

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

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

8

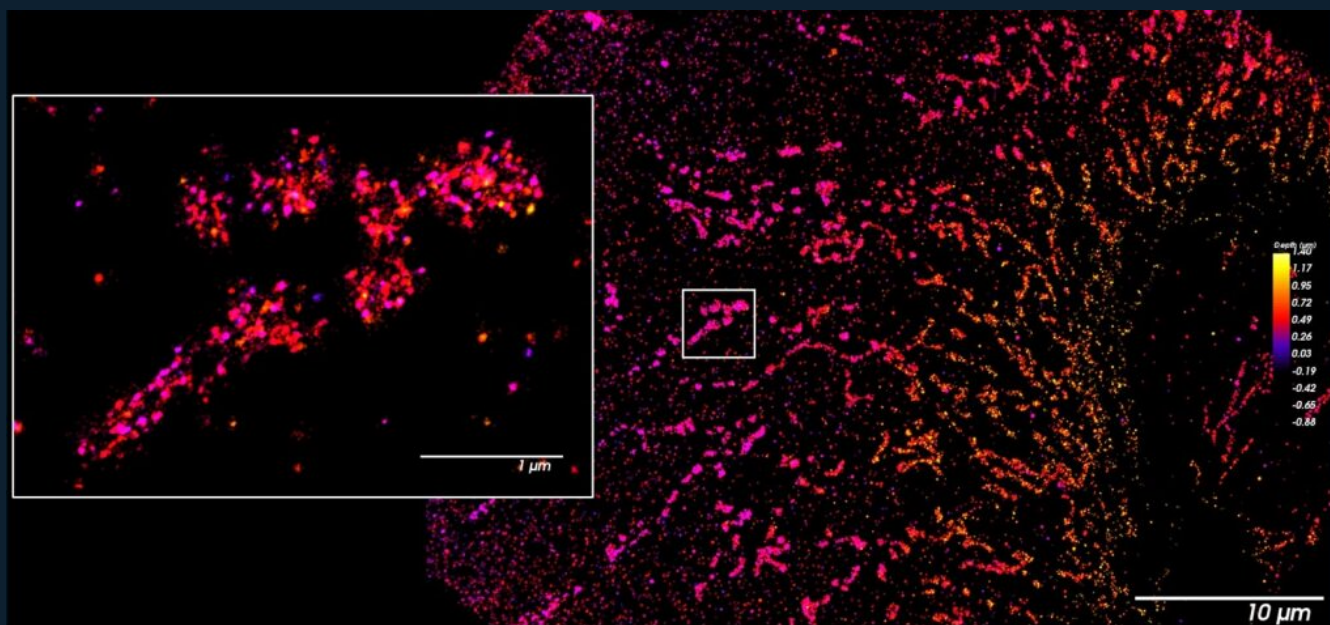
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000040 | 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 Goat 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-000040 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-000040 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	3; 45 Texas Red; 50 Cy5; 49 DAPI; 540 - 580; 625 - 655; 335 - 383; 593 - 668
Widefield SIM — Apotome	405/DAPI; 555	2; 45 Texas Red; 49 DAPI; 540 - 580; 335-383; 593 - 668; 420-470; 553
Confocal	405/DAPI; 488; 555	3; None; 561nm @0.6%; 488nm @0.5%; 405nm @0.5%; 553; 493; 353
Airyscan	405/DAPI; 488; 555	3; None; 560nm @0.2%; 488nm @0.1%; 405nm @1.0%; 553; 5493; 353
SIM	405/DAPI; 488; 532; 555	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820 #2-SR; T3-Axiocam 820 #2-SR; 561; 405
SIM ²	405/DAPI; 488; 555	2; 561nm @4.0%; 405nm @10.0%; 561; 405; 515; 561.000000; 405.000000
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 705, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 200ms; 500ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; 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; 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: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 794 X 1091; Image Size (Pixels): 794 X 1091; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 200ms; 60ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @6%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

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.

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: 16 Slices (1.05µm); Channels: 3; Scaling (Per Pixel): 0.248µm X 0.248µm X 0.070µm; Image size (Pixels): 512 X 512; Image Size (Pixels): 512 X 512; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsp-PMT 2; GaAsp-PMT 1; Detector Type: GaAsp-PMT. Timing: Pixel Time: 1.52µs; Frame Time: 1.86s. Illumination: Laser Wavelength: 561nm @0.6%; 488nm @0.5%. Optical/scan: Pinhole: 1.00AU / 53µm; 1.00AU / 43µm; Scan Zoom: 0.80; LSM Scan Speed: 8; 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. 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 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: 54 Slices (3.71µm); Channels: 3; Scaling (Per Pixel): 35.30nm X 35.30nm X 70.00nm; Image size (Pixels): 2849 X 2849; Image Size (Pixels): 2849 X 2849; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.73µs; Frame Time: 14.22s. Illumination: Laser Wavelength: 560nm @0.2%; 488nm @0.1%. Optical/scan: Pinhole: 5.00 AU / 266µm; 5.00 AU / 243µm; Scan Zoom: 1.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 10.0 (3D, Auto); Super Resolution 8.6 (3D, Auto). Structured source metadata values: 52.

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.

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: 55 Slices (1.62µm); Channels: 2; Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm; Image size (Pixels): 5031 X 2855; Image Size (Pixels): 5031 X 2855; 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 @7.0%; Illumination Wavelength: 561; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 107%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 532; 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 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: 22 Slices (1.47µm); Channels: 2; Scaling (Per Pixel): 15.65nm X 15.65nm X 70.00nm; Image size (Pixels): 1619 X 1401; Image Size (Pixels): 1619 X 1401; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 60ms; 100ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 561nm @4.0%; 405nm @10.0%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Projection: View Angle 35°, Scale Z 164%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 60.

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; 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: 79.

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): 1268 X 1100; Image Size (Pixels): 1268 X 1100; 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-000040 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): 35.30nm X 35.30nm X 70.00nm -> 0.03530 μ m X 0.03530 μ m X 0.07000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2849 X 2849; Image Size (Scaled)=100.57 μ m X 100.57 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 50.00nm -> 0.01320 μ m X 0.01320 μ m X 0.02945 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)=5031 X 2855; Image Size (Scaled)=66.39 μ m X 37.67 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

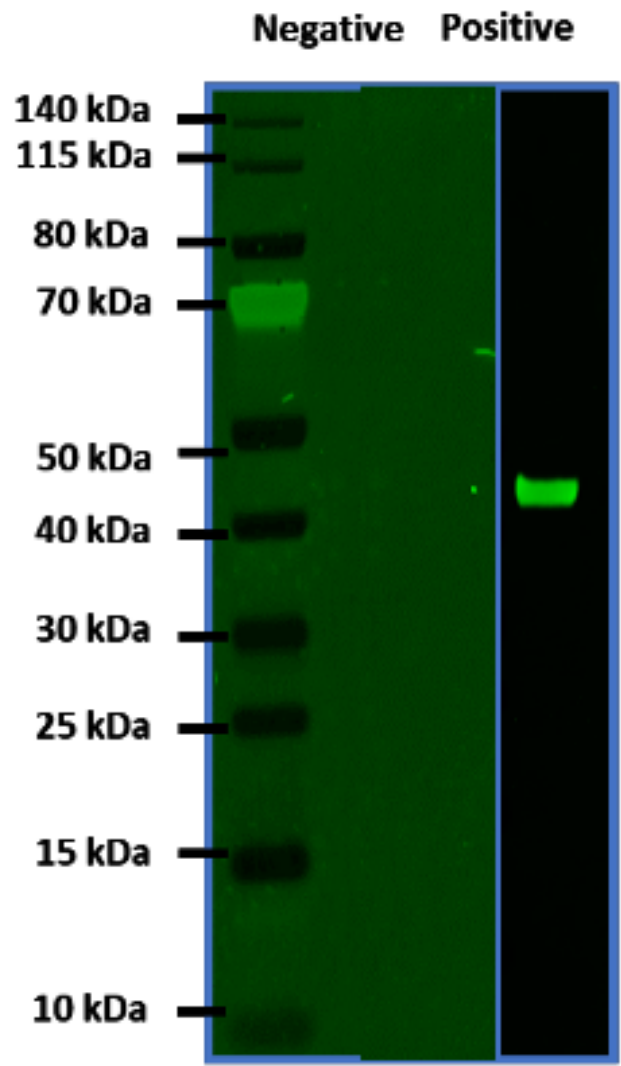
This dossier preserves the complete available evidence progression for SA-000040. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra 7	PRESERVED
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	L2
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 Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000040

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™ 555 Affinity Purified Goat anti-Mouse F(ab)₂ was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence

Laser = 532nm

Emission Filter = 572±28nm

Detector = Avalanche Photodiode

Intensity = L2

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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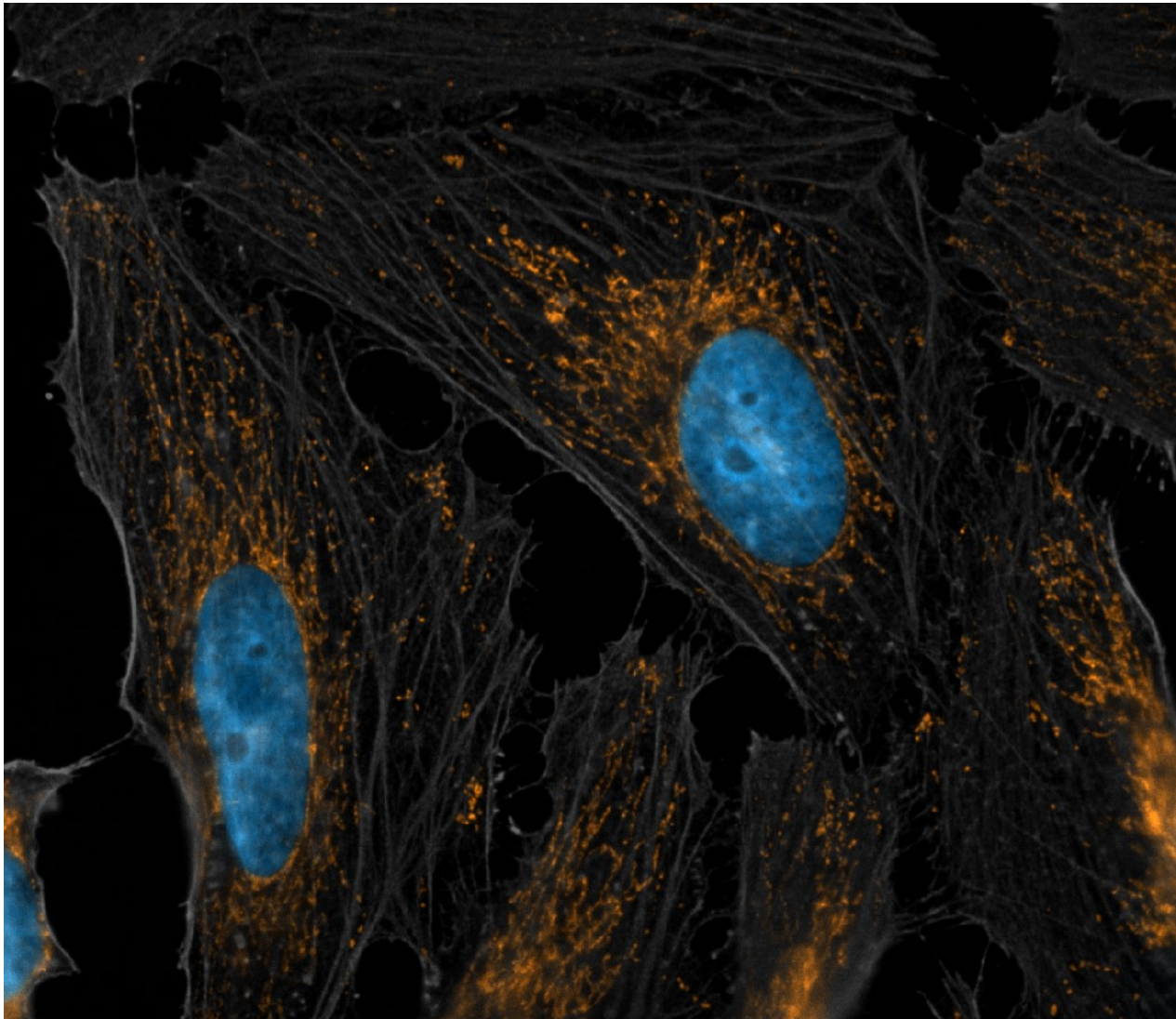
SOURCE PROVENANCE

Catalog	SA-000040
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

SOURCE PROVENANCE

Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D4A22B5B092D8251E513CC25D8A598252A498FD2AF25E3C295C2AFA6D4DAE891
Method PDF SHA-256	0DB509BAE13C20FEDE9A7659151481034FC5B20EBB22BEF18FF9D894B0D54587

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555; 680
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
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 49 DAPI
Beam Splitter	585 660 395
Filter Excitation Wavelength	540 - 580 625 - 655 335 - 383
Filter Emission Wavelength	593 - 668 665 - 715 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @30% X-Cite Xylis Lamp Intensity @15%
Exposure Time	200ms 500ms 50ms
Excitation Wavelength	553 679 353
Emission Wavelength	568 702 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.88µm 1.09µm 0.72µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

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

Cat ID# - SA 000040

Identifyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000071

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MTC02), 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, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18µm X 112.59µm

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 @10%

Exposure Time = 200ms

Excitation Wavelength = 553

Emission Wavelength = 568

Effective NA = 1.4

Imaging Device = Axiocam 705

Camera Adapter = 1X

Depth of Focus = 0.88µm

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 @30%
 Exposure Time = 500ms
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 1.09µm
 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 @15%
 Exposure Time = 50ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 2,2

Post Processing

Identifyn® SRM Alexa Fluor™ 680 was pseudo-colored in Zen Software from red, the default color, to dark grey to provide visual contrast between the 555/680 channels.

Image Correction

None

Image by J.K. Bennett

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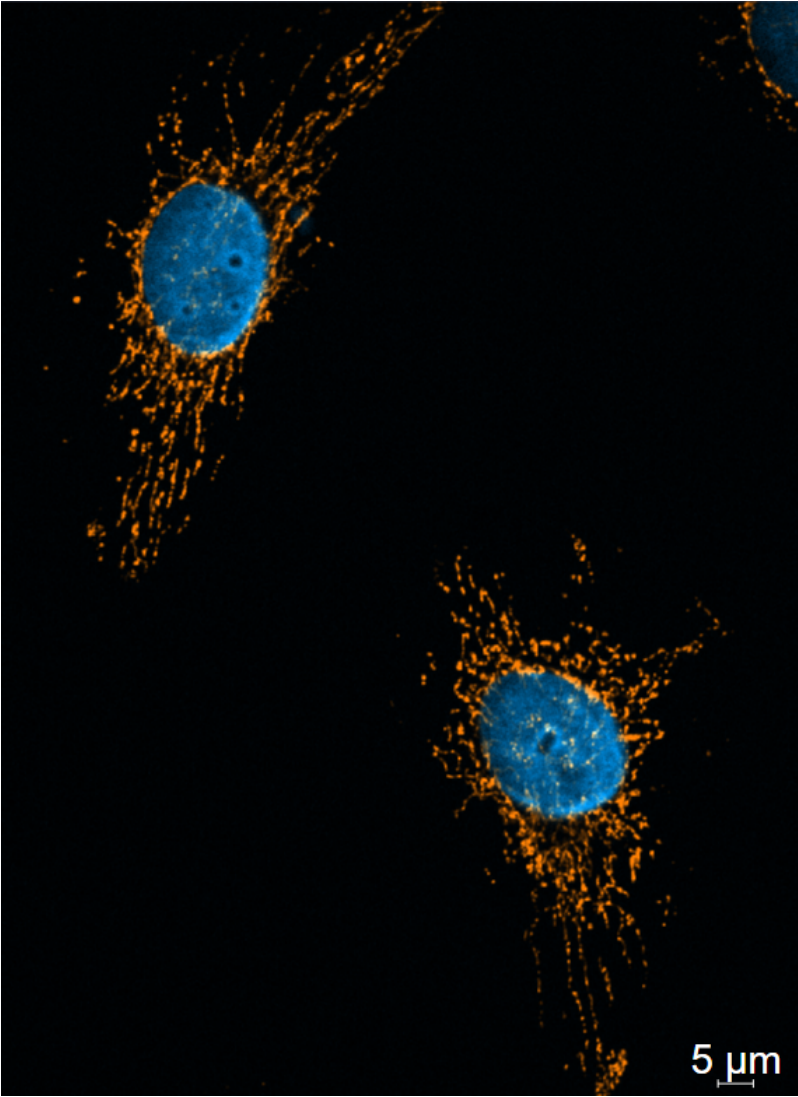
SOURCE PROVENANCE

Catalog	SA-000040
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SOURCE PROVENANCE

Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705, and X-Cite Xylys Lamp was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	03B3D3EB9299F8691EB1250A0FD781BB288869DA0D8C842B8A245A413096D0B8
Method PDF SHA-256	96A88D627D01A22DCEFA5B6BB6DBE92EFB9574EA72215B7CB2BB1E4A2E5601C3

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
METADATA VALUES	32

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
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	794 X 1091
Image Size (Scaled)	113.43µm X 155.86µm
Bit Depth	14 Bit
Image Center Position	X: 98.97mm, Y:49.83mm
Stage Position	X: 98.97mm, Y:49.83mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	45 Texas Red 49 DAPI
Beam Splitter	585 395
Filter Excitation Wavelength	540 - 580 335-383
Filter Emission Wavelength	593 - 668 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @6%
Exposure Time	200ms 60ms
Excitation Wavelength	553 353
Emission Wavelength	568 465
Effective NA	1.25
Imaging Device	Axiocam 807
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 Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000040

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® 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) = 794 X 1091

Image Size (Scaled) = 113.43µm X 155.86µm

Bit Depth = 14 Bit

Image Center Position = X: 98.97mm, Y:49.83mm

Stage Position = X: 98.97mm, Y:49.83mm

ROI Center Offset = X: 1.14µm, 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 @25%

Exposure Time = 200ms

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

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 @6%
 Exposure Time = 60ms
 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 Corrector, Fourier Filter, and Deconvolution were NOT used.

Image Corrections

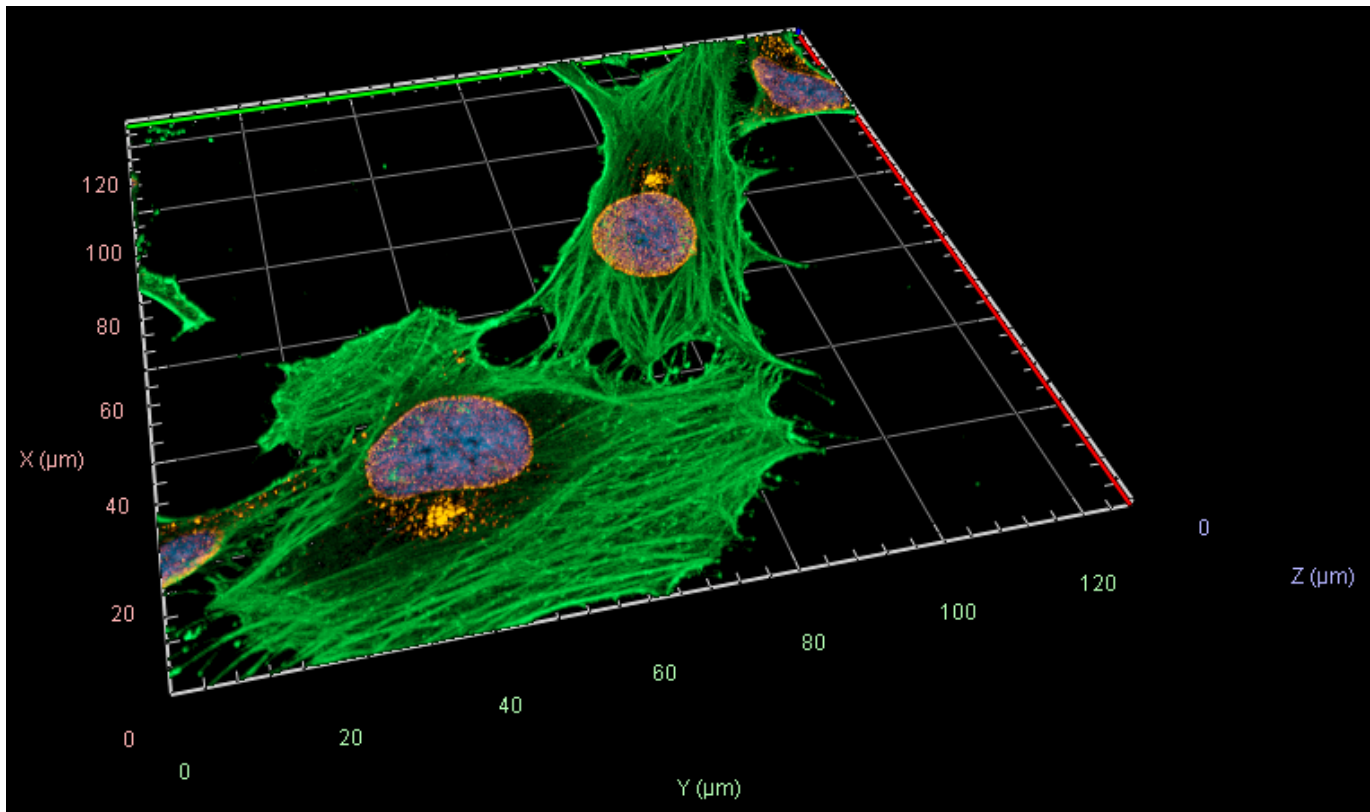
None

Image by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	SA-000040
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	32
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	29E3501E21C3C2DF28F432B428BAA969A748610B93A6F5F529FB50610D80E326
Method PDF SHA-256	C4F92019EEF6A2FD4ACF2FA51A6526A34552095C93A01D6E941F72BBDCBED986

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	16 Slices (1.05µm)
Channels	3
Scaling (Per Pixel)	0.248µm X 0.248µm X 0.070µm
Image Size (Pixels)	512 X 512
Image Size (Scaled)	126.77µm X 126.77µm
Bit Depth	16 Bit
Image Center Position	X: 61.28mm, 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 / 53µm 1.00AU / 43µm 1.00AU / 36µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.6% 488nm @0.5% 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	0.80
Rotation	43.07°
Sampling	0.29
Pixel Time	1.52µs
Frame Time	1.86s
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	564 - 700 410 - 546 400 - 466
Imaging Device	GaAsp-PMT 2 GaAsp-PMT 1
Detector Type	GaAsp-PMT
Detector Gain	650 V 700 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (555), Threshold 2% (488), Threshold 1% (DAPI), Ramp 0% (555), Ramp 0% (488), Ramp 0% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness100%, 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 Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000040

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

Cat ID# - SA 000022

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® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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

Microscope

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

Z Stack - (3D)

Z-Stack = 16 Slices (1.05µm)

Channels = 3

Scaling (Per Pixel) = 0.248µm X 0.248µm X 0.070µm

Image Size (Pixels) = 512 X 512

Image Size (Scaled) = 126.77µm X 126.77µm

Bit Depth = 16 Bit

Image Center Position = X: 61.28mm, 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 / 53µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @0.6%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.80
 Rotation = 43.07°
 Sampling = 0.29
 Pixel Time = 1.52µs
 Frame Time = 1.86s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 564 - 700
 Imaging Device = GaAsp-PMT 2
 Detector Type = GaAsp-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 43µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.80
 Rotation = 43.07°
 Sampling = 0.29
 Pixel Time = 1.52µs
 Frame Time = 1.86s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 410 - 546
 Imaging Device = GaAsP-PMT 1
 Detector Type = GaAsP-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 36µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 0.80
 Rotation = 43.07°
 Sampling = 0.29
 Pixel Time = 1.52µs
 Frame Time = 1.86s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400 - 466
 Imaging Device = GaAsP-PMT 1
 Detector Type = GaAsP-PMT
 Detector Gain = 700 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (555), Threshold 2% (488), Threshold 1% (DAPI), Ramp 0% (555), Ramp 0% (488), Ramp 0% (DAPI), Maximum 100% all channels
 Background = Black
 Light = Brightness100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

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SOURCE PROVENANCE

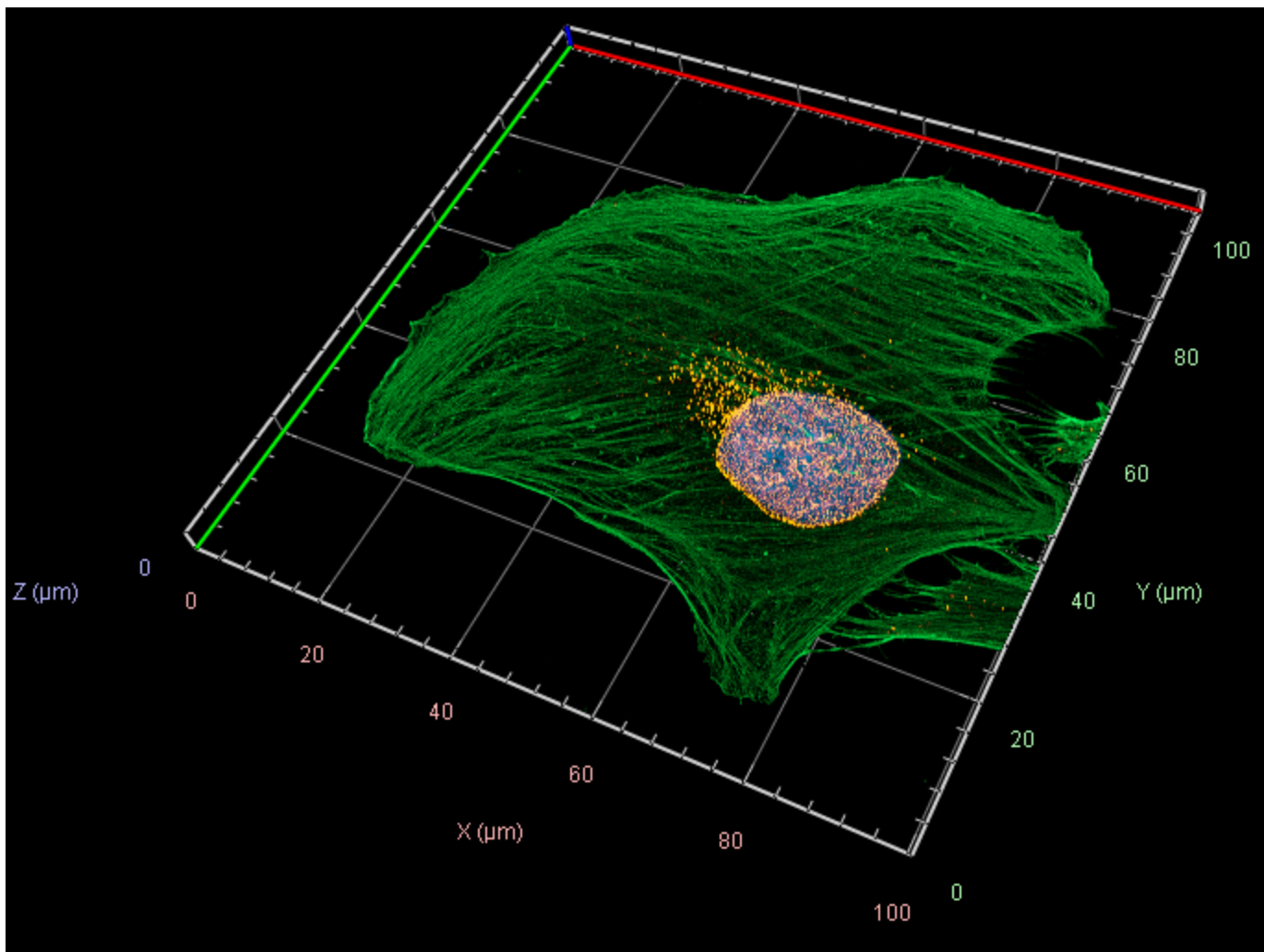
Catalog

SA-000040

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	7E1D8CE73B86FADD35E832F5433A96C5A252CF183AD5C4C45F6C01C843C86E96
Method PDF SHA-256	FC8B354608F30AEF78950BCC30AE8552F4A5CB54C69D5A58D3F97C67B01BD184

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	52

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	54 Slices (3.71µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.07000 µm
Image Size (Pixels)	2849 X 2849
Image Size (Scaled)	100.57µm X 100.57µm
Bit Depth	16 Bit
Image Center Position	X: 60.85mm, Y: 55.44mm
Stage Position	X: 60.85mm, Y: 55.44mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 266µm 5.00 AU / 243µm 5.00 AU / 182µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	560nm @0.2% 488nm @0.1% 405nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	-120.58°
Sampling	2.00
Pixel Time	0.73µs
Frame Time	14.22s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	553 5493 353
Emission Wavelength	568 517 465
Effective NA	1.4
Detection Wavelength	570-700 500-600 400-467
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 10.0 (3D, Auto) Super Resolution 8.6 (3D, Auto) Super Resolution 9.2 (3D, Auto)
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	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

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

Cat ID# - SA 000040

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

Cat ID# - SA 000022

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® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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

Microscope

Zeiss LSM 900 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 = 54 Slices (3.71µm)

Channels = 3

Scaling (Per Pixel) = 35.30nm X 35.30nm X 70.00nm

Image Size (Pixels) = 2849 X 2849

Image Size (Scaled) = 100.57µm X 100.57µm

Bit Depth = 16 Bit

Image Center Position = X: 60.85mm, Y: 55.44mm

Stage Position = X: 60.85mm, Y: 55.44mm

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

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 266µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 560nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = -120.58°
 Sampling = 2.00
 Pixel Time = 0.73µs
 Frame Time = 14.22s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 570-700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 243µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.1%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = -120.58°
 Sampling = 2.00
 Pixel Time = 0.73µs
 Frame Time = 14.22s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 5493
 Emission Wavelength = 517
 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 8.6 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 182µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = -120.58°
 Sampling = 2.00
 Pixel Time = 0.73µs
 Frame Time = 14.22s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400-467
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.2 (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% (555), Threshold 1% (488), Threshold 1% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

None

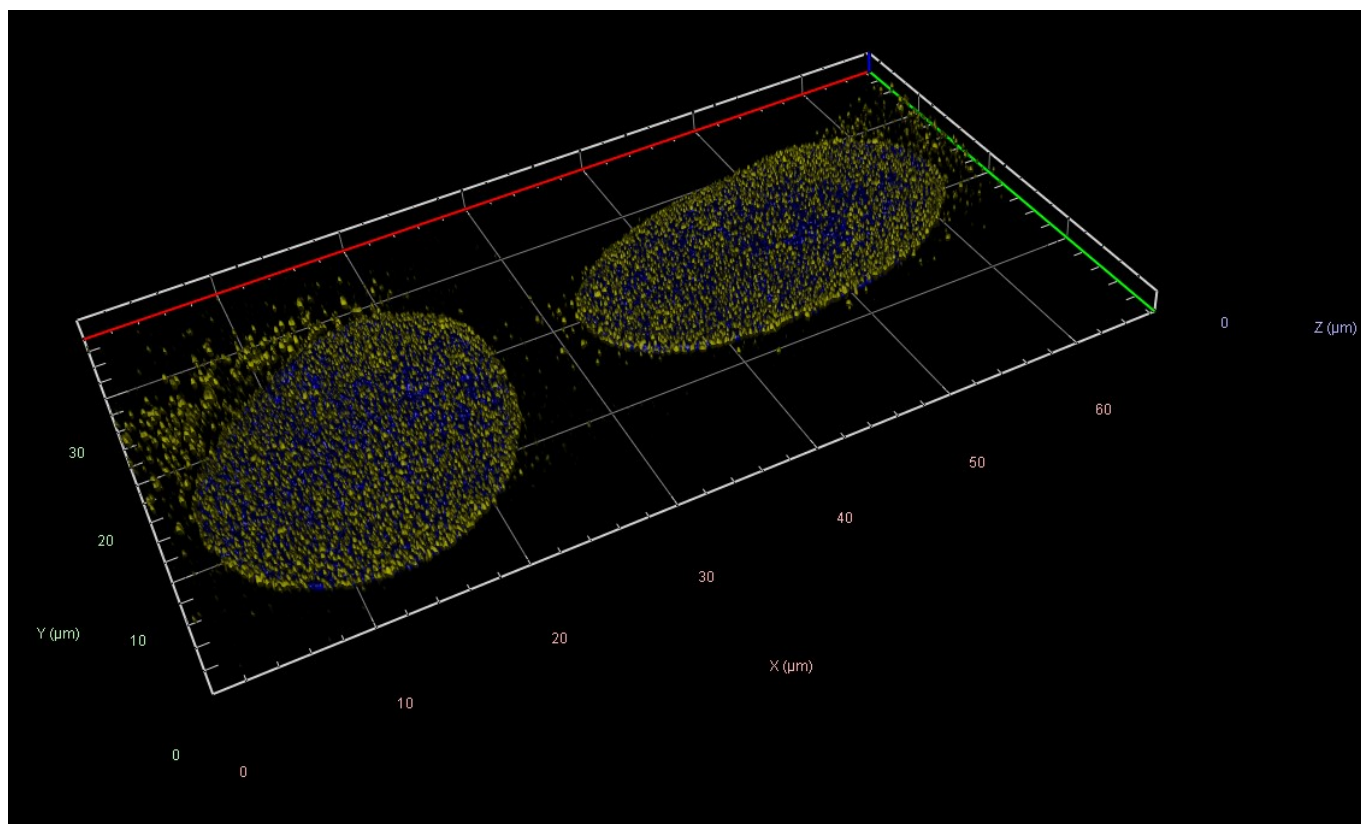
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000040
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	52
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7957934755D687A1707395AF1F9E567D7CFA132A970EDEB75B21BD1E9AE601E2
Method PDF SHA-256	8B3991C74C8D5A8DB6B904CAC0A20CF8CB1884AF17D7C3988FFDD9B082734EF

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	MCF-7
DETECTED DYES	405/DAPI; 488; 532; 555
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	55 Slices (1.62µm)
Channels	2
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.02945 µm
Image Size (Pixels)	5031 X 2855
Image Size (Scaled)	66.39µm X 37.67µm
Bit Depth	16 Bit
Image Center Position	X: 54.19mm, Y: 34.73mm
Stage Position	X: 54.19mm, Y: 34.73mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	561 405
Channel Name	T2-Axiocam 820 #2-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	561 405
Emission Wavelength	597 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	100ms 50ms
Depth of Focus	0.92µm 0.69µm
Binning Mode	2,2
Laser Wavelength	561nm @0.9% 405nm @7.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.8410 (532), 8.5010 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Volume Projection	Precise
Appearance	Threshold 4% (555), Threshold 4% (DAPI), Ramp 96% (555), Ramp 96% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness100%, Azimuth 77°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 107%, 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 Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000040

MCF7 Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 = 55 Slices (1.62µm)

Channels = 2

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

Image Size (Pixels) = 5031 X 2855

Image Size (Scaled) = 66.39µm X 37.67µm

Bit Depth = 16 Bit

Image Center Position = X: 54.19mm, Y: 34.73mm

Stage Position = X: 54.19mm, Y: 34.73mm

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 = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 561
 Emission Wavelength = 597
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 100ms
 Depth of Focus = 0.92µm
 Binning Mode = 2,2
 Laser Wavelength = 561nm @0.9%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating 32.0µm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @7.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D

Result Sampling = 4

Process Sampling = N/A

Channels = 2

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.8410 (532), 8.5010 (DAPI)

Fitting = Fast Fit

Histogram = Scale to Min/Max

Baseline = Cut

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 4% (555), Threshold 4% (DAPI), Ramp 96% (555), Ramp 96% (DAPI), Maximum 100% all channels

Background = Black

Light = Brightness100%, Azimuth 77°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 107%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

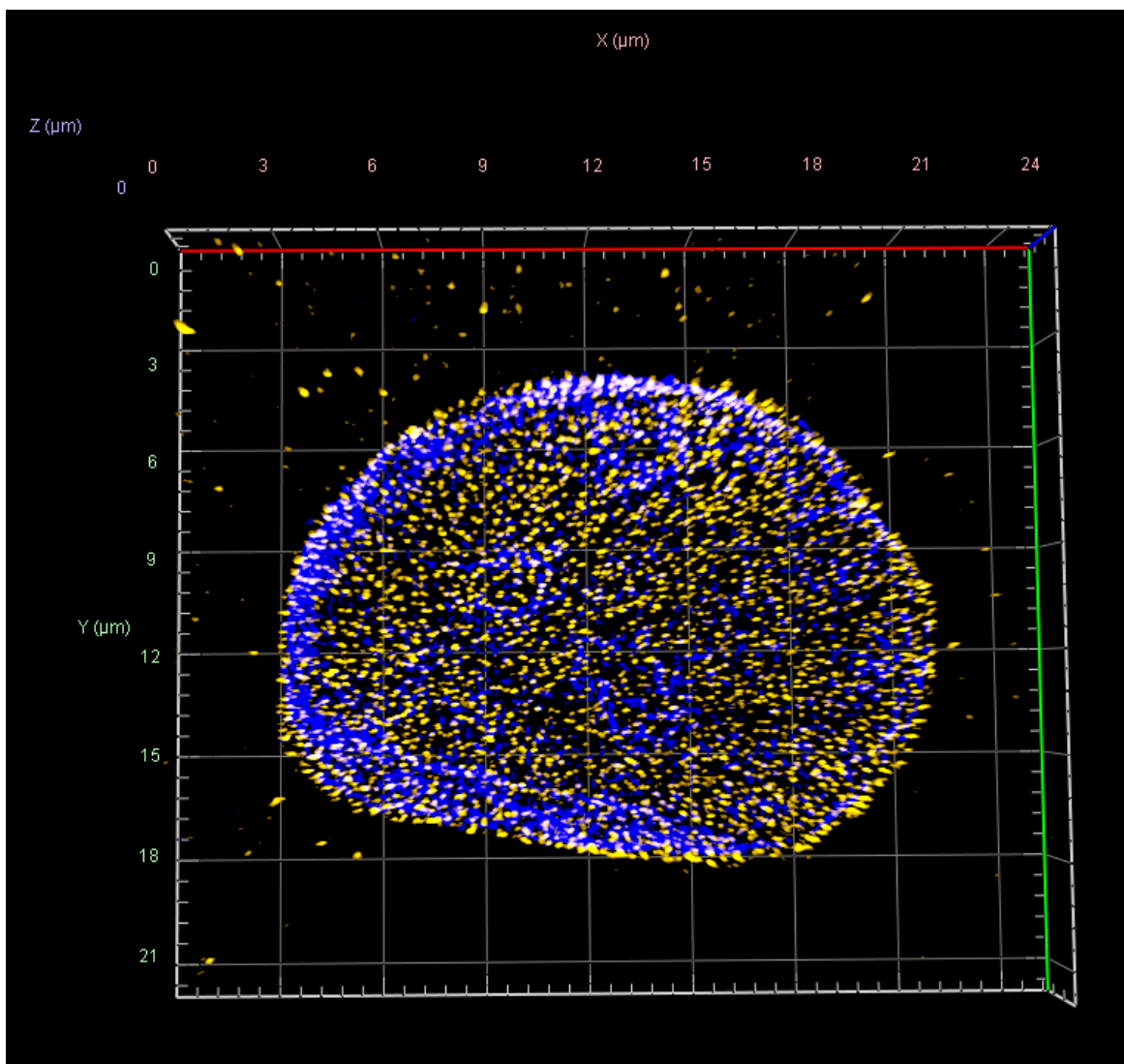
Image by P. Raut

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000040
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	MCF-7
Image SHA-256	B24BDE4EB4D693F193BF3F650A0D0CD7AFA64BB6473480C6C044216F1A0F42CC
Method PDF SHA-256	81A67B61D5FA519534323643B6EA76112EDE6E490A13B1CAEDEE84EFB5D5262F

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	MCF-7
DETECTED DYES	405/DAPI; 488; 555
METADATA VALUES	60

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 555	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
Calibration Marker Positions	Marker 1: 0.00µm, 0.00µm, Marker 2: 0.00µm, 0.00µm, Marker 3 0.00µm, 0.00µm
Z-Stack	22 Slices (1.47µm)
Channels	2
Scaling (Per Pixel)	15.65nm X 15.65nm X 70.00nm
Image Size (Pixels)	1619 X 1401
Image Size (Scaled)	23.34µm X 21.93µm
Bit Depth	16 Bit
Image Center Position	X: 7.12mm, Y: -2.84mm
Stage Position	X: 7.12mm, Y: -2.84mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @4.0% 405nm @10.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	561 405
Emission Wavelength	515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	60ms 100ms
Depth of Focus	0.73µm
Binning Mode	1x1
Detector Type	Camera

FIELD	SOURCE-DOCUMENTED VALUE
Depth Offset	0
Detector Digital Gain	0.00
Channel	561.000000 405.000000
Grating Period (µm)	27.500000 23.000000
Processing	3D
Input SNR	Medium Low
Iterations	16 15
Regularization Weight	0.0650000 0.0800000
Processing Sampling	4
Output Sampling	4
Filter	Median
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 8% (555), Threshold 12% (405) Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 130%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 164%, 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 Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000040

MCF7 Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 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 555 = 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 = 22 Slices (1.47µm)

Channels = 2

Scaling (Per Pixel) = 15.65nm X 15.65nm X 70.00nm

Image Size (Pixels) = 1619 X 1401

Image Size (Scaled) = 23.34µm X 21.93µm

Bit Depth = 16 Bit

Image Center Position = X: 7.12mm, Y: -2.84mm

Stage Position = X: 7.12mm, Y: -2.84mm

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

555 -

Contrast Method = Fluorescence
 Laser Wavelength = 561nm @4.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 561
 Emission Wavelength = 515
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 60ms
 Depth of Focus = 0.73µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 561.000000
Grating Period (µm): 27.500000

SIM2

Processing: 3D
 Input SNR: Medium
 Iterations: 16
 Regularization Weight: 0.0650000
 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 @10.0%
 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 = 100ms
 Depth of Focus = 0.73µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 405.000000
Grating Period (µm): 23.000000

SIM2
 Processing: 3D
 Input SNR: Low
 Iterations: 15
 Regularization Weight: 0.0800000
 Processing Sampling: 4
 Output Sampling: 4
 Filter: Median
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 8% (555), Threshold 12% (405) Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 130%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 164%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

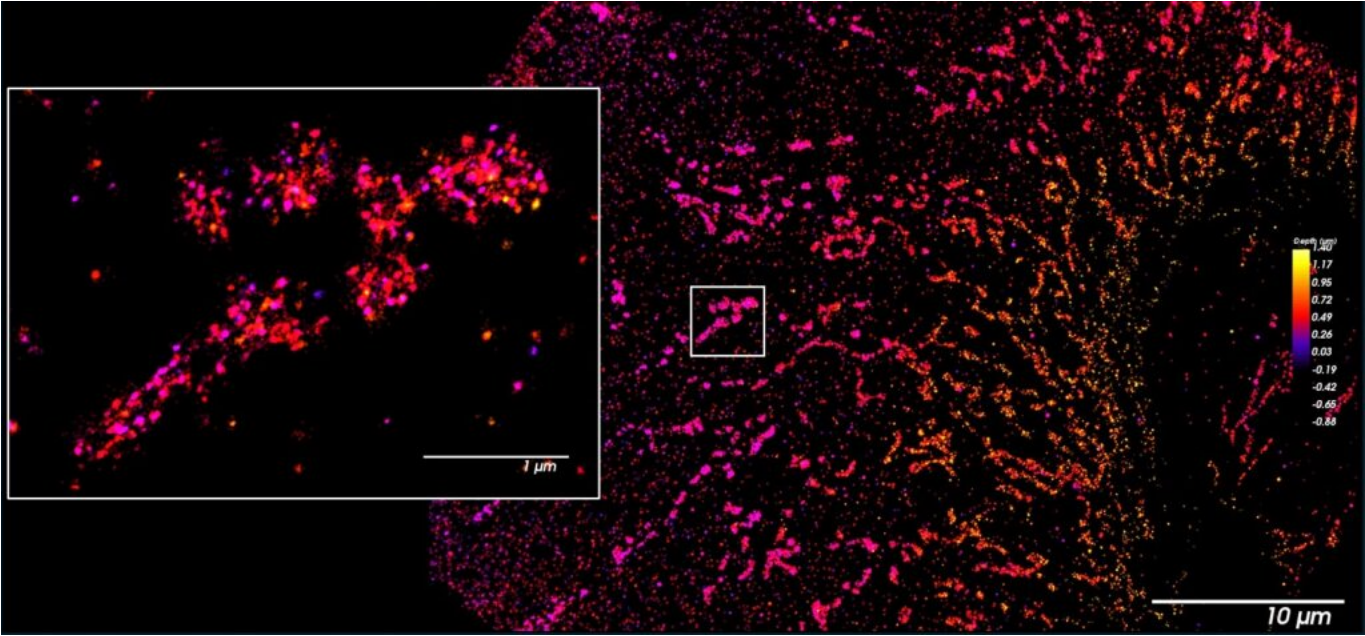
Image by P. Raut

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000040
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	60
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	MCF-7
Image SHA-256	06CDB24079D9B00976A23B5EE2C19664A2D2A05023E1DF80BB63D9B83B02D823
Method PDF SHA-256	92CE580C6AF588AAF0844EAB991964B1335D6258E37AD8CBF383409989ACF1A5

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	555
METADATA VALUES	79

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	150 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: 148.2; End: 148.8
SuperRes 555nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 500
Z Positions	3 using Steps: 0.2µm (200nm)
Cycles	20
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
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.09µm (900nm)
Maximum Particle Count to Form Cluster	20
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	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 Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000040

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, Mitochondria Antibody (MA5-12017), 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: 150 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: 148.2; End: 148.8

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: 500

Z Positions: 3 using Steps: 0.2µm (200nm)

Cycles: 20

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 - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.09µm (900nm)

Maximum Particle Count to Form Cluster: 20

Computer Hulls: Not Selected

Compute Density Isosurfaces: Not Selected

Edit Settings: All Probes

Compute: Selected

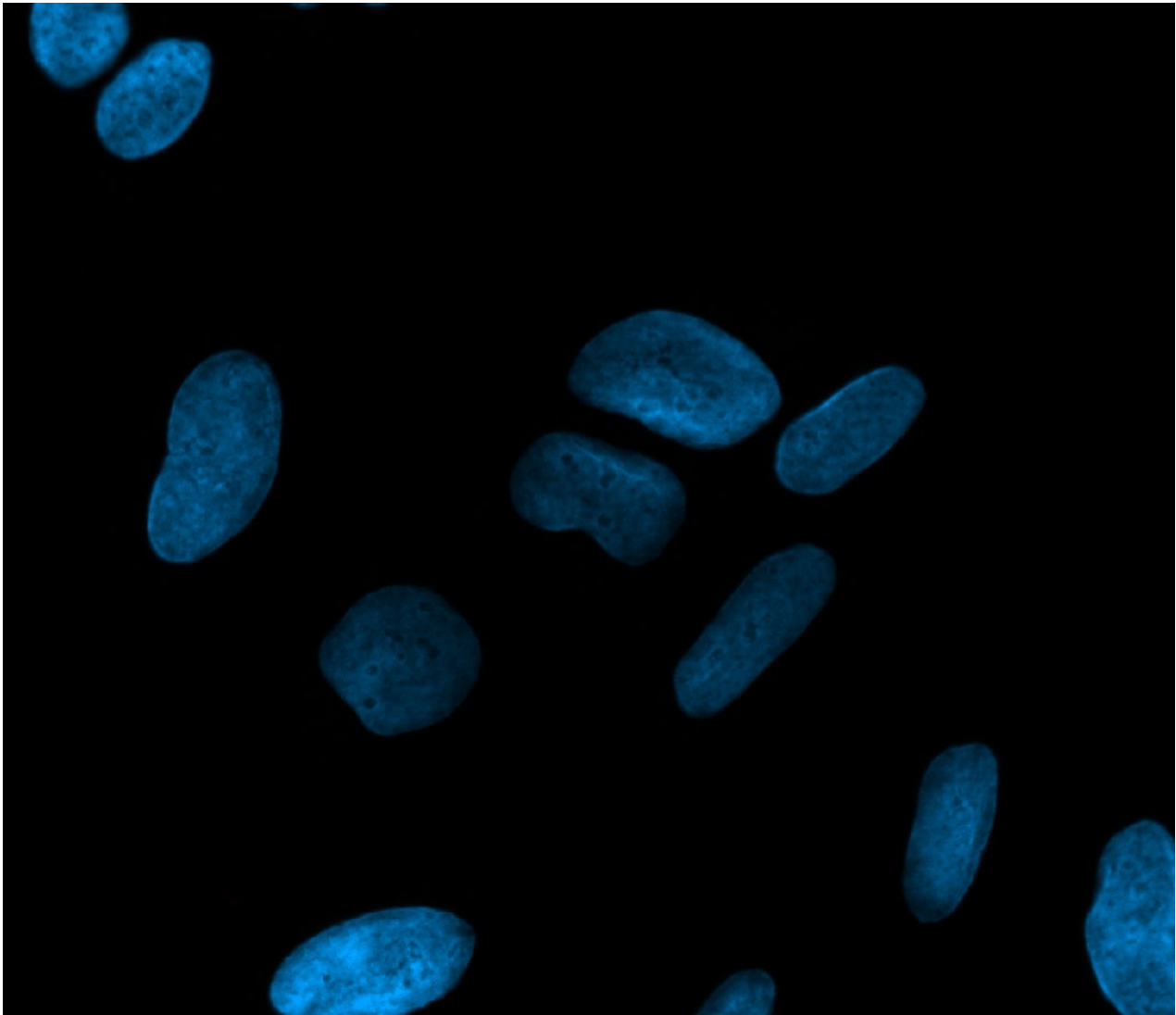
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

Image by S. Thakur

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SOURCE PROVENANCE	
Catalog	SA-000040
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	79
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F439348DD78703B050B2FBEF9207B83BA83A74F22AF672063ED4256056AB60D7
Method PDF SHA-256	9102AB4DC75D7F4854BF51CDCBEFFCAAC803D94BC765F8E0031C5A9A30E85674

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)	1268 X 1100
Image Size (Scaled)	184.14µm X 157.14µm
Bit Depth	14 Bit
Image Center Position	X: 49.46mm, Y: 50.86mm
Stage Position	X: 49.46mm, Y: 50.86mm
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 Goat Anti-Mouse, F(ab')₂ (H + L)

Cat ID# - SA 000040

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) = 1268 X 1100

Image Size (Scaled) = 184.14µm X 157.14µm

Bit Depth = 14 Bit

Image Center Position = X: 49.46mm, Y: 50.86mm

Stage Position = X: 49.46mm, Y: 50.86mm

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-000040
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	E28363A8C788FA07FD9ACC15DC345CE7D1053EFB789B5928B1D0960EAD40AE35
Method PDF SHA-256	F866F9EE93C3D2D1ECB34F648478C711CA9840F9B0AA4C6A15B572CE4ABA926F