

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8

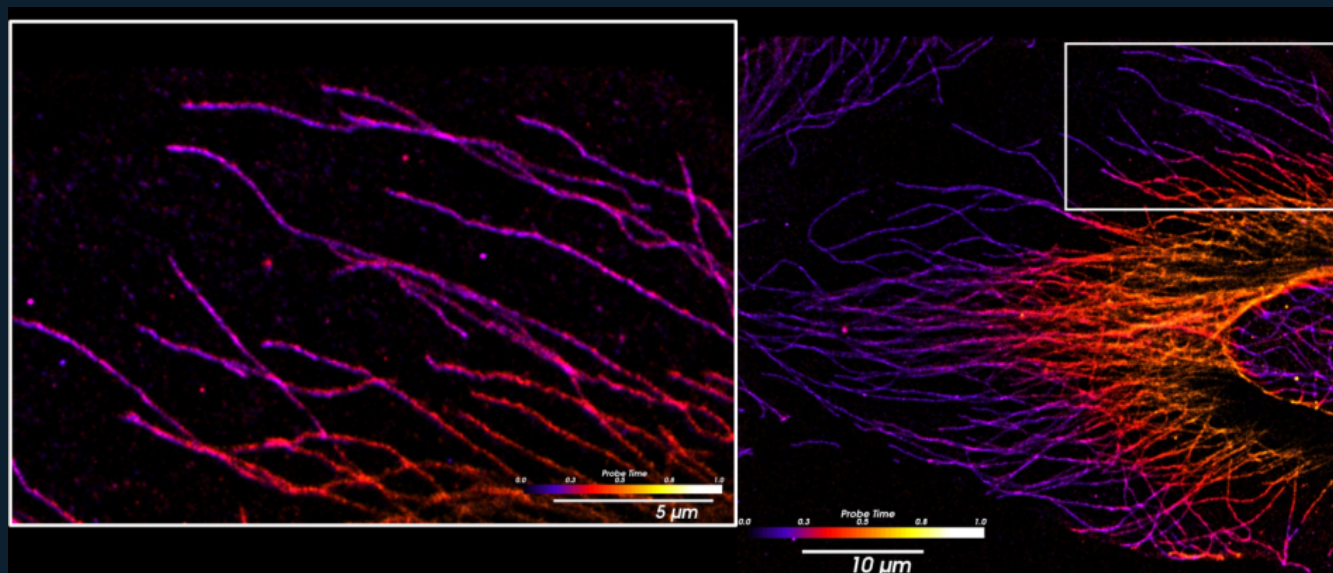
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000064 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 647 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 -> Single-molecule STORM
-> Control

BENNETT ASSESSMENT

The preserved SA-000064 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved SA-000064 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 647	3; 50 Cy5; AF532-49014; 49 DAPI; 625 - 655; 515 - 545; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 647	2; 50 Cy5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Confocal	405/DAPI; 488; 532; 647	3; None; 640nm @0.7%; 488nm @0.7%; 405nm @0.5%; 653; 528; 353
Airyscan	405/DAPI; 488; 532; 647	3; None; 640nm @0.4%; 488nm @0.8%; 405nm @1.0%; 653; 528; 353
SIM	405/DAPI; 488; 647	2; 642nm @1.50%; 405nm @7.0%; 642; 405; 655; 515; 642.000000
SIM ²	405/DAPI; 488; 532; 647	3; 642nm @1.80%; 488nm @7.0%; 405nm @7.0%; 642; 488; 405; 655
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 647	2; 50 Cy5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 1388 X 1040; Image Size (Pixels): 1388 X 1040; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 300ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @50%; X-Cite Xylis Lamp Intensity @30%. 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; 532; 555; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 12 Slices (2.20µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.1423µm X 0.200µm; Image size (Pixels): 883 X741; Image Size (Pixels): 883 X741; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 200ms; 20ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @40%; X-Cite Xylis Lamp Intensity @8%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 74°, Scale Z 65%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 70.57nm X 70.57nm; Image size (Pixels): 1437 X 1437; Image Size (Pixels): 1437 X 1437; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-PMT 2; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.54µs; Frame Time: 20.90s. Illumination: Laser Wavelength: 640nm @0.7%; 488nm @0.7%. Optical/scan: Pinhole: 1.00AU / 57µm; 1.00AU / 46µm; Scan Zoom: 1.0; LSM Scan Speed: 8; Scan Direction: Bidirectional; Effective NA: 1.4. Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: 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: 97 Slices (4.80µm); Channels: 3; Scaling (Per Pixel): 35.29nm X 35.29nm X 50.00nm; Image size (Pixels): 1627 X 1620; Image Size (Pixels): 1627 X 1620; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.55µs; Frame Time: 4.74s. Illumination: Laser Wavelength: 640nm @0.4%; 488nm @0.8%. Optical/scan: Pinhole: 5.00 AU / 282µm; 5.00 AU / 230µm; Scan Zoom: 1.5; 1.15; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 59°, 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 9.2 (3D, Auto); Super Resolution 9.3 (3D, Auto). Structured source metadata values: 54.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / 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: 19Slices (1.505µm); Channels: 2; Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm; Image size (Pixels): 1572 X 2228; Image Size (Pixels): 1572 X 2228; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 40ms; 60ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 642nm @1.50%; 405nm @7.0%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Projection: View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 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 7, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra 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: 19 Slices (1.08µm); Channels: 3; Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm; Image size (Pixels): 5007 X 2939; Image Size (Pixels): 5007 X 2939; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 40ms; 100ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 642nm @1.80%; 488nm @7.0%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Projection: View Angle 35°, Scale Z 70%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 65.

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; 532; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected.

Structured source metadata values: 71.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 987 X 767; Image Size (Pixels): 987 X 767; 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; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed antibody-control guidance that a no-primary or related control bounds background but is not a complete specificity system.

INTERPRETIVE LIMIT

A control bounds the recorded comparison only; it does not establish universal specificity, zero background, zero cross-reactivity, or absence of free dye.

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000064 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.
- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Confocal / Scaling (Per Pixel): 70.57nm X 70.57nm -> 0.07057 μ m X 0.07057 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)=1437 X 1437; Image Size (Scaled)=101.41 μ m X 101.41 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.29nm X 35.29nm X 50.00nm -> 0.03529 μ m X 0.03529 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)=1627 X 1620; Image Size (Scaled)=57.41 μ m X 57.16 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm -> 0.03130 μ m X 0.03130 μ m X 0.07921 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)=1572 X 2228; Image Size (Scaled)=49.21 μ m X 69.74 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): 31.30nm X 31.30nm 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)=5007 X 2939; Image Size (Scaled)=78.37 μ m X 46.00 μ m; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

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

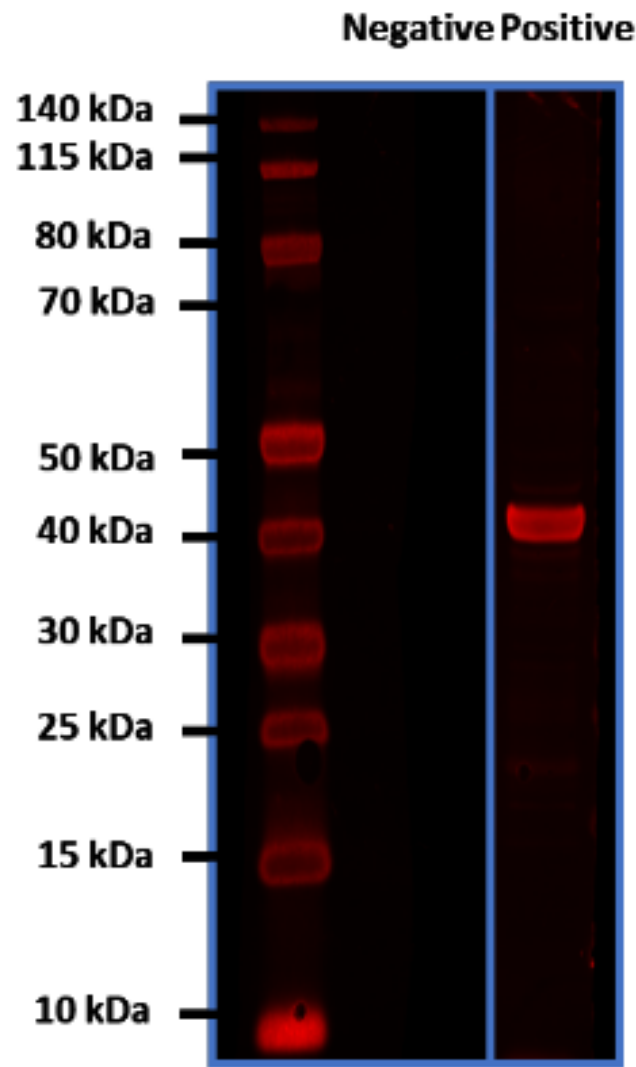
This dossier preserves the complete available evidence progression for SA-000064. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 7	PRESERVED
7	SIM ²	ZEISS Elyra 7	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	638nm
Emission Filter	676±37nm
Detector	Avalanche Photodiode
Intensity	2
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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)
Cat ID# - SA 000064

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™ 647 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 = 638nm
Emission Filter = 676±37nm
Detector = Avalanche Photodiode
Intensity = 2
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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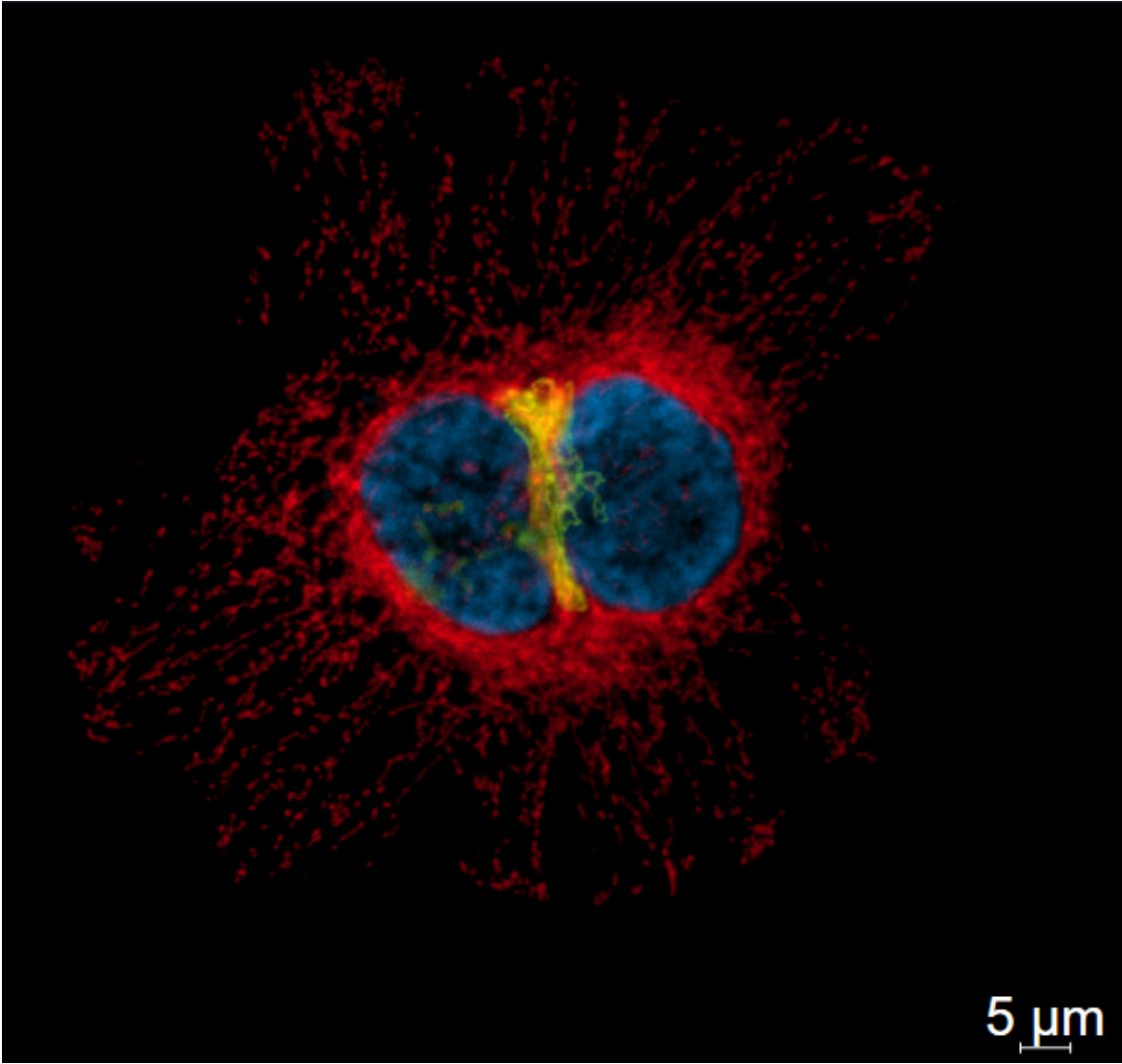
SOURCE PROVENANCE

Catalog	SA-000064
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	3A4A1F9A97F073107AF67F5325C8A0D5B1405C34C2445E8C78F6D860B896F10F
Method PDF SHA-256	36DDB85F261E26B31F97F9C24355894B133C0BBCAC24776D54239EE33738BB9F

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 647
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 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	3
Scaling (Per Pixel)	0.102µm X 0.102µm
Image size (Pixels)	1388 X 1040
Image Size (Scaled)	142.10µm X 106.48µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	50 Cy5 AF532-49014 49 DAPI
Beam Splitter	660 550 395
Filter Excitation Wavelength	625 - 655 515 - 545 335 - 383
Filter Emission Wavelength	665 - 715 555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @50% X-Cite Xylis Lamp Intensity @30% X-Cite Xylis Lamp Intensity @15%
Exposure Time	300ms 100ms 80ms
Excitation Wavelength	653 528 353
Emission Wavelength	668 553 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	1.03µm 0.86µm 0.72µm
Binning Mode	1,1

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000030

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® 532 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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio 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 = 3

Scaling (Per Pixel) = 0.102µm X 0.102µm

Image size (Pixels) = 1388 X 1040

Image Size (Scaled) = 142.10µm X 106.48µm

Bit Depth = 12 Bit

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

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

647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @50%

Exposure Time = 300ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 1.03µm

Binning Mode = 1,1

532 -

Reflector = AF532-49014
 Beam Splitter = 550
 Filter Excitation Wavelength = 515 - 545
 Filter Emission Wavelength = 555 - 595
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @30%
 Exposure Time = 100ms
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Imaging Device = Axiocam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.86µm
 Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15%
 Exposure Time = 80ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 1,1

Post Processing

None

Image Correction

None

Image by E.F. Simon

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SOURCE PROVENANCE

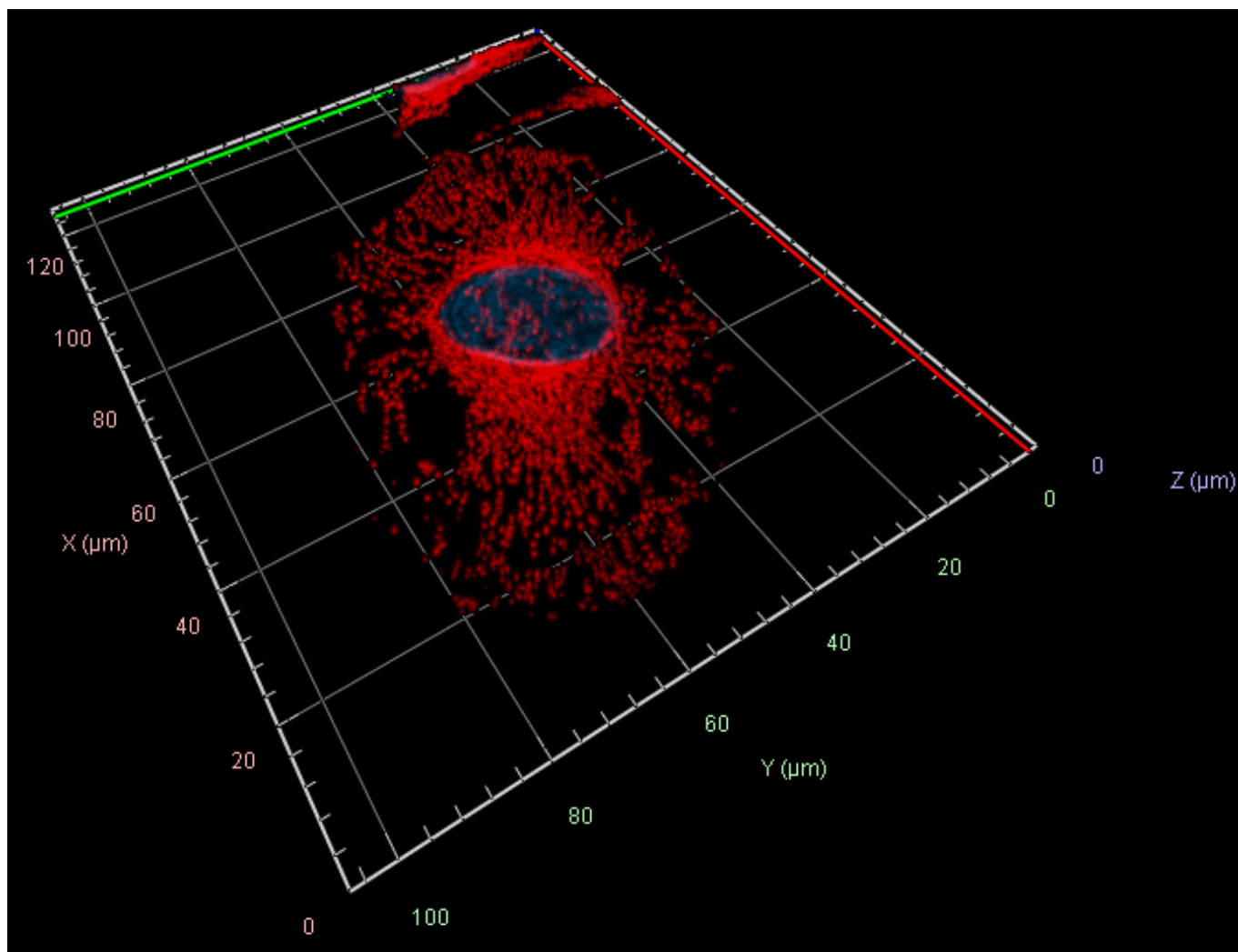
Catalog

SA-000064

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 MR R3, 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	99A730E1D3DF8B960E95F6544F70B3F4DF05B67AECC02B77D3535CF1137D44FE
Method PDF SHA-256	7B31212EB0215DAEA834696C858100EE5D1017230F8EB26FF99A0BFD626BA353

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; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	12 Slices (2.20µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.1423µm X 0.200µm
Image Size (Pixels)	883 X741
Image Size (Scaled)	126.14µm X 105.86µm
Bit Depth	14 Bit
Image Center Position	X: 51.64mm, Y: 54.07µm
Stage Position	X: 51.64mm, Y: 54.07µm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50 Cy5 49 DAPI
Beam Splitter	660 395
Filter Excitation Wavelength	625 - 655 335 - 383
Filter Emission Wavelength	665 - 715 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @40% X-Cite Xylis Lamp Intensity @8%
Exposure Time	200ms 20ms
Excitation Wavelength	653 353
Emission Wavelength	668 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.30µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 3% (647), Threshold 5% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 63%, Enabled Tone Mapping
Projection	View Angle 74°, Scale Z 65%, 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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Mitochondria Antibody (MA5-12017), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 12 Slices (2.20µm)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.1423µm X 0.200µm

Image Size (Pixels) = 883 X741

Image Size (Scaled) = 126.14µm X 105.86µm

Bit Depth = 14 Bit

Image Center Position = X: 51.64mm, Y: 54.07µm

Stage Position = X: 51.64mm, Y: 54.07µm

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

647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

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

Exposure Time = 200ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = Axiocam 807

Camera Adapter = 1X

Depth of Focus = 1.30µm

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%
 Exposure Time = 20ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 0.90µm
 Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 3% (647), Threshold 5% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 63%, Enabled Tone Mapping
 Projection = View Angle 74°, Scale Z 65%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by E.F. Simon

Copyright 2026 GMD12 LLC.

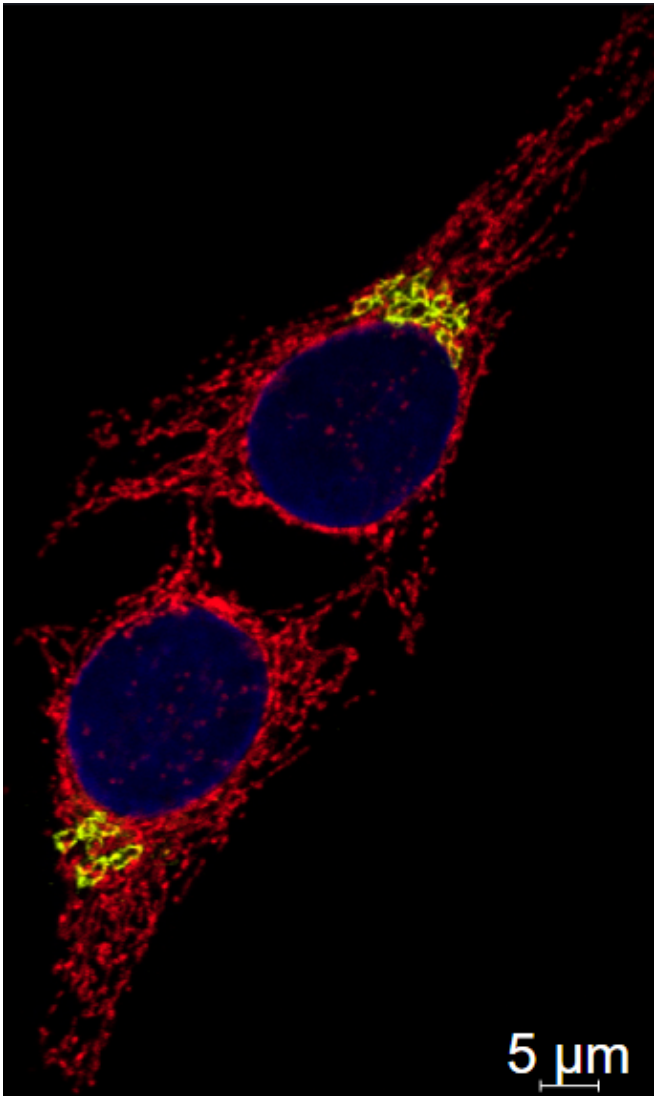
SOURCE PROVENANCE

Catalog	SA-000064
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	5D29F208833DC8405A46AAE22B71B9EE15F882C29CA175A3B531D08E127C0407
Method PDF SHA-256	322A06D5B198A89D85E649CDE5CA12CEBF27407FA40F3D1E68BAA80F8AD1BB6A

ORIGINAL IMAGE EVIDENCE / CONFOCAL



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

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Channels	3
Scaling (Per Pixel)	0.07057 μm X 0.07057 μm
Image Size (Pixels)	1437 X 1437
Image Size (Scaled)	101.41 μm X 101.41 μm
Bit Depth	16 Bit
Image Center Position	X: 72.97mm, Y: 55.57mm
Stage Position	X: 72.97mm, Y: 55.57mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 57 μm 1.00AU / 46 μm 1.00AU / 37 μm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @0.7% 488nm @0.7% 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	0°
Sampling	1.00
Pixel Time	0.54 μs
Frame Time	20.90s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653 528 353
Emission Wavelength	668 553 465
Effective NA	1.4
Detection Wavelength	656 - 700 495 - 599 410 - 470
Imaging Device	GaAsp-PMT 2 Airyscan GaAsp-PMT 1
Detector Type	GaAsp-PMT
Detector Gain	650 V 700 V
Detector Offset	0

FIELD	SOURCE-DOCUMENTED VALUE
Detector Digital Gain	1.0

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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

Identifyfyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000030

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. Identifyfyn® 532 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. Identifyfyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 70.57nm X 70.57nm

Image Size (Pixels) = 1437 X 1437

Image Size (Scaled) = 101.41µm X 101.41µm

Bit Depth = 16 Bit

Image Center Position = X: 72.97mm, Y: 55.57mm

Stage Position = X: 72.97mm, Y: 55.57mm

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

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 57µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.7%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.54µs
 Frame Time = 20.90s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 16
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 656 - 700
 Imaging Device = GaAsp-PMT 2
 Detector Type = GaAsp-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 46µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.7%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.54µs
 Frame Time = 20.90s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 16
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Detection Wavelength = 495 - 599
 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 = 1.0
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.54µs
 Frame Time = 20.90s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 16
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410 - 470
 Imaging Device = GaAsP-PMT 1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

Post Processing

None

Image Corrections

None

Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

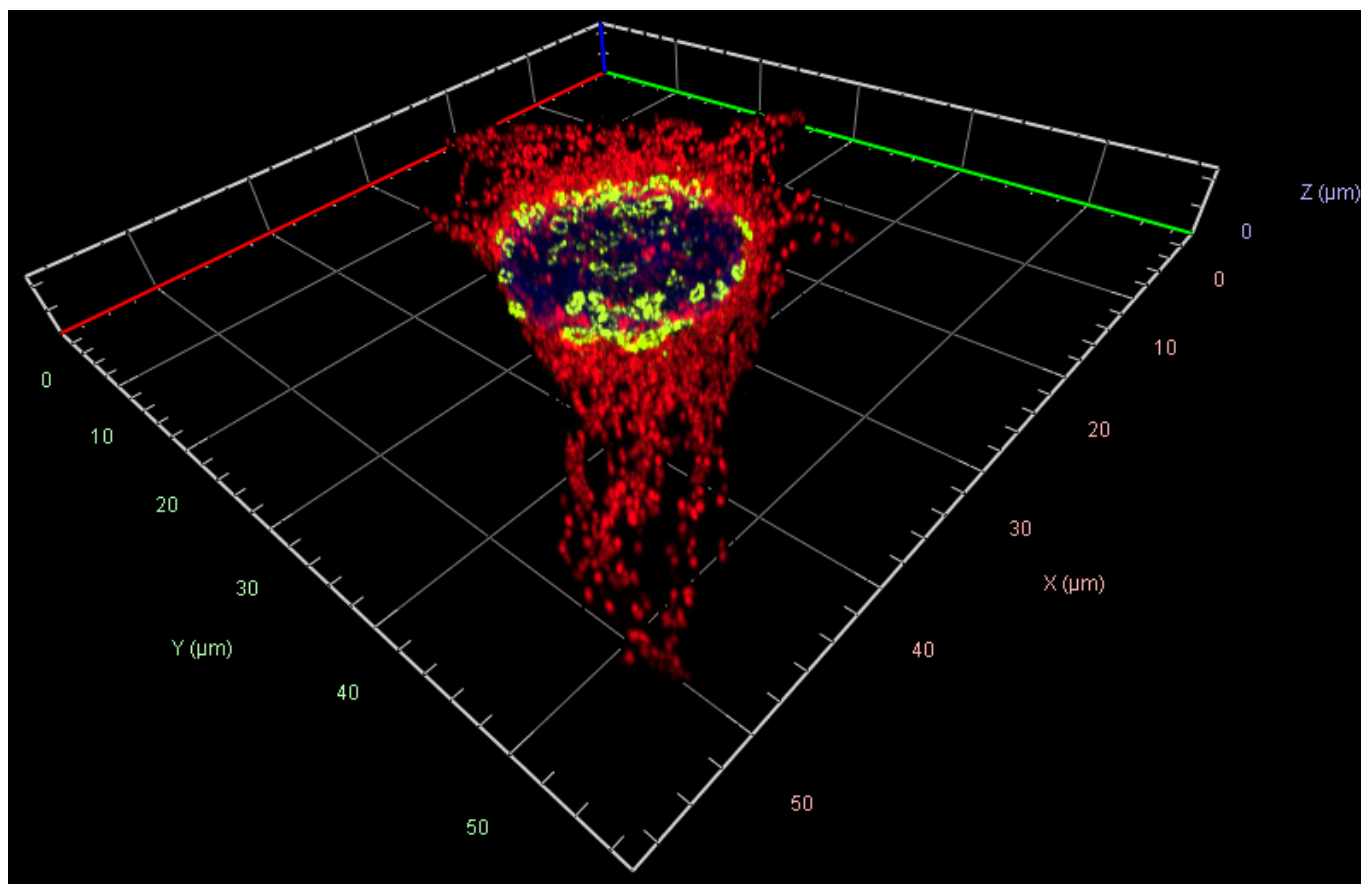
SOURCE PROVENANCE

Catalog	SA-000064
Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:

SOURCE PROVENANCE

Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D4FAD56485E9F7B000B7B0452F7DC31116821D2D58A82CD2B29F885A7CCC1E4D
Method PDF SHA-256	1E07061D1310BD55A0C4B8F94B2BDE9FEB07436B9E4D96EF3572C75419AA9304

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	54

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	97 Slices (4.80µm)
Channels	3
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.05000 µm
Image Size (Pixels)	1627 X 1620
Image Size (Scaled)	57.41µm X 57.16µm
Bit Depth	16 Bit
Image Center Position	X: 75.05mm, Y: 75.05mm
Stage Position	X: 75.05mm, Y: 75.05mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 282µm 5.00 AU / 230µm 5.00 AU / 181µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @0.4% 488nm @0.8% 405nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.5 1.15
Rotation	0°
Sampling	2.00
Pixel Time	0.55µs
Frame Time	4.74s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	653 528 353
Emission Wavelength	668 553 465
Effective NA	1.4
Detection Wavelength	650-700 500-600 400-462
Imaging Device	AiryScan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V 800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 9.2 (3D, Auto) Super Resolution 9.3 (3D, Auto) Super Resolution 8.4 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 135%, did NOT Enabled Tone Mapping
Projection	View Angle 59°, 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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000030

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® 532 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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 = 97 Slices (4.80µm)

Channels = 3

Scaling (Per Pixel) = 35.29nm X 35.29nm X 50.00nm

Image Size (Pixels) = 1627 X 1620

Image Size (Scaled) = 57.41µm X 57.16µm

Bit Depth = 16 Bit

Image Center Position = X: 75.05mm, Y: 75.05mm

Stage Position = X: 75.05mm, Y: 75.05mm

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

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 282µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.55µs
Frame Time = 4.74s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 650-700
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.2 (3D, Auto)

532 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 230µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.8%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.15
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.55µs
 Frame Time = 4.74s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 528
 Emission Wavelength = 553
 Effective NA = 1.4
 Detection Wavelength = 500-600
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.3 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 181µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.5
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.55µs
 Frame Time = 4.74s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400-462
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.4 (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% (647), Threshold 1% (532), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 135%, did NOT Enabled Tone Mapping
 Projection = View Angle 59°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

None

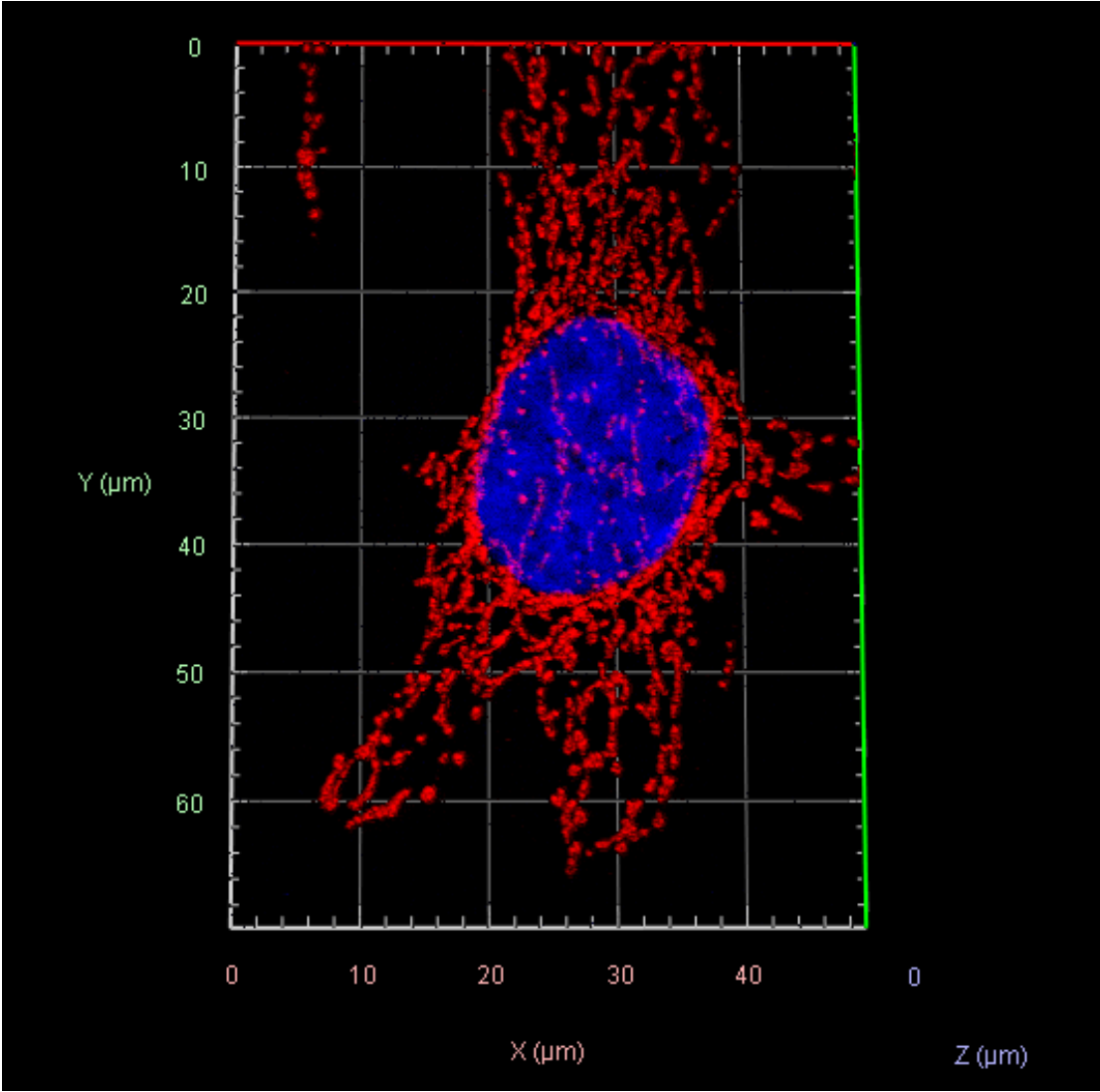
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000064
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	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A464832E492AB8E57ADFCBA6504591F644E49A85A0765A4C5ACD306049F128D2
Method PDF SHA-256	039D5F8BC903690E4EB9D46894CF39F70DB1018C47A9E463AF3A8435BC2137EE

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
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 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 647	Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter	SBS LP 560
Beam Splitter 405	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	19Slices (1.505µm)
Channels	2
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.07921 µm
Image Size (Pixels)	1572 X 2228
Image Size (Scaled)	49.21µm X 69.74µm
Bit Depth	16 Bit
Image Center Position	X: 614.00mm, Y: 5.61mm
Stage Position	X: 614.00mm, Y: 5.61mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	642nm @1.50% 405nm @7.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	642 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	40ms 60ms
Depth of Focus	0.93µm 0.73µm
Binning Mode	1x1
Detector Type	Camera

FIELD	SOURCE-DOCUMENTED VALUE
Depth Offset	0
Detector Digital Gain	0.00
Channel	642.000000 405.000000
Grating Period (µm)	32.000000 36.500000
Processing	3D
Auto Sharpness	Yes
Sharpness	10.721398 11.082407
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 121%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)
Cat ID# - SA 000064

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Mitochondria Antibody (MA5-12017), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 647 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

Beam Splitter 405 = 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 = 19Slices (1.505µm)

Channels = 2

Scaling (Per Pixel) = 31.30nm X 31.30nm X 60.00nm

Image Size (Pixels) = 1572 X 2228

Image Size (Scaled) = 49.21µm X 69.74µm

Bit Depth = 16 Bit

Image Center Position = X: 614.00mm, Y: 5.61mm

Stage Position = X: 614.00mm, Y: 5.61mm

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

647 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @1.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 642
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 40ms
 Depth of Focus = 0.93µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 642.000000
Grating Period (µm): 32.000000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 10.721398
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @7.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 405
 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 - SIM Processing

Channel = 405.000000
Grating Period (µm): 36.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 11.082407
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (647), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 121%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

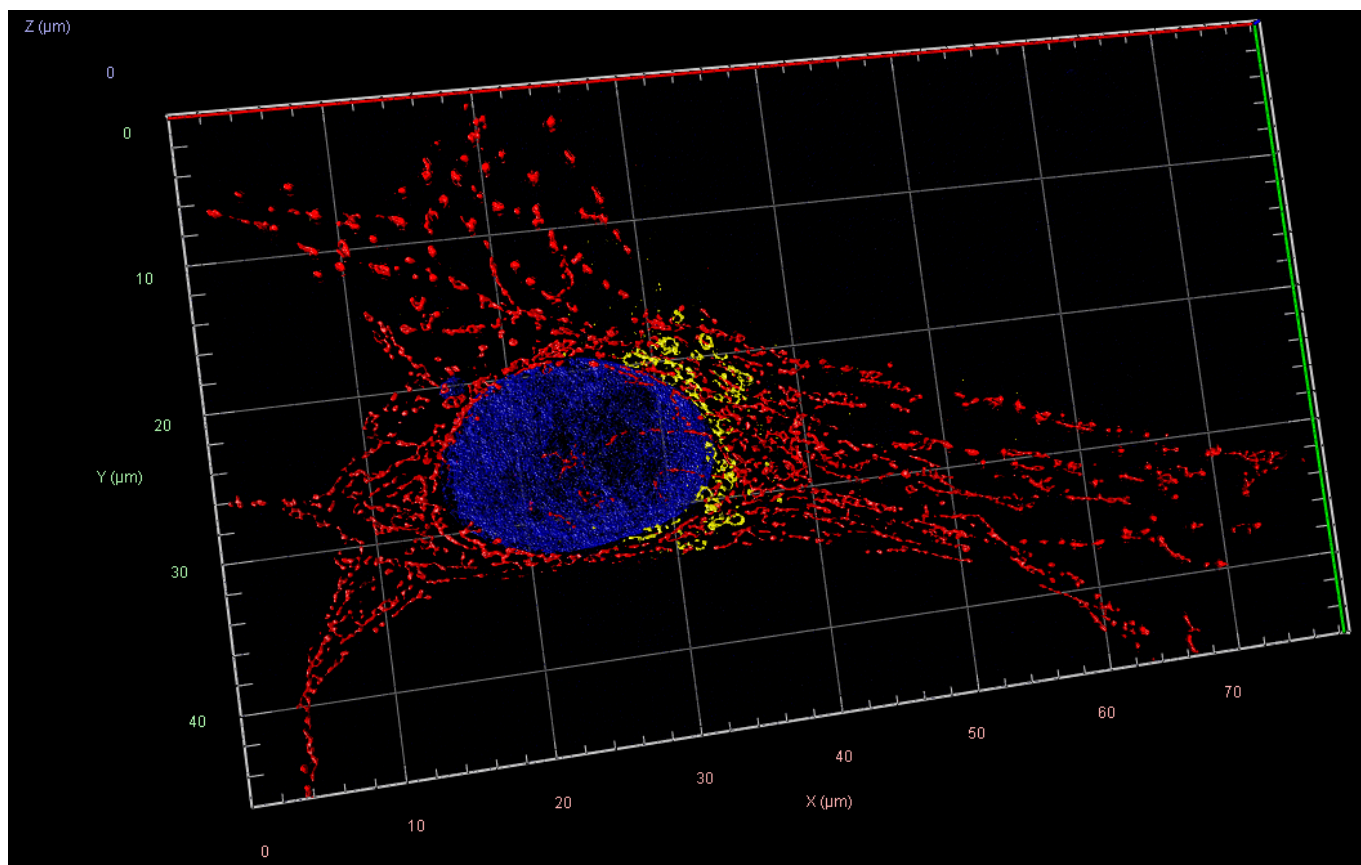
None

Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000064
Stage	SIM
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	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	777746D9171FC2ACBDE95DA5679671C6146CA721E40E38ABA8E3FB9F48B31323
Method PDF SHA-256	C88B67C684EABEE30D321889191014D2565C4EDB13771CD3255E04214B773633

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	65

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 647	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 640
Beam Splitter 532	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Beam Splitter 405	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	19 Slices (1.08µm)
Channels	3
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.06000 µm
Image Size (Pixels)	5007 X 2939
Image Size (Scaled)	78.37µm X 46.00µm
Bit Depth	16 Bit
Image Center Position	X: -1.74mm, Y: 9.69mm
Stage Position	X: -1.74mm, Y: 9.69mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	642nm @1.80% 488nm @7.0% 405nm @7.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	642 488 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	40ms 100ms 60ms
Depth of Focus	0.93µm 0.73µm
Binning Mode	1x1

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	Camera
Depth Offset	0
Detector Digital Gain	0.00
Channel	642.000000 488.000000 (532) 405.000000
Grating Period (µm)	32.000000 42.000000
Processing	3D
Input SNR	Medium
Iterations	16 5
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 Volume Projection	Precise
Appearance	Threshold 8% (647), Threshold 8% (532), Threshold 0% (DAPI), Ramp 92% (647), Ramp 92% (532), Ramp 98% (DAPI), Maximum 100% both channels
Background	Black
Light	Brightness 135%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 70%, 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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000030

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® 532 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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss 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 647 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642

Dual Camera Beam Splitter = SBS LP 640

Beam Splitter 532 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642

Dual Camera Beam Splitter = SBS LP 640

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

Dual Camera Beam Splitter = SBS LP 640

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 = 19 Slices (1.08µm)

Channels = 3

Scaling (Per Pixel) = 31.30nm X 31.30nm X 60.00nm

Image Size (Pixels) = 5007 X 2939

Image Size (Scaled) = 78.37µm X 46.00µm

Bit Depth = 16 Bit

Image Center Position = X: -1.74mm, Y: 9.69mm

Stage Position = X: -1.74mm, Y: 9.69mm

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

647 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @1.80%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 642
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 40ms
 Depth of Focus = 0.93µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 642.000000
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

532 -

Contrast Method = Fluorescence
 Laser Wavelength = 488nm @7.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 488
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 100ms
 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 (532)
Grating Period (µm): 32.000000

SIM2

Processing: 3D
 Input SNR: Medium
 Iterations: 5
 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

DAPI -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @7.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 405
 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 = 405.000000
Grating Period (µm): 42.000000

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

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 8% (647), Threshold 8% (532), Threshold 0% (DAPI), Ramp 92% (647), Ramp 92% (532), Ramp 98% (DAPI), Maximum 100% both channels
 Background = Black
 Light = Brightness 135%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 70%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

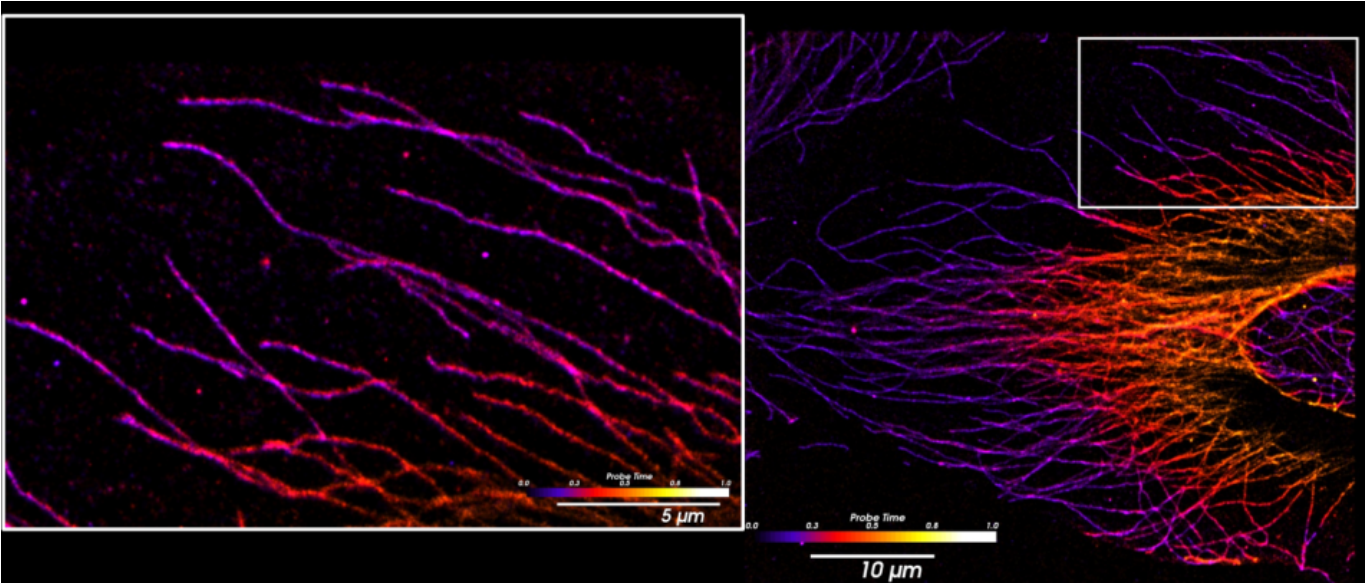
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000064
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	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F8138B5EDA5E182405CAB1ECF99C27FE4A0C79CB2825A8132DA3B3EBB74D2581
Method PDF SHA-256	AF443421CB4FFD054A4C244DF02EC340E08451C86D2BB49B6226C0042ABC47C2

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	152 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (635 nm Dichroic))	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5084 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 150.2; End: 151.8
SuperRes 6nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 500
Z Positions	18 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	50%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 20
Sizing Mode	Radial precision
Particle size	5nm
Color by	Depth
Color Table	Single
Opacity Mode	Constant; Opacity 1: 0.05
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	10 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	20nm
Smoothing	8.00
PSF Z offset	-450 to +450
Radial precision	25
Axial precision	60

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™ 647 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000064

Hela cells were grown on μ-Slide 8 Well high Glass Bottom Ibidi chamber, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

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

Calibration -

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

<https://identifyn.com/storm-calibration>

Image Acquisition -

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

Configuration Tab - Capture Configuration

Number of Probes: 1

Number of Simultaneous Probes: 1

Number of Reference Probes: 1

Camera: Both Cameras

Color Channel: Both Channels

Probe Capture Mode: Interleaved

Z -Stack Capture Mode: Interleaved

Multiple Location Capture: Not selected

Post Record Reference: Not Selected

Fluidics: Not Selected

Autofocus Drift Correction: On

Microscope configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of Sample

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

Piezo Current: 152 µm

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

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
 Reference Probe 1 Laser control (640 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

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

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

Autofocus - Calibration Values

Current Position" -0.979

Current Intensity: 4.5

Calibration Value: -5084 nm/unit

Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50µm is Selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 150.2; End: 151.8

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

SuperRes 6nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

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

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms

Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 500

Z Positions: 18 using Steps: 0.2µm (200nm)

Cycles: 1

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 50%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 20

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
 Sizing Mode: Radial precision
 Particle size: 5nm
 Color by: Depth
 Color Table: Single
 Opacity Mode: Constant; Opacity 1: 0.05
 Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
 Time Intervals: 10 Intervals
 Pixel Size: 20nm
 Smoothing: 8.00

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

PSF Z offset: -450 to +450

Radial precision: 25

Axial precision: 60

The other parameters were not applied.

Statistical analysis - NA

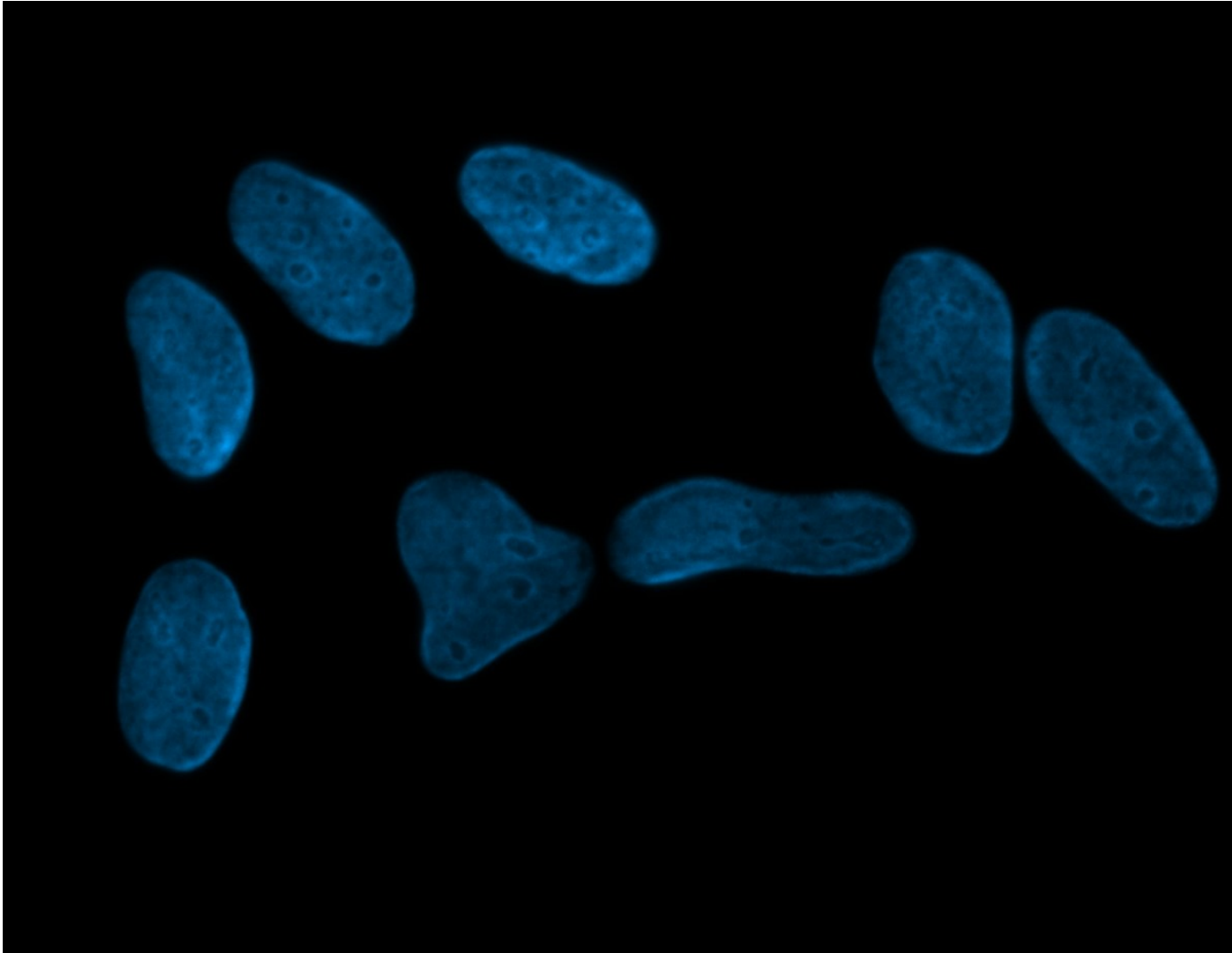
Image by S. Thakur

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SOURCE PROVENANCE

Catalog	SA-000064
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8E18810C7DCB5DF8D3DCAF5F1AF06F1A20334B7CBCCB85E94E7B3A72AECDE9BD
Method PDF SHA-256	89E8BA6E5CC67B6E7F525AC25E5007E950267FFCE811C48863313AED1999A06A

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
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)	987 X 767
Image Size (Scaled)	141.00µm X 109.57µm
Bit Depth	14 Bit
Image Center Position	X: 45.78mm, Y: 53.84mm
Stage Position	X: 45.78mm, Y: 53.84mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50 Cy5 49 DAPI
Beam Splitter	660 395
Filter Excitation Wavelength	625 - 655 335 - 383
Filter Emission Wavelength	665 - 715 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	653 353
Emission Wavelength	668 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.30µm 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™ 647 Affinity Purified Donkey Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000064

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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) = 987 X 767

Image Size (Scaled) = 141.00µm X 109.57µm

Bit Depth = 14 Bit

Image Center Position = X: 45.78mm, Y: 53.84mm

Stage Position = X: 45.78mm, Y: 53.84mm

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

647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

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

Exposure Time = 200ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.30µm

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-000064
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
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	B0826BAD11108532B3FAB0226A88D26EC47067955968BEF3F287D73379435256
Method PDF SHA-256	807E2624074DC0FFDEFA3507D0FF3A8F41A49EE8DF0C482073756338F73E210F