

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

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

8

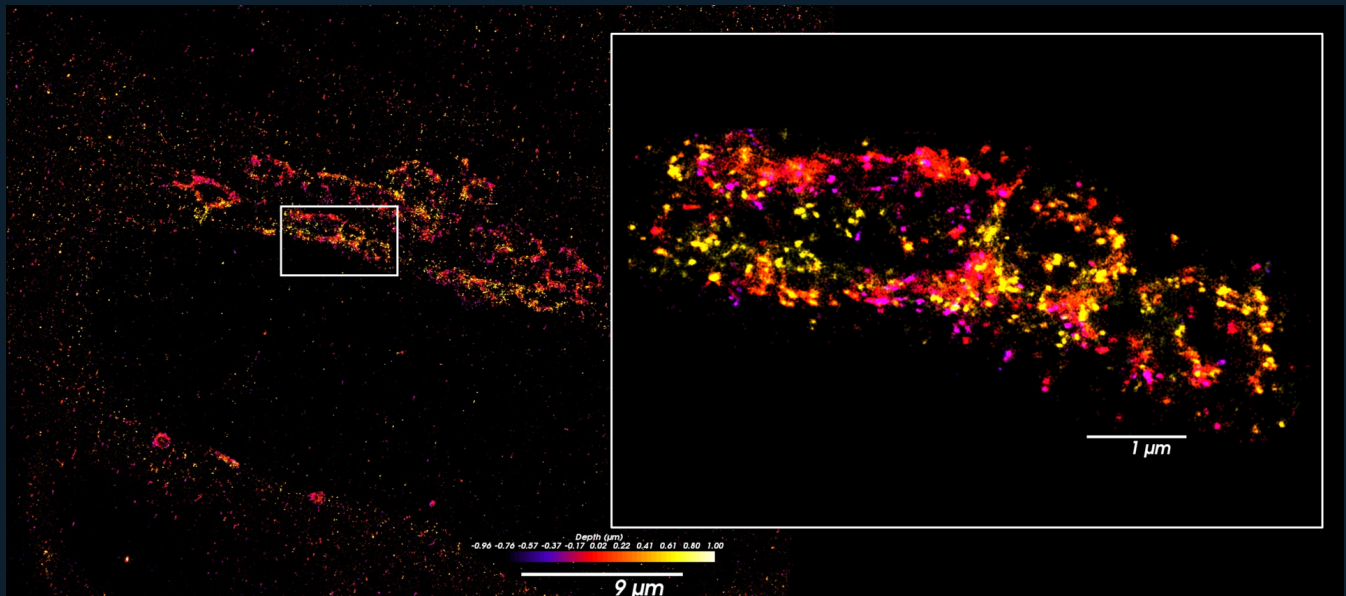
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000043 | 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™ 555 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 -> Single-molecule STORM
-> Control

BENNETT ASSESSMENT

The preserved SA-000043 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-000043 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	555	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 680	2; 45 Texas Red; 50 Cy5; 540 - 580; 625 - 655; 593 - 668; 665 - 715; 553
Widefield SIM — Apotome	405/DAPI; 555; 680	3; AF532-49014; 50 Cy5; 49 DAPI; 515 - 545; 625 - 655; 335-383; 555 - 595
Confocal	405/DAPI; 488; 555	3; None; 561nm @0.2%; 488nm @0.1%; 405nm @1.0%; 553; 493; 353
Airyscan	405/DAPI; 488; 555; 680; 750	2; None; 561nm @1.00%; 640nm @3.30%; 553; 679; 568; 702
SIM	405/DAPI; 488; 555	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820 #2-SR; 561
SIM ²	405/DAPI; 488; 555	2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 #2-SR; T2-Axiocam 820 #2-SR; 561
Single-molecule STORM	555	Both Channels
Control	405/DAPI; 555	2; AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 553

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 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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: 2; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 539 X 550; Image Size (Pixels): 539 X 550; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 75ms; 450ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @25%. 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; 555; 680.

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: 17 Slices (3.20µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 206 X 259; Image Size (Pixels): 206 X 259; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 150ms; 300ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @8.0%; X-Cite Xylis Lamp Intensity @13.0%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 53°, 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: 47.

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; 555; 680.

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: Z-Stack: 52 Slices (2.55µm); Channels: 3; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.050µm; Image size (Pixels): 1105 X 1105; Image Size (Pixels): 1105 X 1105; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-PMT 1; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.70µs; Frame Time: 8.05s. Illumination: Laser Wavelength: 561nm @0.2%; 488nm @0.1%. Optical/scan: Pinhole: 1.00AU / 50µm; 1.00AU / 44µm; Scan Zoom: 1.3; LSM Scan Speed: 8; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 56°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

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; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 82 Slices (4.05µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 50.00nm; Image size (Pixels): 954 X 771; Image Size (Pixels): 954 X 771; 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: 561nm @1.00%; 640nm @3.30%. Optical/scan: Pinhole: 5.00AU / 245µm; 5.00AU / 282µm; Scan Zoom: 1.5; LSM Scan Speed: 7; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 9.6 (3D, Auto); Super Resolution 9.2 (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; 555; 680; 750.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 38 Slices (1.85µm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 2577 X 2577; Image Size (Pixels): 2577 X 2577; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @0.9%; 405nm @6.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 49°, Scale Z 52%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 56.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 62 Slices (3.05µm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 683 X 806; Image Size (Pixels): 683 X 806; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 561nm @0.9%; 405nm @6.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 34°, Scale Z 162%, 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 SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 555.

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 555.

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): 1260 X 722; Image Size (Pixels): 1260 X 722; 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; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000043 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.29nm 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)=954 X 771; Image Size (Scaled)=33.66 μ m X 27.21 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): 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)=2577 X 2577; Image Size (Scaled)=43.53 μ m X 43.53 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): 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)=683 X 806; Image Size (Scaled)=11.54 μ m X 13.61 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

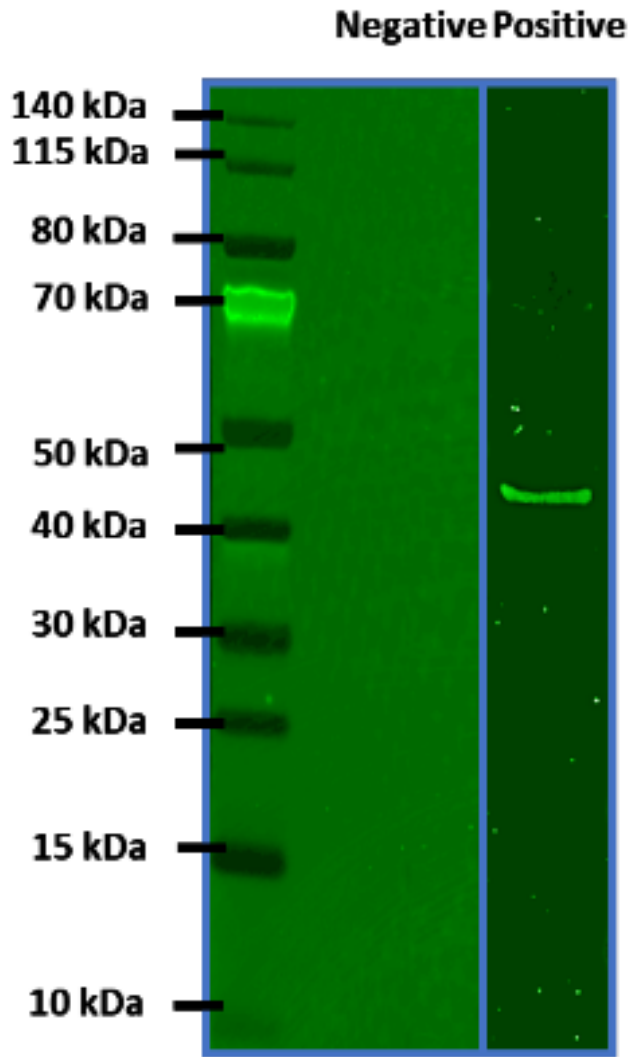
This dossier preserves the complete available evidence progression for SA-000043. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra	PRESERVED
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	555
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	572±28nm
Detector	Avalanche Photodiode
Intensity	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™ 555 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000043

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™ 555 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 = 572±28nm

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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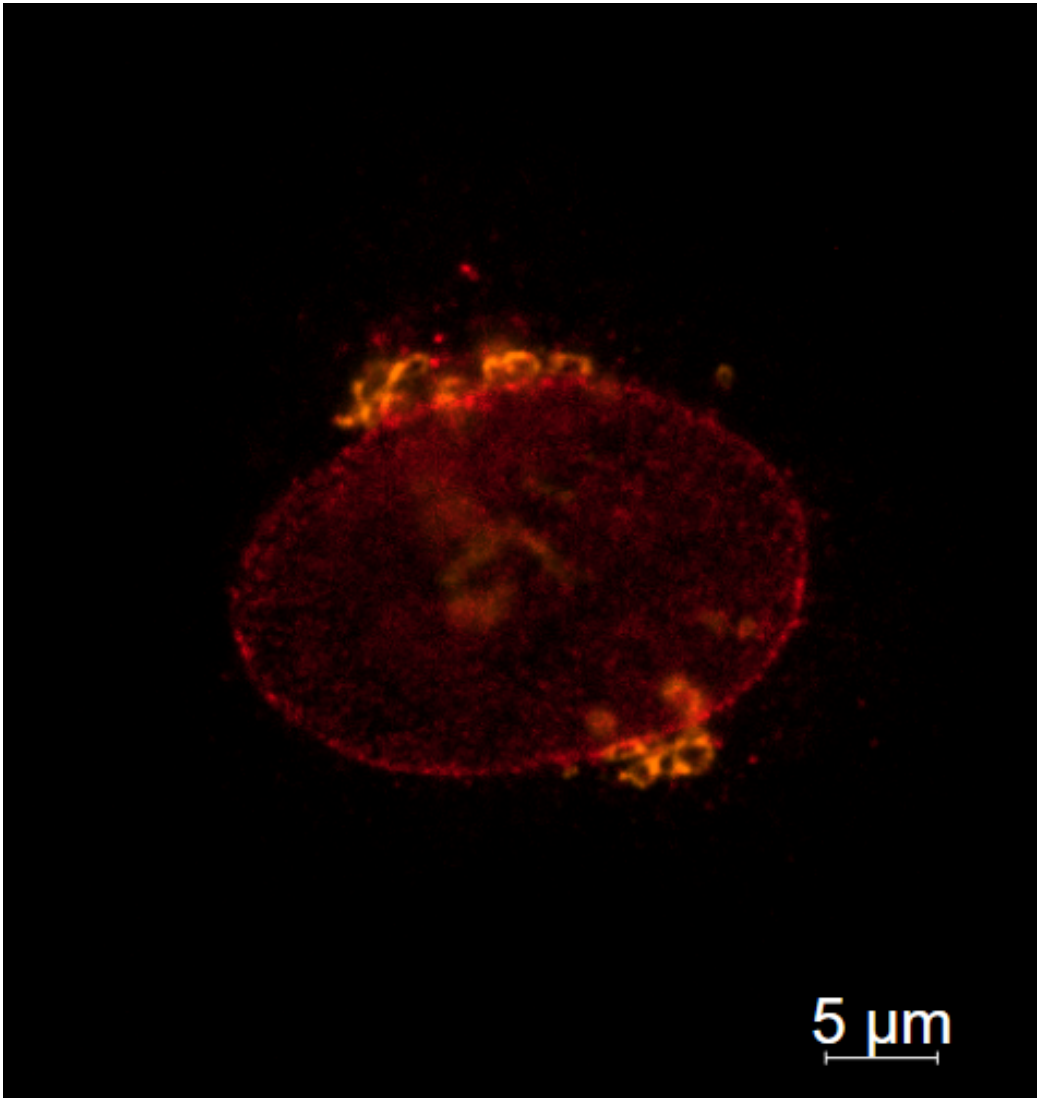
SOURCE PROVENANCE

Catalog	SA-000043
Stage	Western blot

SOURCE PROVENANCE

Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AADC0D061EF56FECDF746A836F3723C5C624D7A60D53308048F9626B7EE0157F
Method PDF SHA-256	3A3A27F01075B7E580C562DBCCA23838ACC3CB3602390B976E5511A7A01FE9C1

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 680
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)	539 X 550
Image Size (Scaled)	55.18µm X 56.31µm
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 50 Cy5
Beam Splitter	585 660
Filter Excitation Wavelength	540 - 580 625 - 655
Filter Emission Wavelength	593 - 668 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @25%
Exposure Time	75ms 450ms
Excitation Wavelength	553 679
Emission Wavelength	568 702
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.88µm 1.09µm
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™ 555 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000043

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000073

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody that followed this, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 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) = 539 X 550

Image Size (Scaled) = 55.18µm X 56.31µm

Bit Depth = 12 Bit

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

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

555 -

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 = 75ms

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 0.88µm

Binning Mode = 1,1

680 -

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 = 450ms
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Imaging Device = AxioCam MR R3
 Camera Adapter = 1X
 Depth of Focus = 1.09µm
 Binning Mode = 1,1

Post Processing

None

Image Correction

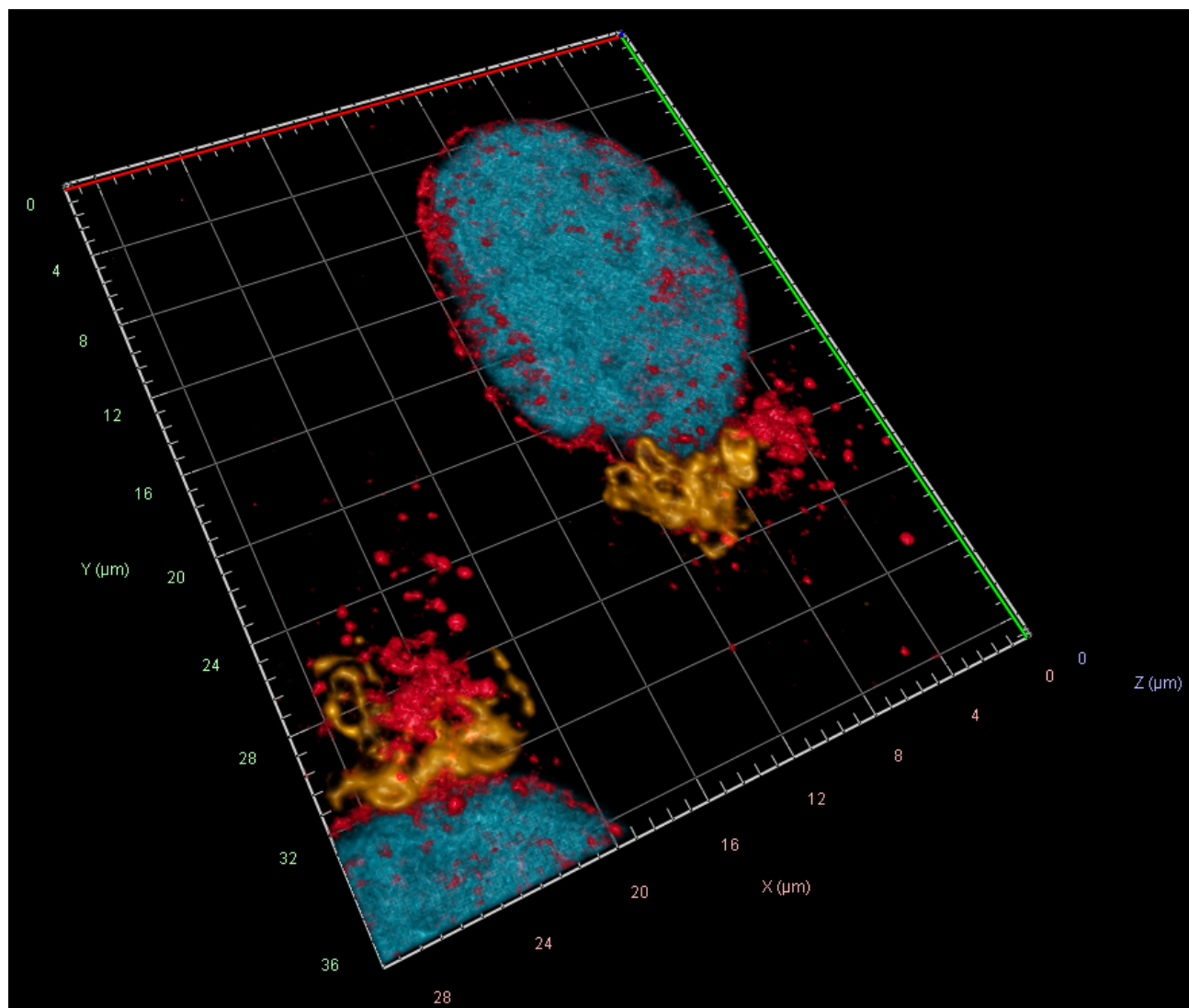
None

Image by E.F. Simon

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SOURCE PROVENANCE	
Catalog	SA-000043
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	BD73B7C211A8A22E0BF919966F570232BCE8E283196CBBC15C8D58B268E2E4BE
Method PDF SHA-256	8F18376DD319D10A8B68C82834CB393B022F6F6425DFA1EBD765AC255F269420

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; 555; 680
METADATA VALUES	47

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	17 Slices (3.20µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	206 X 259
Image Size (Scaled)	29.43µm X 37.00µm
Bit Depth	14 Bit
Image Center Position	X: 89.49mm, Y: 47.01mm
Stage Position	X: 89.49mm, Y: 47.01mm
ROI Center Offset	X: -20.57, Y: -6.29µm
Reflector	AF532-49014 50 Cy5 49 DAPI
Beam Splitter	550 660 395
Filter Excitation Wavelength	515 - 545 625 - 655 335-383
Filter Emission Wavelength	555 - 595 665 - 715 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @8.0% X-Cite Xylis Lamp Intensity @13.0% X-Cite Xylis Lamp Intensity = 8.0%
Exposure Time	150ms 300ms 20ms
Excitation Wavelength	553 679 353
Emission Wavelength	568 702 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.10µm 1.36µm 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 17% (680), Threshold 14% (555), Threshold 8% (DAPI) Ramp 50% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 130%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 53°, 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™ 555 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000043

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000073

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody that followed this, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 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 = 17 Slices (3.20µm)

Channels = 3

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

Image Size (Pixels) = 206 X 259

Image Size (Scaled) = 29.43µm X 37.00µm

Bit Depth = 14 Bit

Image Center Position = X: 89.49mm, Y: 47.01mm

Stage Position = X: 89.49mm, Y: 47.01mm

ROI Center Offset = X: -20.57, Y: -6.29µm

555 -

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 @8.0%
 Exposure Time = 150ms
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 1.10µm
 Binning Mode = 2,2

680 -

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 @13.0%
 Exposure Time = 300ms
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 1.36µm
 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 = 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 Volume Projection = Precise

Appearance = Threshold 17% (680), Threshold 14% (555), Threshold 8% (DAPI) Ramp 50% all channels, Maximum 100% all channels

Background = Black

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

Projection = View Angle 53°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

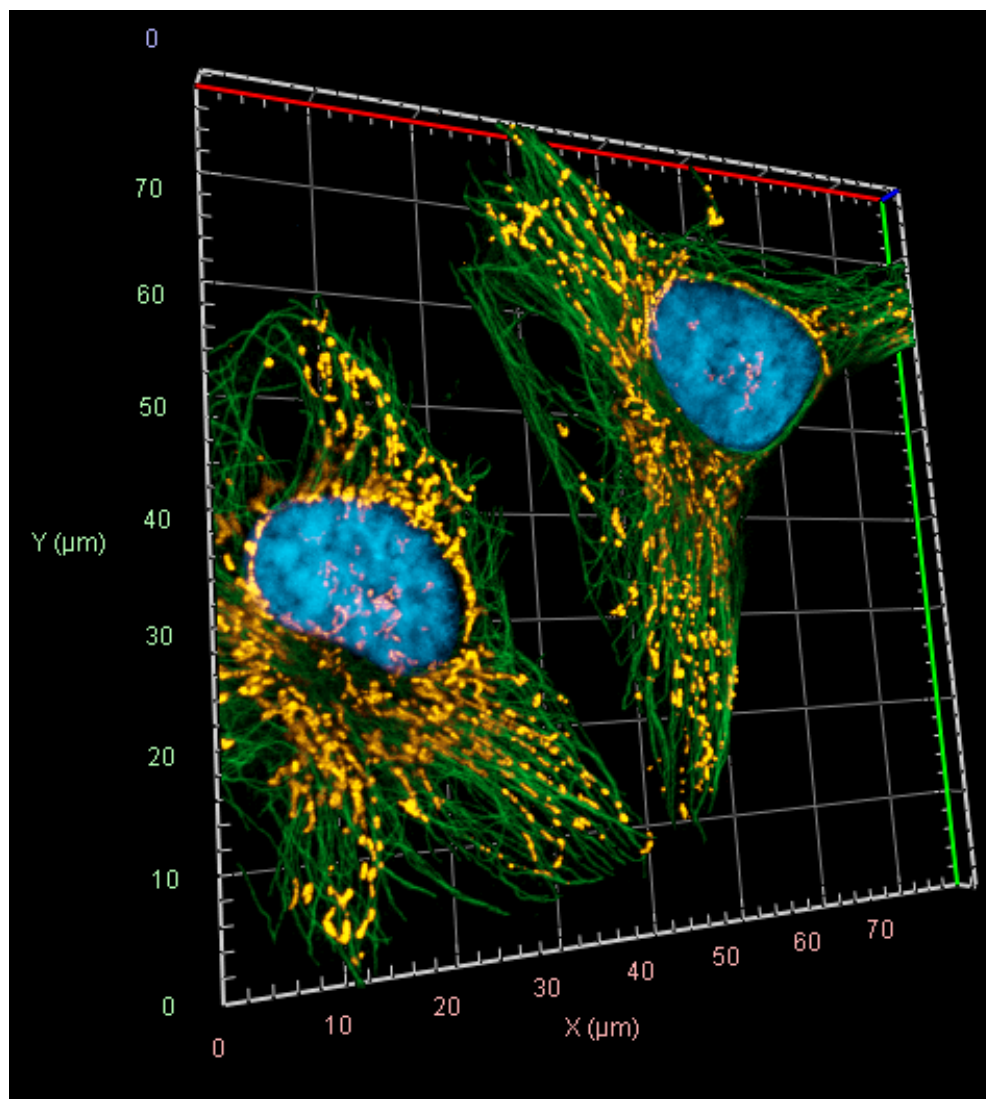
Image by B.T. Bennett

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SOURCE PROVENANCE

Catalog	SA-000043
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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	98A34E942F788C5073CAC38BD7F0057F40943D9308B3E5EC15C6338DFD60056C
Method PDF SHA-256	08B28C91219FA7F97CE4C469BA8F37AAB21225F78A081BF6C10C980807B0CC10

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	52 Slices (2.55µm)
Channels	3
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.050µm
Image Size (Pixels)	1105 X 1105
Image Size (Scaled)	78.01µm X 78.01µm
Bit Depth	16 Bit
Image Center Position	X: 70.77mm, Y: 52.23mm
Stage Position	X: 61.28mm, Y: 52.23mm
ROI Center Offset	X: -0.94µm, Y: -0.94µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 50µm 1.00AU / 44µm 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.2% 488nm @0.1% 405nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	0°
Sampling	1.00
Pixel Time	0.70µs
Frame Time	8.05s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	553 493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	568 - 630 498 - 550 410 - 470
Imaging Device	Airyscan GaAsp-PMT 1
Detector Type	GaAsp-PMT
Detector Gain	700 V 650 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (555), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness130%, Enabled Tone Mapping
Projection	View Angle 56°, 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™ 555 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000043

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific

Cat ID# - SA 000012

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 Rabbit Recombinant Antibody (MTC02,2860R), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 52 Slices (2.55µm)

Channels = 3

Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.050µm

Image Size (Pixels) = 1105 X 1105

Image Size (Scaled) = 78.01µm X 78.01µm

Bit Depth = 16 Bit

Image Center Position = X: 70.77mm, Y: 52.23mm

Stage Position = X: 61.28mm, Y: 52.23mm

ROI Center Offset = X: -0.94µm, Y: -0.94µm

555 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 50µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.70µs
Frame Time = 8.05s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 553
Emission Wavelength = 568
Effective NA = 1.4
Detection Wavelength = 568 - 630
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 700 V
Detector Offset = 0
Detector Digital Gain = 1.0

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 44µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.1%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.70µs
 Frame Time = 8.05s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 498 - 550
 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 @1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.70µs
 Frame Time = 8.05s
 LSM Scan Speed = 8
 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-PMT 1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (555), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness130%, Enabled Tone Mapping
 Projection = View Angle 56°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

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SOURCE PROVENANCE

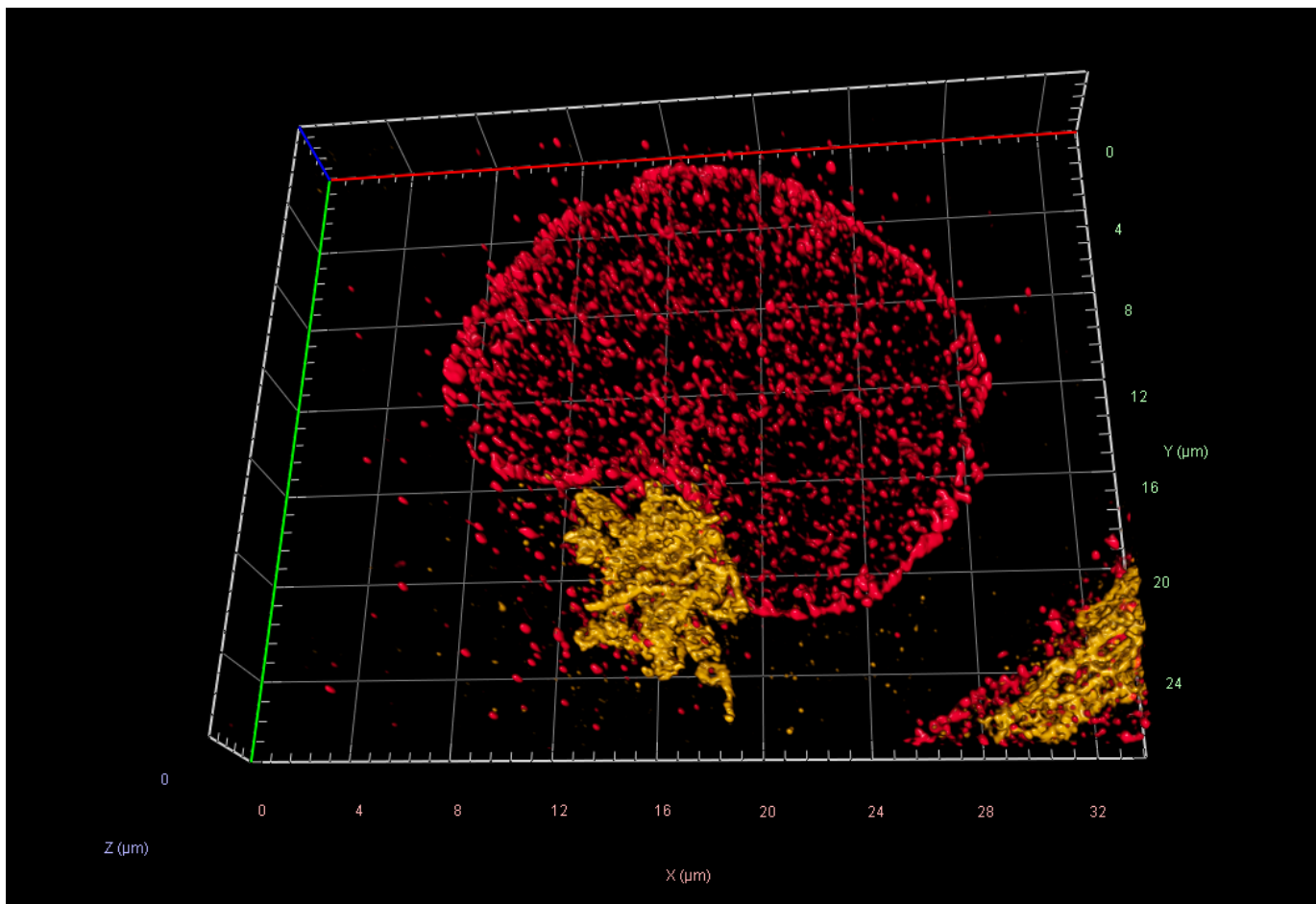
Catalog

SA-000043

SOURCE PROVENANCE

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:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9A6C280F4036777D2645472218AA33A208D9BDD700D78E90E171AD51CDB96E94
Method PDF SHA-256	F00164B3395D49A43696F2160BFC552A1D4A8A50497F304B35E9457B36E4542D

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 680; 750
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 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	82 Slices (4.05µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.05000 µm
Image Size (Pixels)	954 X 771
Image Size (Scaled)	33.66µm X 27.21µm
Bit Depth	16 Bit
Image Center Position	X: 68.46mm, Y: 49.07mm
Stage Position	X: 68.46mm, Y: 49.07mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 245µm 5.00AU / 282µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @1.00% 640nm @3.30%
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
Excitation Wavelength	553 679
Emission Wavelength	568 702
Effective NA	1.4
Detection Wavelength	550 - 610 656 - 700
Imaging Device	Airyscan
Detector Type	GaAsp-PMT
Detector Gain	800 V 750 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 9.6 (3D, Auto) Super Resolution 9.2 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 19%, Ramp 81%, Maximum 100%
Background	Black
Light	Brightness 127%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000043

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000073

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody that followed this, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 82 Slices (4.05µm)

Channels = 2

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

Image Size (Pixels) = 954 X 771

Image Size (Scaled) = 33.66µm X 27.21µm

Bit Depth = 16 Bit

Image Center Position = X: 68.46mm, Y: 49.07mm

Stage Position = X: 68.46mm, Y: 49.07mm

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

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 245µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @1.00%
 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 = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 550 - 610
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.6 (3D, Auto)

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 282μm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @3.30%
 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 = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Detection Wavelength = 656 - 700
 Imaging Device = Airyscan
 Detector Type = GaAsp-PMT
 Detector Gain = 750 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.2 (3D, Auto)

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 19%, Ramp 81%, Maximum 100%
 Background = Black
 Light = Brightness127%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

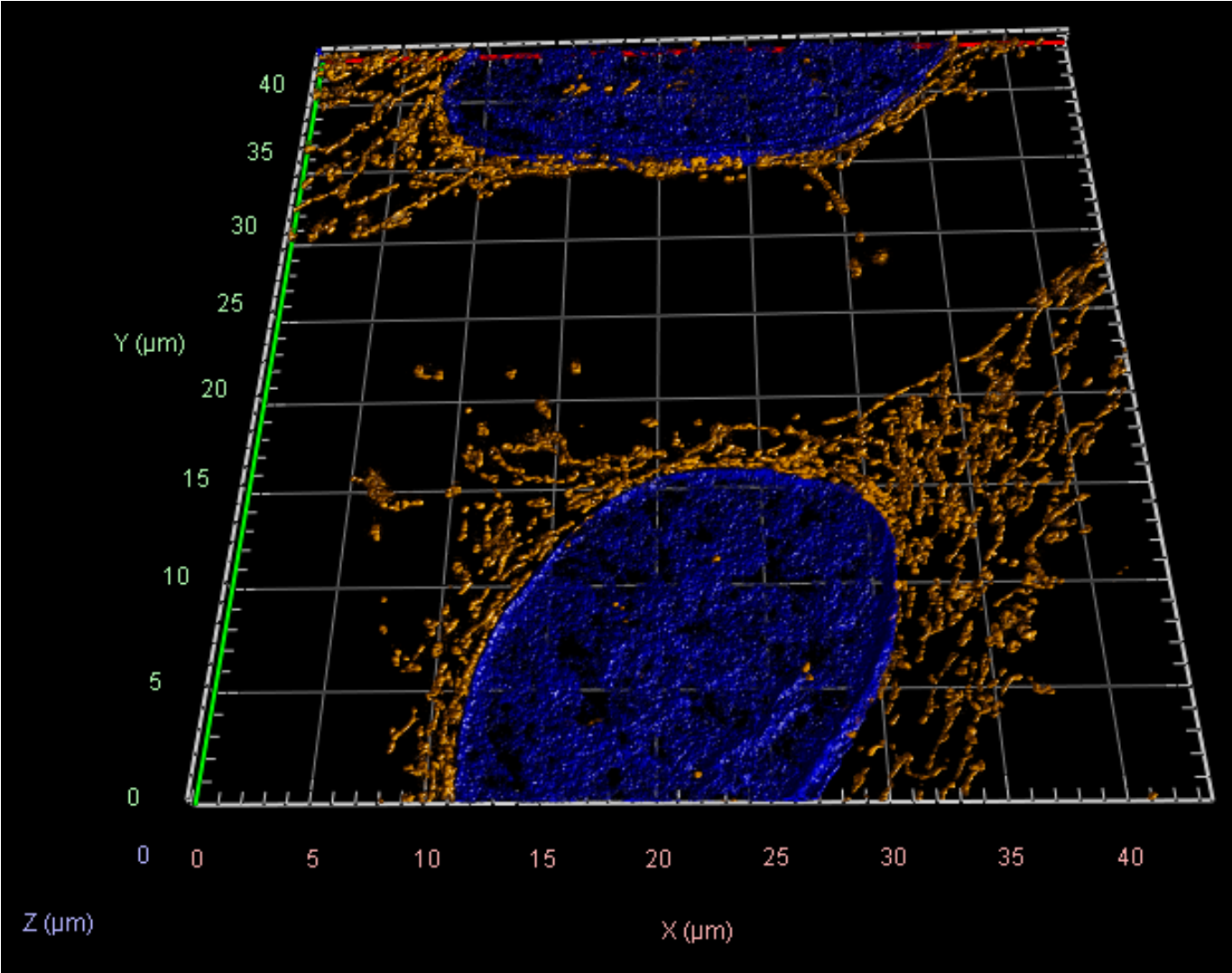
Catalog

SA-000043

SOURCE PROVENANCE

Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	54522D6C33C11C18C14AC4C86E8901F3664C74C9BCF066A5B86CADA60324E78B
Method PDF SHA-256	D8A5BFC03A0DEF5407C8E077300C910F50DB92002904688EC089F7FC5CA03405

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	38 Slices (1.85µm)
Channels	2 1
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	2577 X 2577
Image Size (Scaled)	43.53µm X 43.53µm
Bit Depth	16 Bit
Image Center Position	X: 60.79mm, Y: 33.95mm
Stage Position	X: 60.79mm, Y: 33.95mm
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 T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	583 525
Effective NA	1.4
Detection Wavelength	503 - 546, 576 - 590 503-546, 576-590
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms 50ms
Depth of Focus	0.90µm 0.81µm
Binning Mode	2,2
Laser Wavelength	561nm @0.9% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	2
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.5251
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Volume Projection	Precise
Appearance	Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% (555), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness170%, Azimuth 32°, Elongation -34°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 49°, Scale Z 52%, 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™ 555 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)
Cat ID# - SA 000043

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 overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 555 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 = 38 Slices (1.85µm)

Channels = 2

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

Image Size (Pixels) = 2577 X 2577

Image Size (Scaled) = 43.53µm X 43.53µm

Bit Depth = 16 Bit

Image Center Position = X: 60.79mm, Y: 33.95mm

Stage Position = X: 60.79mm, Y: 33.95mm

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

555 -

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 = 583
 Effective NA = 1.4
 Detection Wavelength = 503 - 546, 576 - 590
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 100ms
 Depth of Focus = 0.90µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @0.9%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 27.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 503-546, 576-590
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D

Result Sampling = 4

Process Sampling = 2

Channels = 1

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 10.5251

Input SNR = N/A

Regularization Weight = N/A

Iterations = N/A

Filter = N/A

Fitting = N/A

Normalization = Automatic

Experimental PSF = N/A

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% (555), Ramp 98% (DAPI), Maximum 100% all channels

Background = Black

Light = Brightness170%, Azimuth 32°, Elongation -34°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 49°, Scale Z 52%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

Copyright 2026 GMD12 LLC.

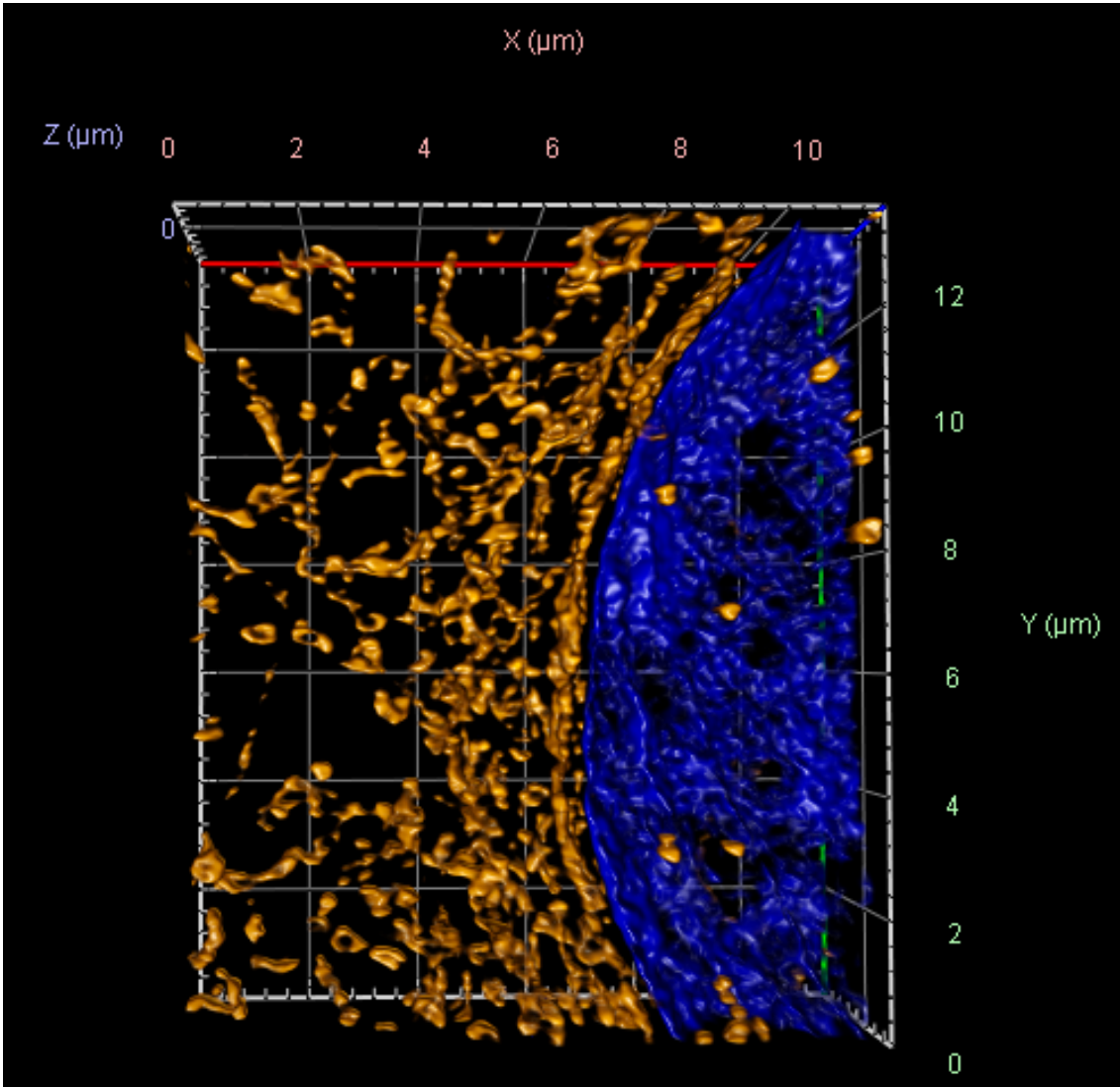
SOURCE PROVENANCE	
Catalog	SA-000043
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	87D6D304BDA9916DB9C1116DA28AEA7E6DB5D120CEDC508FED218C3E8E9FEFA8

SOURCE PROVENANCE

Method PDF SHA-256

F19A52F8E1BA5A272DB9CC00D5C899E0B0FFB65AF0C872776AACBABC6741E09F

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	56

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	62 Slices (3.05µm)
Channels	2 1
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	683 X 806
Image Size (Scaled)	11.54µm X 13.61µm
Bit Depth	16 Bit
Image Center Position	X: 56.43mm, Y: 34.69mm
Stage Position	X: 56.43mm, Y: 34.69mm
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 T2-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	583 525
Effective NA	1.4
Detection Wavelength	503 - 546, 576 - 590 503-546, 576-590
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms 50ms
Depth of Focus	0.90µm 0.81µm
Binning Mode	2,2
Laser Wavelength	561nm @0.9% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	10.5797
Input SNR	N/A
Regularization Weight	N/A
Iterations	N/A
Filter	N/A
Fitting	N/A
Normalization	Automatic
Experimental PSF	N/A
3D Volume Projection	Precise
Appearance	Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% (555), Ramp 98% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness158%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 34°, Scale Z 162%, 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™ 555 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000043

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 overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 555 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 = 62 Slices (3.05µm)

Channels = 2

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

Image Size (Pixels) = 683 X 806

Image Size (Scaled) = 11.54µm X 13.61µm

Bit Depth = 16 Bit

Image Center Position = X: 56.43mm, Y: 34.69mm

Stage Position = X: 56.43mm, Y: 34.69mm

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

555 -

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 = 583
 Effective NA = 1.4
 Detection Wavelength = 503 - 546, 576 - 590
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 100ms
 Depth of Focus = 0.90µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @0.9%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 27.5µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 503-546, 576-590
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D

Result Sampling = 4

Process Sampling = 4

Channels = 1

Algorithm = SIM2

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 10.5797

Input SNR = N/A

Regularization Weight = N/A

Iterations = N/A

Filter = N/A

Fitting = N/A

Normalization = Automatic

Experimental PSF = N/A

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 2% (555), Threshold 2% (DAPI), Ramp 98% (555), Ramp 98% (DAPI), Maximum 100% all channels

Background = Black

Light = Brightness158%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 34°, Scale Z 162%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

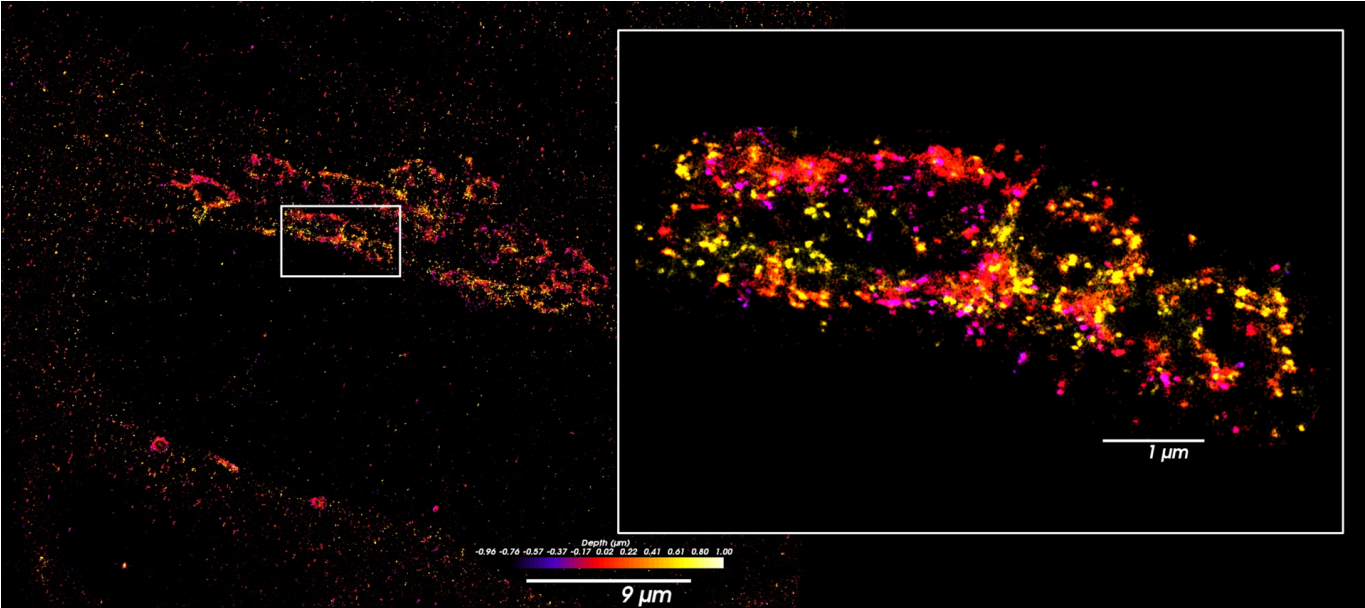
Catalog	SA-000043
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	63FA3D47D2DAFA0D7B420975EF89D0988AB7AFBFCCFD453D696F874D4C18623A

SOURCE PROVENANCE

Method PDF SHA-256

A136DB101BE64485FFF3D40042137FDBCA5D9CA58B17A5BC68EA12D086C4A87C

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	555
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: 555 nm (635 nm Dichroic); First reference probe: 555 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	178.9 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 555nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (555 nm (635 nm Dichroic))	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 555nm" 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: 180.2; End: 180.2
SuperRes 555nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 30000
Z Positions	1 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	Constant
Particle size	25nm
Color by	Depth
Color Table	Single
Opacity Mode	Constant; Opacity 1: 0.7
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™ 555 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000043

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, Golgi GOLPH2 Polyclonal Antibody, 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® 555 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: 555 nm (635 nm Dichroic); First reference probe: 555 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: 178.9 µm

Probe 1 Laser control (555 nm (635 nm Dichroic))

Widefield 555nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
 Reference Probe 1 Laser control (555 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

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

Widefield Laser: "Widefield 555nm" 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: 180.2; End: 180.2

Probe 1 Laser control (555 nm (635 nm Dichroic)

SuperRes 555nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control (555 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: 30000

Z Positions: 1 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 (555 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: Constant
 Particle size: 25nm
 Color by: Depth
 Color Table: Single
 Opacity Mode: Constant; Opacity 1: 0.7
 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

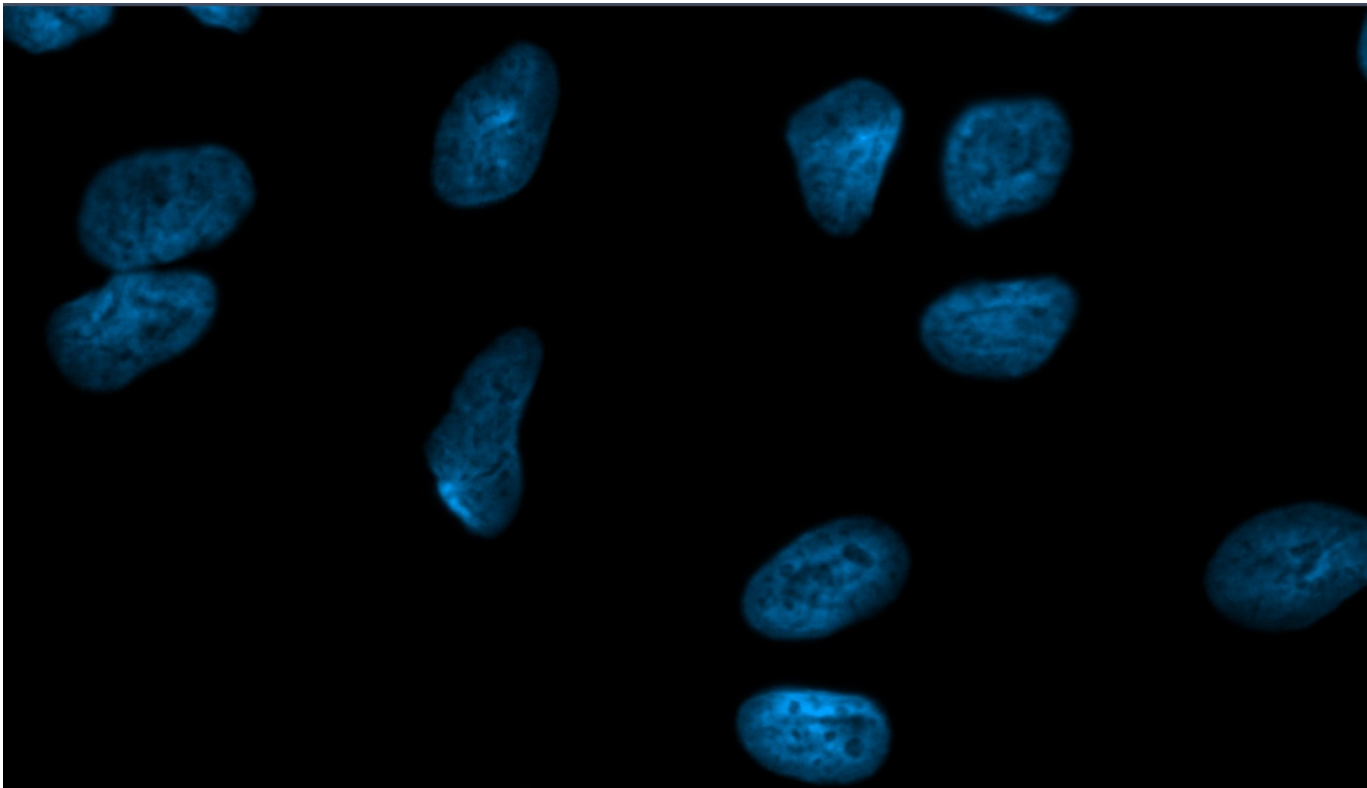
Image by S. Thakur

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SOURCE PROVENANCE

Catalog	SA-000043
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	5247B5FB7800186A93F4A374A09C00F83B4D411BCB1AE7261F44DA83B07014E9
Method PDF SHA-256	EBD13B9729FEB58DC02550BB5EE96D0A1054AC2B4EF7DF5B3054FCACF50444A2

ORIGINAL IMAGE EVIDENCE / CONTROL



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

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1260 X 722
Image Size (Scaled)	180.00µm X 103.14µm
Bit Depth	14 Bit
Image Center Position	X: 50.12mm, Y: 50.76mm
Stage Position	X: 50.12mm, Y: 50.76mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	AF532-49014 49 DAPI
Beam Splitter	550 395
Filter Excitation Wavelength	515 - 545 335 - 383
Filter Emission Wavelength	555 - 595 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	553 353
Emission Wavelength	568 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.10µm 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™ 555 Affinity Purified Donkey Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000043

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® 555 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) = 1260 X 722

Image Size (Scaled) = 180.00µm X 103.14µm

Bit Depth = 14 Bit

Image Center Position = X: 50.12mm, Y: 50.76mm

Stage Position = X: 50.12mm, Y: 50.76mm

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

555 -

Reflector = AF532-49014

Beam Splitter = 550

Filter Excitation Wavelength = 515 - 545

Filter Emission Wavelength = 555 - 595

Contrast Method = Fluorescence

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

Exposure Time = 200ms

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.10µm

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-000043
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	8570937E0FD24FDECB7F6C5523D604D896DB351D92AAF36CA2A9D3FD5AD34A12
Method PDF SHA-256	FA7F502BB6487185BD3DCDF4B35EB68EEB67B25E8E01573D60BD66717E6632C8