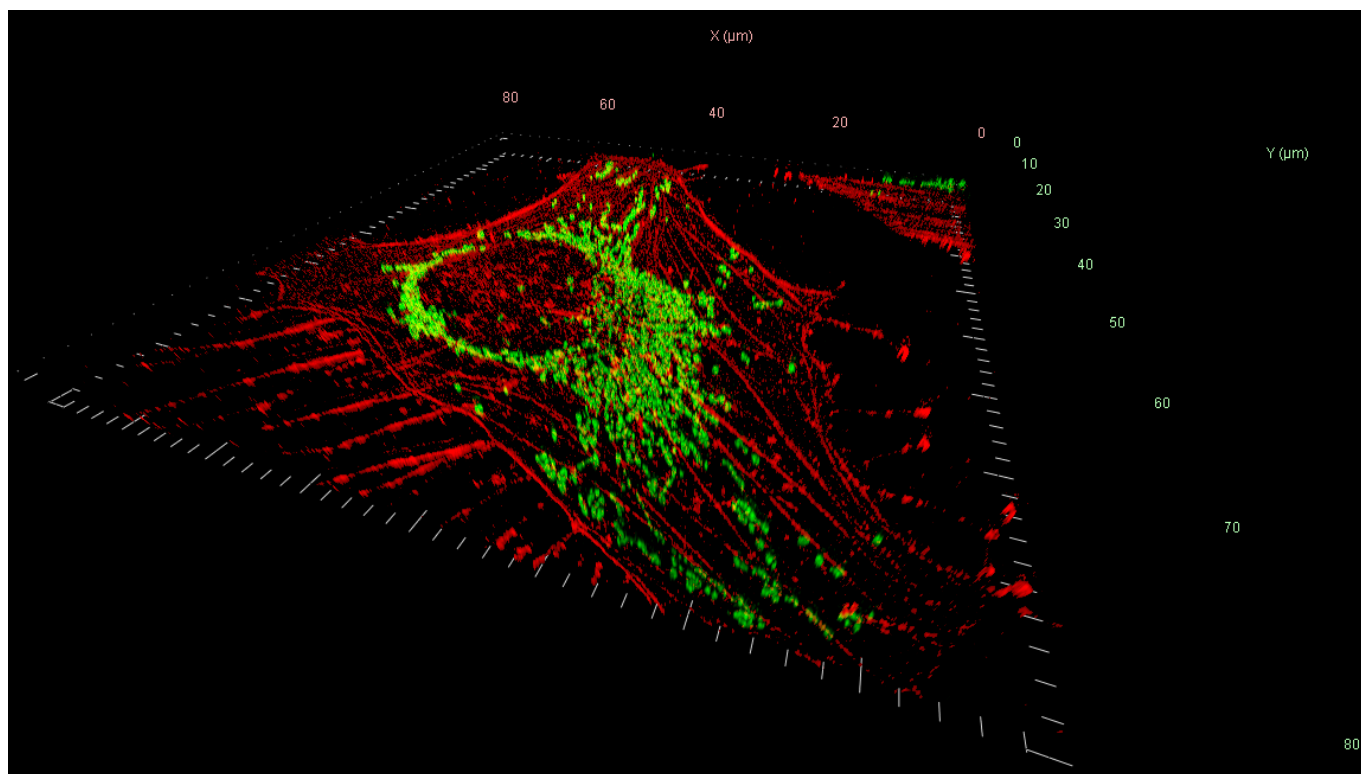
None

Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000054
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DA904C1B83A32AAECDA597D7E9A70A61DE1844ED004DC4C7AB63D5241CC4707D
Method PDF SHA-256	E34F20A3E1B6FA06DF5B10C904D4D5DBA4D44239094ACC58467805F51F6C1D38

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	39Slices (2.66µm)
Channels	2
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.07000 µm
Image Size (Pixels)	5120 X 5120
Image Size (Scaled)	80.14µm X 80.14µm
Bit Depth	16 Bit
Image Center Position	X: -2.37mm, Y: -2.97mm
Stage Position	X: -2.37mm, Y: -2.97mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @4.0% 488nm @2.60%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	561 488
Emission Wavelength	515 655
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	60ms 65ms
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 488.000000
Grating Period (µm)	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
Detection Wavelength	655 - 1000
3D Maximum Projection	Precise
Appearance	Threshold 8% (594), Threshold 1% (488) Ramp 8% (594), Ramp 0% (488), Maximum 100% both channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 90°, Scale Z 130%, 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-Rabbit F(ab')₂ (H + L)
Cat ID# - SA 000054

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Rabbit Anti-Mouse F(ab')₂ (H + L)
Cat ID# - SA 000021

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® 488 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® 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 = 39Slices (2.66µm)

Channels = 2
Scaling (Per Pixel) = 15.65nm X 15.65nm X 70.00nm
Image Size (Pixels) = 5120 X 5120
Image Size (Scaled) = 80.14µm X 80.14µm
Bit Depth = 16 Bit
Image Center Position = X: -2.37mm, Y: -2.97mm
Stage Position = X: -2.37mm, Y: -2.97mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Contrast Method = Fluorescence
 Laser Wavelength = 561nm @4.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 561
 Emission Wavelength = 515
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 60ms
 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
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

488 -

Contrast Method = Fluorescence
 Laser Wavelength = 488nm @2.60%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 488
 Emission Wavelength = 655
 Effective NA = 1.46
 Detection Wavelength = 655 - 1000
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 65ms
 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 Maximum Projection = Precise

Appearance = Threshold 8% (594), Threshold 1% (488) Ramp 8% (594), Ramp 0% (488), Maximum 100% both channels

Background = Black

Light = Brightness 100%, Enabled Tone Mapping

Projection = View Angle 90°, Scale Z 130%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

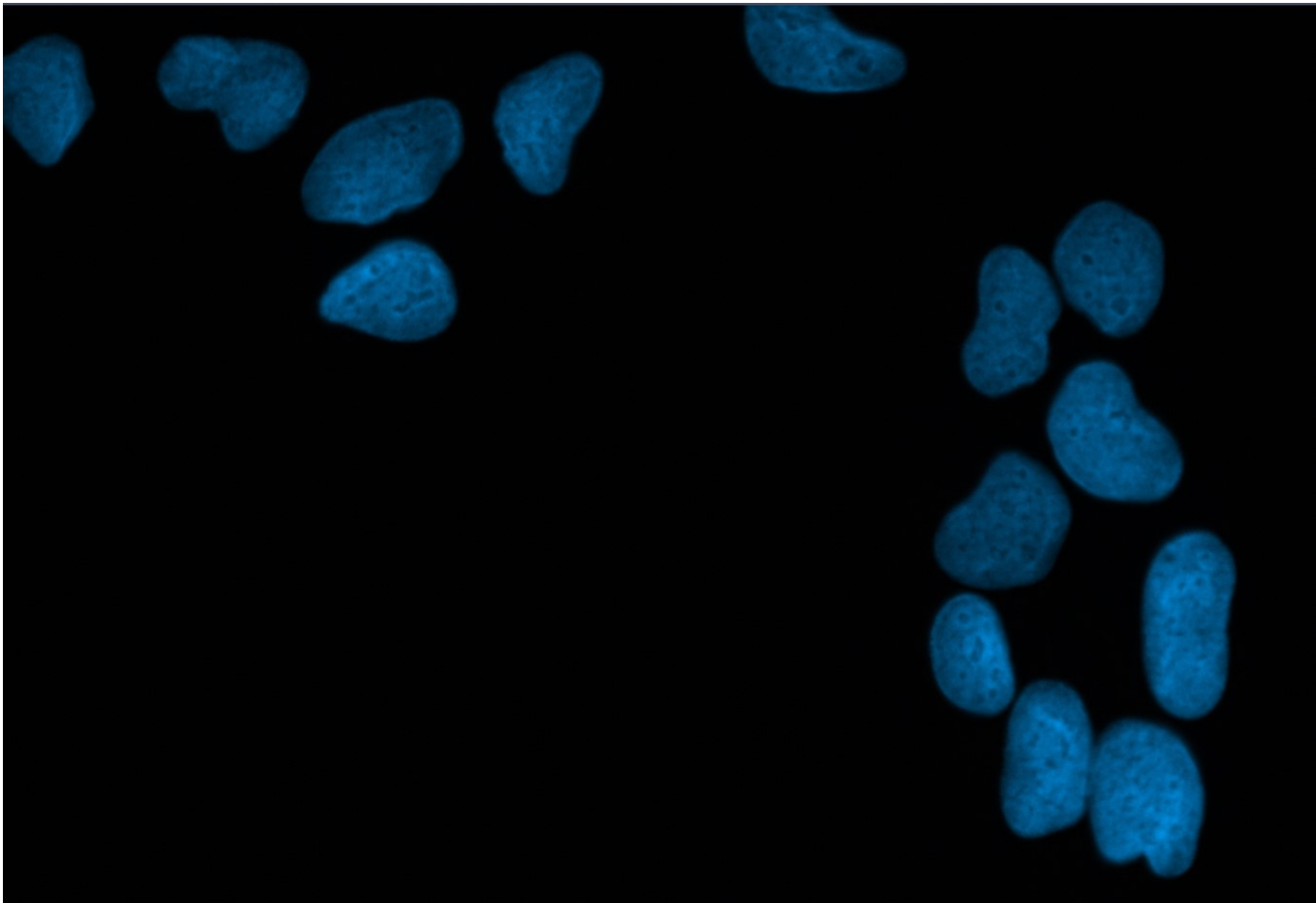
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000054
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	A95EAC26D53040827620F57CE7DC5A12660E012A5EFB4296ABB36FC1F0E03278
Method PDF SHA-256	CFCA13F5D56B4F02C103FCAA044E9DFDCAFF0721B227ADD44F298C7BB5401A3D

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)	1374 X 941
Image Size (Scaled)	196.29µm X 134.43µm
Bit Depth	14 Bit
Image Center Position	X: 52.66mm, Y: 46.89mm
Stage Position	X: 52.66mm, Y: 46.89mm
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-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000054

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) = 1374 X 941

Image Size (Scaled) = 196.29µm X 134.43µm

Bit Depth = 14 Bit

Image Center Position = X: 52.66mm, Y: 46.89mm

Stage Position = X: 52.66mm, Y: 46.89mm

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-000054
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	4DF8D5B7F95644B9CE41DA509C9C3B3528D6FE15F097E32350D44D6306F1B0A6
Method PDF SHA-256	DC39DD59D3C332D55673BD1EFAA96CCB1447A10A61A493ABED858976CF56AFC1