

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

ORDERED EVIDENCE TIERS

SUPPORTING CONTROL

SCIENTIST REVIEW



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™ 680 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-000076 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-000076 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	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555; 680	2; 50 Cy5; 45 Texas Red; 625 - 655; 540 - 580; 665 - 715; 593 - 668; 679
Widefield SIM — Apotome	405/DAPI; 555; 680	2; 50Cy5; AF532-49014; 625 - 655; 515 - 545; 665 - 715; 555 - 595; 679
Confocal	405/DAPI; 488; 555; 680	2; None; 640nm @0.5%; 561nm @1.20%; 679; 553; 702; 568
Airyscan	405/DAPI; 488; 555; 680	2; None; 640nm @2.0%; 561nm @0.9%; 679; 553; 702; 568
SIM	405/DAPI; 488; 555; 680	2; 561nm @1.50%; 642nm @4.0%; 561; 642; 570; 561.000000; 642.000000
SIM ²	405/DAPI; 488; 680	2; 642nm @8.0%; 405nm @10%; 642; 405; 655; 515; 642.000000
Single-molecule STORM	680	Both Channels
Control	405/DAPI; 680	2; 50 Cy5; 49 DAPI; 625 - 655; 335-383; 665 - 715; 420-470; 679

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 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

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): 1188 X 783; Image Size (Pixels): 1188 X 783; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 550ms; 250ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @80%; X-Cite Xylis Lamp Intensity @50%. 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 Xylys Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 989 X 940; Image Size (Pixels): 989 X 940; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 420ms; 100ms. Illumination: Light source: X-Cite Xylys Lamp Intensity @60%; X-Cite Xylys Lamp Intensity @30%. 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; 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: 106 Slices (5.25µm); Channels: 2; Scaling (Per Pixel): 0.098µm X 0.098µm X 0.050µm; Image size (Pixels): 798 X 798; Image Size (Pixels): 798 X 798; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsp-PMT 2; Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.55µs; Frame Time: 3.26s. Illumination: Laser Wavelength: 640nm @0.5%; 561nm @1.20%. Optical/scan: Pinhole: 1.00AU / 57µm; 1.00AU / 49µm; Scan Zoom: 1.3; LSM Scan Speed: 10; 8; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 46.

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; 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

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: 99 Slices (2.94µm); Channels: 2; Scaling (Per Pixel): 48.88nm X 48.88nmµm X 30.00nm; Image size (Pixels): 1572 X 1572; Image Size (Pixels): 1572 X 1572; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.66µs; Frame Time: 3.95s. Illumination: Laser Wavelength: 640nm @2.0%; 561nm @0.9%. Optical/scan: Pinhole: 5.00AU / 282µm; 5.31AU / 250µm; Scan Zoom: 1.3; 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 8.1 (3D, Auto); Super Resolution 8.7 (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.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra 7. Objective: Plan-Apochromat 63X/1.46 oil Korr M27. Platform: Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye: Acquisition: Z-Stack: 139 Slices (8.28µm); Channels: 2; Scaling (Per Pixel): 30.0nm X 30.0nm X 60.00nm; Image size (Pixels): 936 X 906; Image Size (Pixels): 936 X 906; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 50ms; 80ms; Frame Time: 4.80s. Illumination: Laser Wavelength: 561nm @1.50%; 642nm @4.0%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 4; Effective NA: 1.46. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra 7, and records detected dye/channel descriptors as 405/DAPI; 488; 555; 680.

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): 1321X 1080; Image Size (Pixels): 1321X 1080; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 642nm @8.0%; 405nm @10%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Projection: View Angle 55°, 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: 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; 680.

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: 200ms. 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 680.

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): 984 X 997; Image Size (Pixels): 984 X 997; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 300ms; 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; 680.

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-000076 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

SIM / Scaling (Per Pixel): 30.0nm X 30.0nm X 60.00nm -> 0.03130 μ m X 0.03130 μ m X 0.06000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=936 X 906; Image Size (Scaled)=29.30 μ m X 28.36 μ m; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

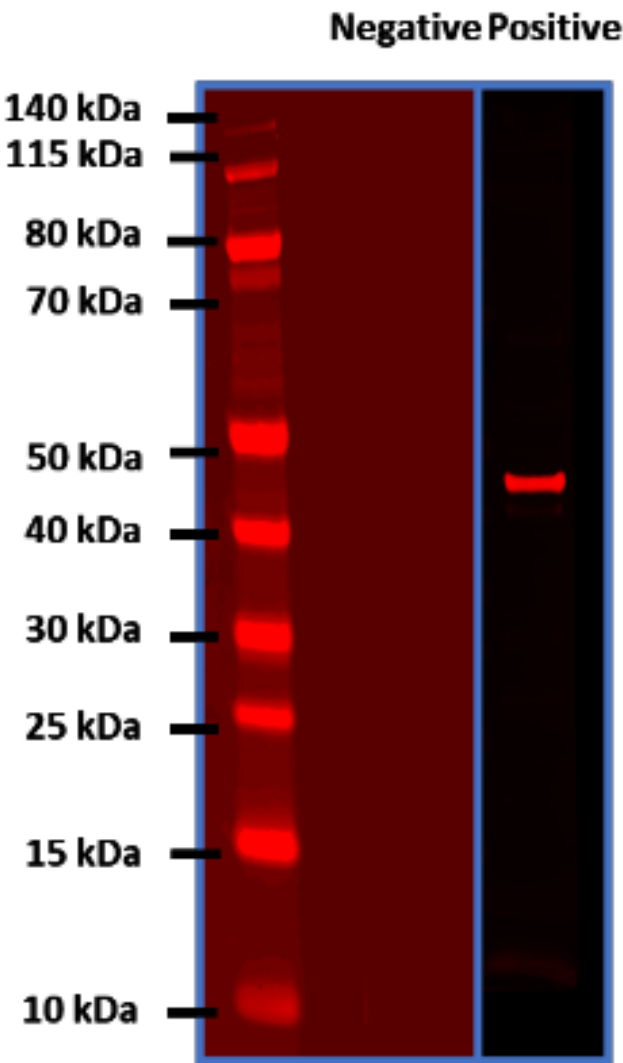
This dossier preserves the complete available evidence progression for SA-000076. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra 7	PRESERVED
7	SIM ²	ZEISS Elyra 7	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	680
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	638nm
Emission Filter	676±37nm
Detector	Avalanche Photodiode
Intensity	2
Pixel size	100 µm
Scan Speed	Medium
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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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™ 680 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 = 638nm

Emission Filter = 676±37nm

Detector = Avalanche Photodiode

Intensity = 2

Pixel size = 100 µm

Scan Speed = Medium

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

Copyright 2026 GMD12 LLC.

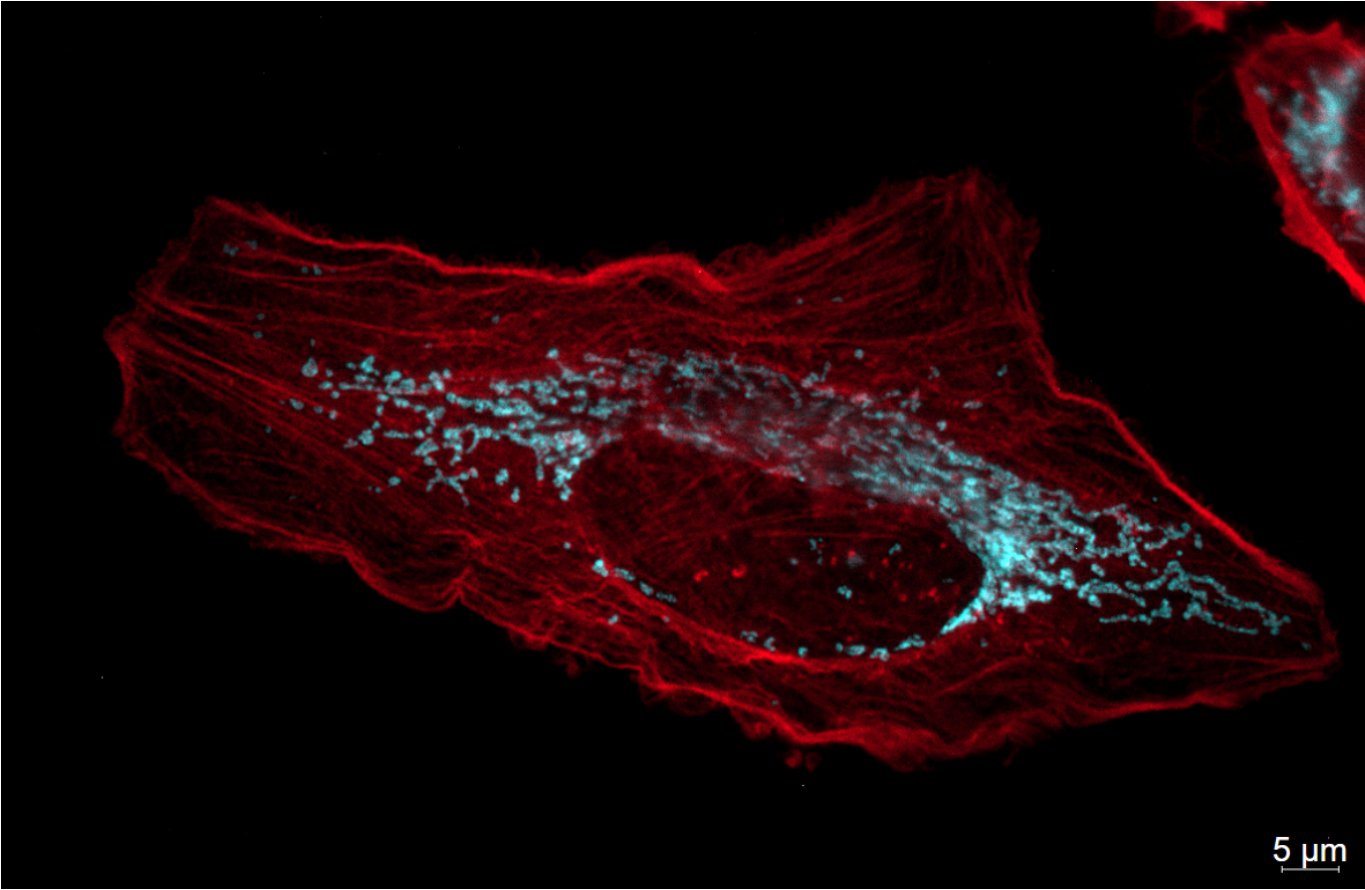
SOURCE PROVENANCE

Catalog	SA-000076
Stage	Western blot
Instrument	Azure Sapphire FL

SOURCE PROVENANCE

Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	28F7F43F1E5D79647943D35FE29428072D3A42C123A05E417F9A70E833A8ECCA
Method PDF SHA-256	068A0EE2203496FADF65E44BEC276C030E882DDEDDBB29FF2E0BEF18124C3A5B

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)	1188 X 783
Image Size (Scaled)	121.63µm X 80.16µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	50 Cy5 45 Texas Red
Beam Splitter	660 585
Filter Excitation Wavelength	625 - 655 540 - 580
Filter Emission Wavelength	665 - 715 593 - 668
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @80% X-Cite Xylis Lamp Intensity @50%
Exposure Time	550ms 250ms
Excitation Wavelength	679 553
Emission Wavelength	702 568
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	1.09µm 0.88µm
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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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

Cat ID# - SA 000044

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 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 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) = 1188 X 783

Image Size (Scaled) = 121.63µm X 80.16µm

Bit Depth = 12 Bit

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

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

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 @80%

Exposure Time = 550ms

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

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 @50%
 Exposure Time = 250ms
 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

Post Processing

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

Image Correction

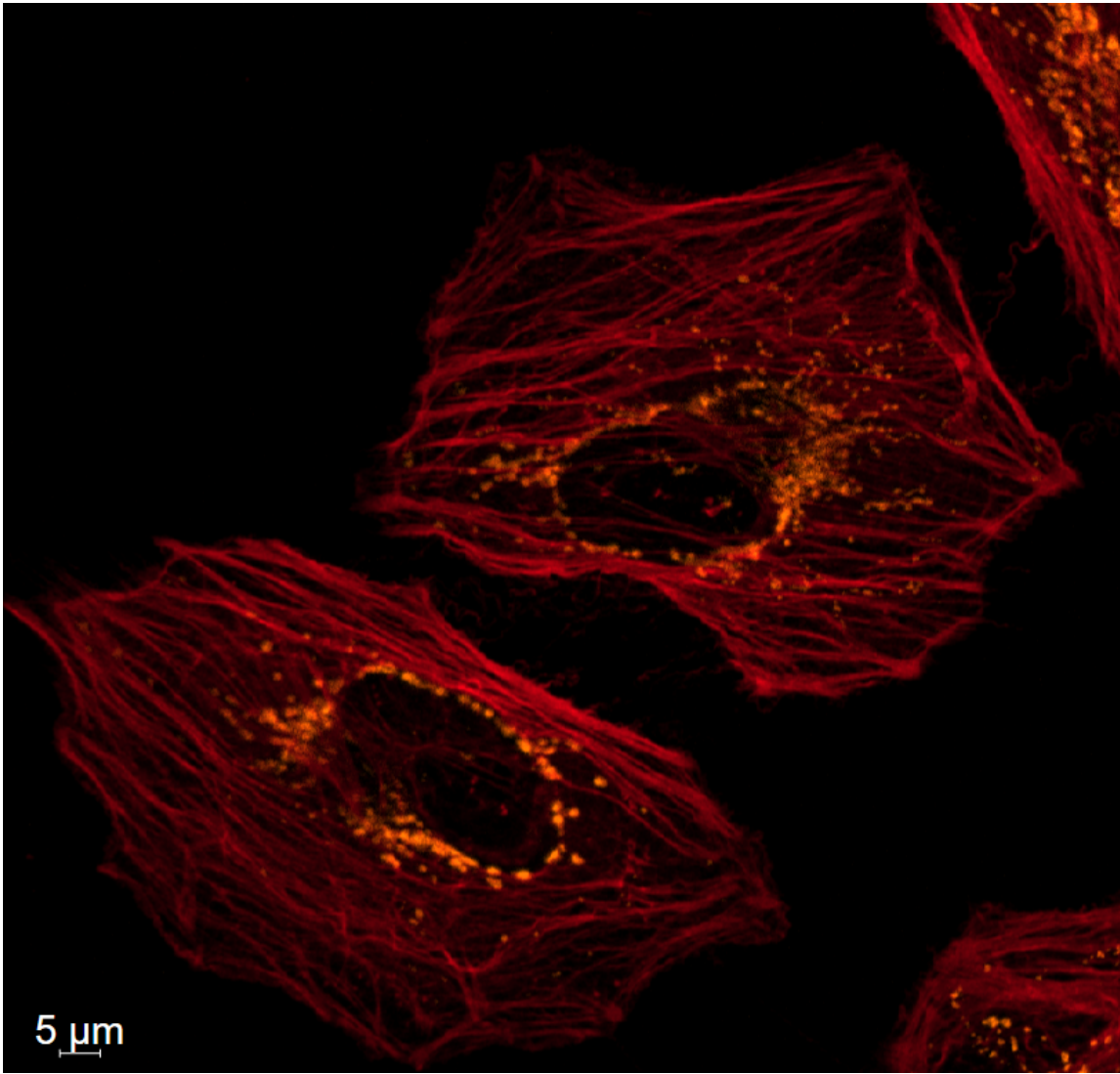
None

Image by E.F. Simon

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SOURCE PROVENANCE	
Catalog	SA-000076
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	97E325776AF720D63926C2D7AEE92040065E65158663EF5CC1BB2796666C784B
Method PDF SHA-256	61429DDFD6D6AC47464C3C678AA588C84EB899B10651DE0664322F8E0CD18AFF

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	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)	989 X 940
Image Size (Scaled)	141.29µm X 134.29µm
Bit Depth	14 Bit
Image Center Position	X: 65.21mm, Y: 46.70mm
Stage Position	X: 65.21mm, Y: 46.70mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50Cy5 AF532-49014
Beam Splitter	660 550
Filter Excitation Wavelength	625 - 655 515 - 545
Filter Emission Wavelength	665 - 715 555 - 595
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @60% X-Cite Xylis Lamp Intensity @30%
Exposure Time	420ms 100ms
Excitation Wavelength	679 553
Emission Wavelength	702 568
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.36µm 1.10µm
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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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

Cat ID# - SA 000044

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 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 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) = 989 X 940

Image Size (Scaled) = 141.29µm X 134.29µm

Bit Depth = 14 Bit

Image Center Position = X: 65.21mm, Y: 46.70mm

Stage Position = X: 65.21mm, Y: 46.70mm

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

680 -

Reflector = 50Cy5
 Beam Splitter = 660
 Filter Excitation Wavelength = 625 - 655
 Filter Emission Wavelength = 665 - 715
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @60%
 Exposure Time = 420ms
 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

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 @30%
 Exposure Time = 100ms
 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

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.

Image Corrections

None

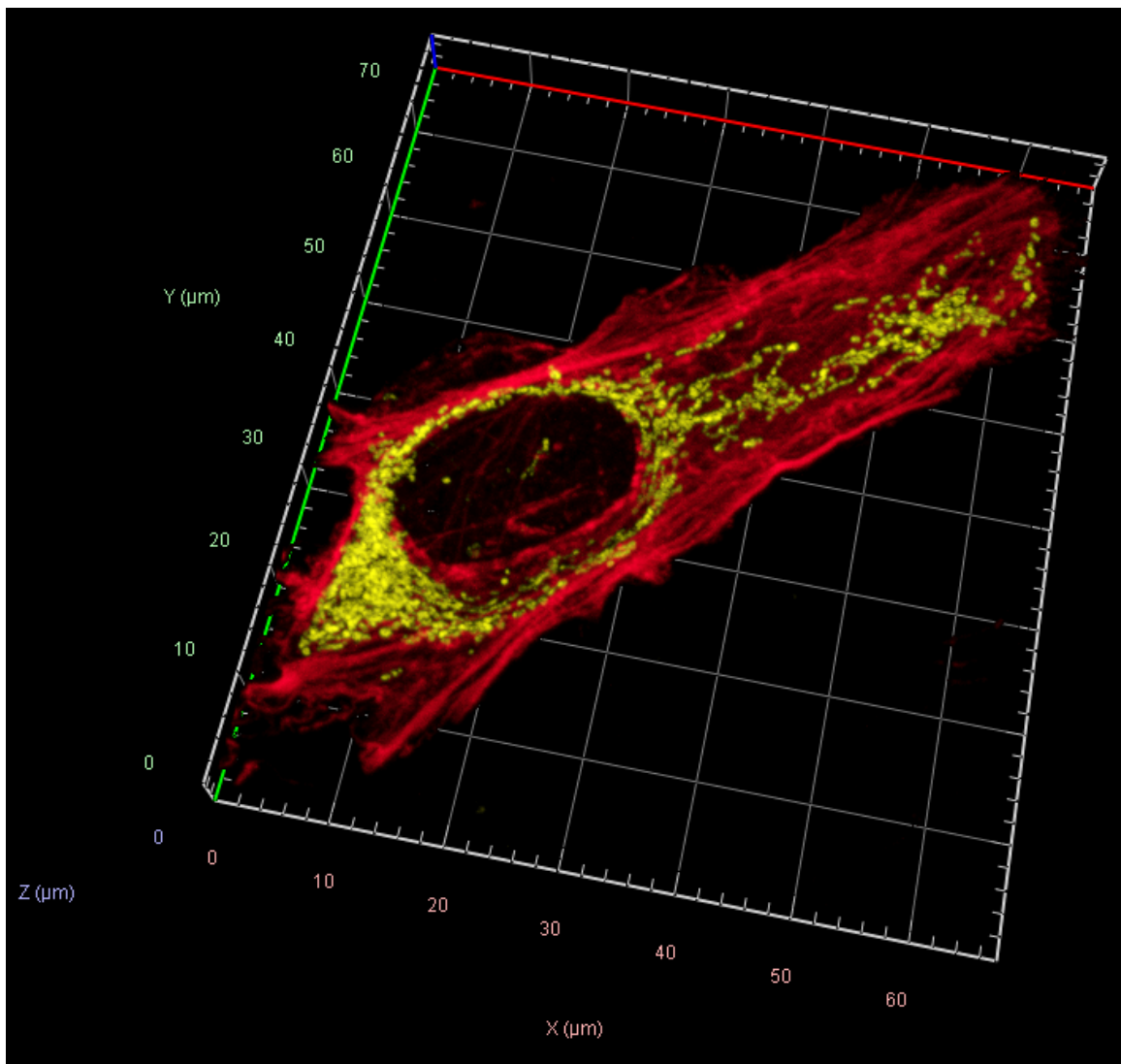
Image by E.F. Simon

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SOURCE PROVENANCE

Catalog	SA-000076
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	3C7CA76EEA38CEA6AFBA3C500140DE5D71270C8DD481239225D2AFDDB2DC1721
Method PDF SHA-256	15AF761188597A1A02D487104C7E5E6AD074FDFEE7F28532DFB3025E5080F796

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 680
METADATA VALUES	46

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	106 Slices (5.25µm)
Channels	2
Scaling (Per Pixel)	0.098µm X 0.098µm X 0.050µm
Image Size (Pixels)	798 X 798
Image Size (Scaled)	78.01µm X 78.01µm
Bit Depth	16 Bit
Image Center Position	X: 72.84mm, Y: 49.32mm
Stage Position	X: 72.84mm, Y: 49.32mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 57µm 1.00AU / 49µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @0.5% 561nm @1.20%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	0°
Sampling	1.00
Pixel Time	0.55µs
Frame Time	3.26s
LSM Scan Speed	10 8
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	679 553
Emission Wavelength	702 568
Effective NA	1.4
Detection Wavelength	656 - 700 545 - 610
Imaging Device	GaAsp-PMT 2 Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	700 V
Detector Offset	0
Detector Digital Gain	1.0
3D Maximum Projection	Precise
Appearance	Threshold 1% (680), Threshold 1% (555), Ramp 0% (680), Ramp 0% (555), 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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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

Cat ID# - SA 000044

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 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 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 = 106 Slices (5.25µm)

Channels = 2

Scaling (Per Pixel) = 0.098µm X 0.098µm X 0.050µm

Image Size (Pixels) = 798 X 798

Image Size (Scaled) = 78.01µm X 78.01µm

Bit Depth = 16 Bit

Image Center Position = X: 72.84mm, Y: 49.32mm

Stage Position = X: 72.84mm, Y: 49.32mm

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

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 57µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.55µs
 Frame Time = 3.26s
 LSM Scan Speed = 10
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Detection Wavelength = 656 - 700
 Imaging Device = GaAsp-PMT 2
 Detector Type = GaAsp-PMT
 Detector Gain = 700 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 49µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @1.20%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.55µs
 Frame Time = 3.26s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 545 - 610
 Imaging Device = Airyscan
 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% (680), Threshold 1% (555), Ramp 0% (680), Ramp 0% (555), 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 J.K. Bennett

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SOURCE PROVENANCE

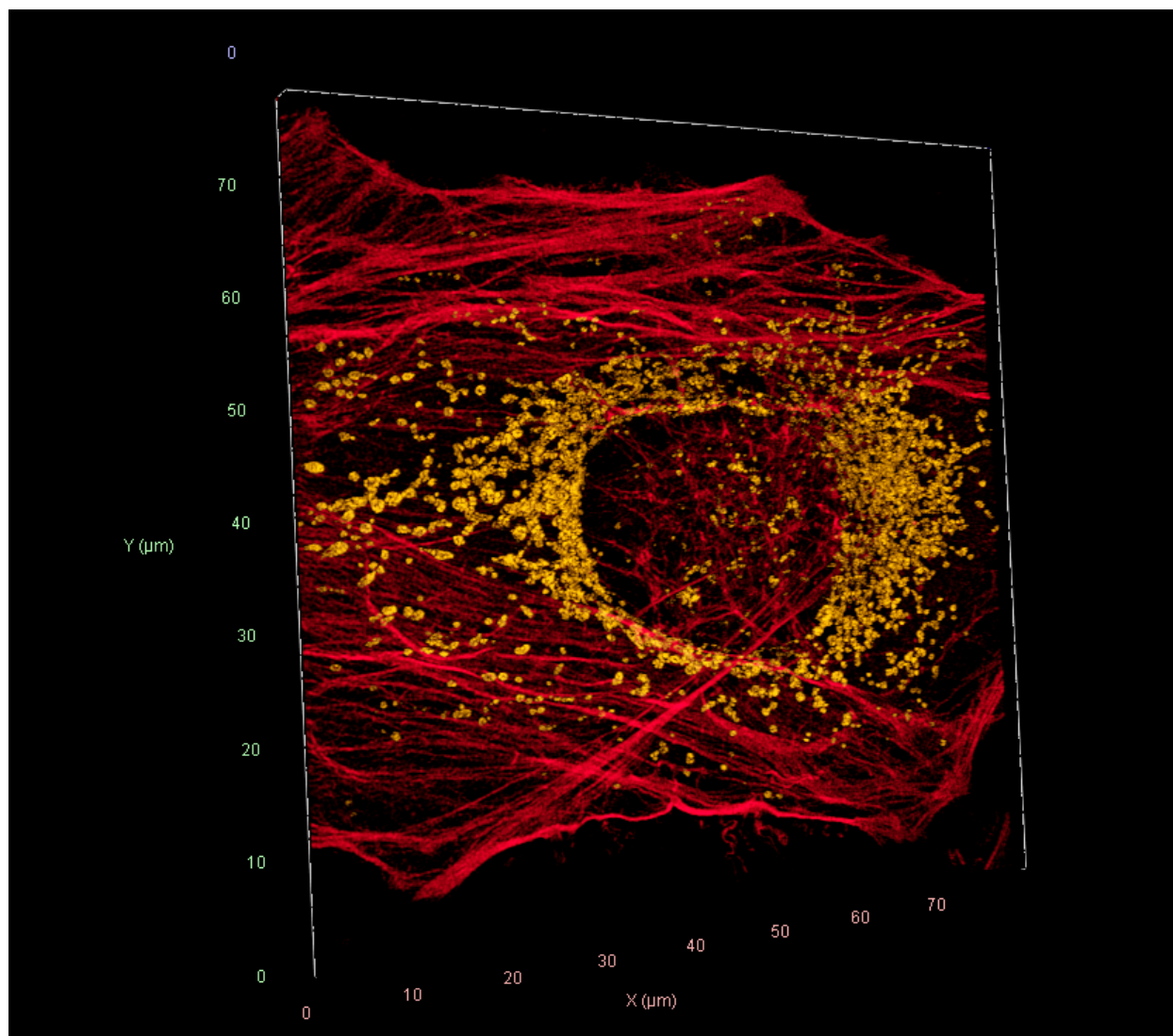
Catalog

SA-000076

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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CA0E5184C4A39801668B411ADF3609567B34BF228D58509394CB2C4561998F12
Method PDF SHA-256	793626D7A92E6792D3A38C7011853E03086DA616511036E33EBF1C50B1B51F52

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 680
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	99 Slices (2.94µm)
Channels	2
Scaling (Per Pixel)	48.88nm X 48.88nmµm X 30.00nm
Image Size (Pixels)	1572 X 1572
Image Size (Scaled)	65.27µm X 57.32µm
Bit Depth	16 Bit
Image Center Position	X: 71.10mm, Y: 54.97mm
Stage Position	X: 71.10mm, Y: 54.97mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 282µm 5.31AU / 250µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	640nm @2.0% 561nm @0.9%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.3
Rotation	0°
Sampling	2.00
Pixel Time	0.66µs
Frame Time	3.95s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	679 553
Emission Wavelength	702 568
Effective NA	1.4
Detection Wavelength	650 - 700 565 - 630
Imaging Device	Airyscan
Detector Type	GaAsp-PMT
Detector Gain	800 V 850 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.1 (3D, Auto) Super Resolution 8.7 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 0% (680), Threshold 1% (555), Ramp 0% all channels, 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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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

Cat ID# - SA 000044

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 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 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 = 99 Slices (2.94µm)

Channels = 2

Scaling (Per Pixel) = 48.88nm X 48.88nmµm X 30.00nm

Image Size (Pixels) = 1572 X 1572

Image Size (Scaled) = 65.27µm X 57.32µm

Bit Depth = 16 Bit

Image Center Position = X: 71.10mm, Y: 54.97mm

Stage Position = X: 71.10mm, Y: 54.97mm

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

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 282µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @2.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66µs
 Frame Time = 3.95s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 2
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Detection Wavelength = 650 - 700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.1 (3D, Auto)

555 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.31AU / 250µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @0.9%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.3
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.66µs
 Frame Time = 3.95s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 2
 Excitation Wavelength = 553
 Emission Wavelength = 568
 Effective NA = 1.4
 Detection Wavelength = 565 - 630
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 8.7 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 0% (680), Threshold 1% (555), Ramp 0% all channels, 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 J.K. Bennett

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SOURCE PROVENANCE

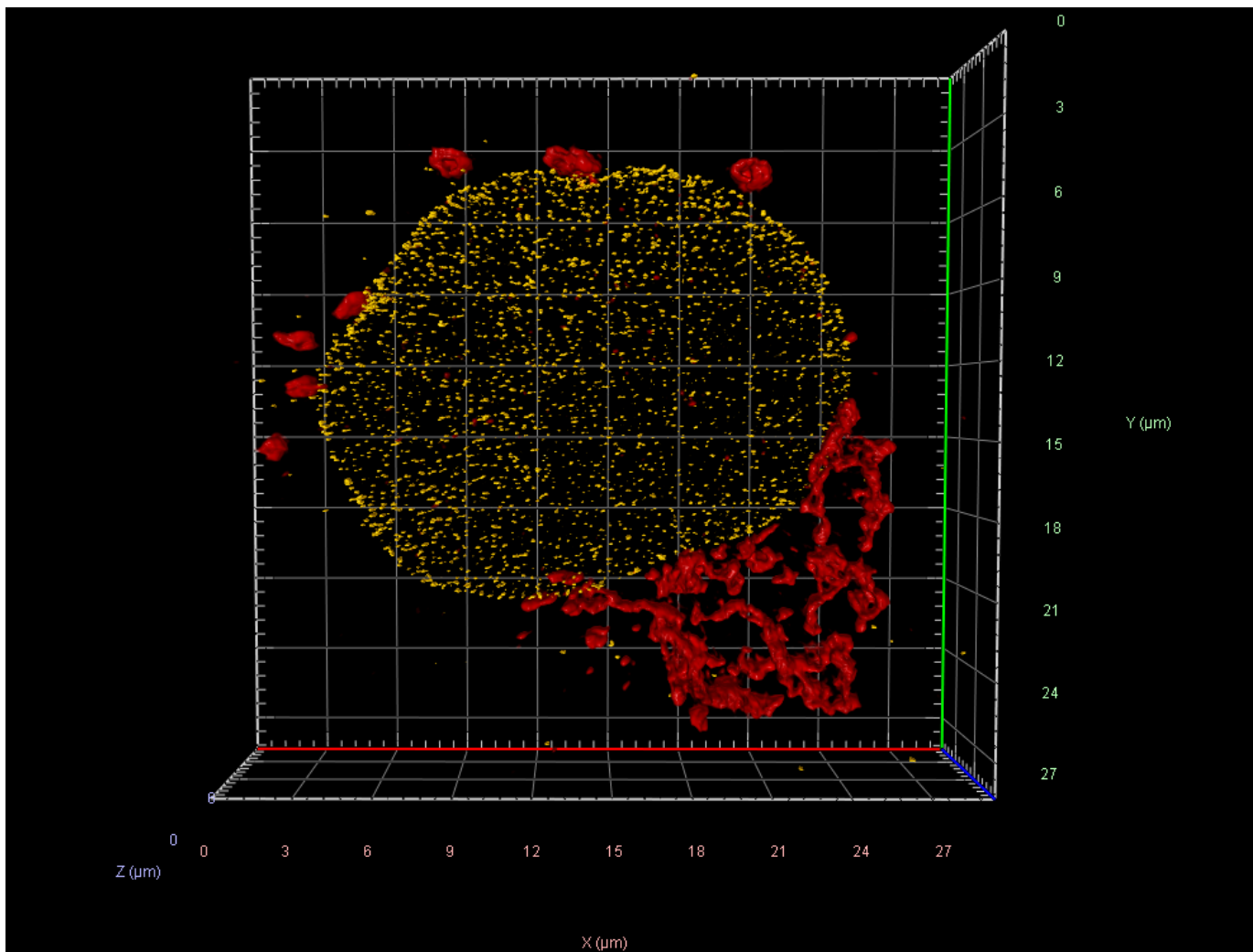
Catalog

SA-000076

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	BB011970783476011922DC4F60A2C002D94488593D5E135E51D7A39C7755EA47
Method PDF SHA-256	D97C604B21BAB1D287AB55A3AD1A4F1C32E193FDB13D5A3190445C144C519397

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 555; 680
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.46 oil Korr M27
Filters	-2147483648
Beam Splitter 555	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 560
Beam Splitter 680	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	139 Slices (8.28µm)
Channels	2
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.06000 µm
Image Size (Pixels)	936 X 906
Image Size (Scaled)	29.30µm X 28.36µm
Bit Depth	16 Bit
Image Center Position	X: -1.25mm, Y: -5.73mm
Stage Position	X: -1.25mm, Y: -5.73mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @1.50% 642nm @4.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	4.80s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	4
Excitation Wavelength	561 642
Emission Wavelength	570
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	50ms 80ms
Depth of Focus	0.81µm 0.80µm
Binning Mode	1x1
Detector Type	Camera

FIELD	SOURCE-DOCUMENTED VALUE
Depth Offset	0
Detector Digital Gain	0.00
Channel	561.000000 642.000000
Grating Period (µm)	27.500000 36.500000
Processing	3D
Auto Sharpness	Yes
Sharpness	12.773677 10.158410
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Volume Projection	Precise
Appearance	Threshold 39% (555), Threshold 16% (680) Ramp 39% (555), Ramp 84% (680), Maximum 100% both channels
Background	Black
Light	Brightness 110%, 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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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

Cat ID# - SA 000042

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 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® 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 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 560

Beam Splitter 680 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642

Dual Camera Beam Splitter = SBS LP 560

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

Z Stack - (3D)

Z-Stack = 139 Slices (8.28µm)

Channels = 2

Scaling (Per Pixel) = 30.0nm X 30.0nm X 60.00nm

Image Size (Pixels) = 936 X 906

Image Size (Scaled) = 29.30µm X 28.36µm

Bit Depth = 16 Bit

Image Center Position = X: -1.25mm, Y: -5.73mm

Stage Position = X: -1.25mm, Y: -5.73mm

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

555 -

Contrast Method = Fluorescence
 Laser Wavelength = 561nm @1.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 4.80s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 4
 Excitation Wavelength = 561
 Emission Wavelength = 570
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 50ms
 Depth of Focus = 0.81µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 561.000000

Grating Period (µm): 27.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 12.773677
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

680 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @4.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 4.80s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 4
 Excitation Wavelength = 642
 Emission Wavelength = 570
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 80ms
 Depth of Focus = 0.80µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 642.000000

Grating Period (µm): 36.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 10.158410
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 39% (555), Threshold 16% (680) Ramp 39% (555), Ramp 84% (680), Maximum 100% both channels
 Background = Black
 Light = Brightness 110%, 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

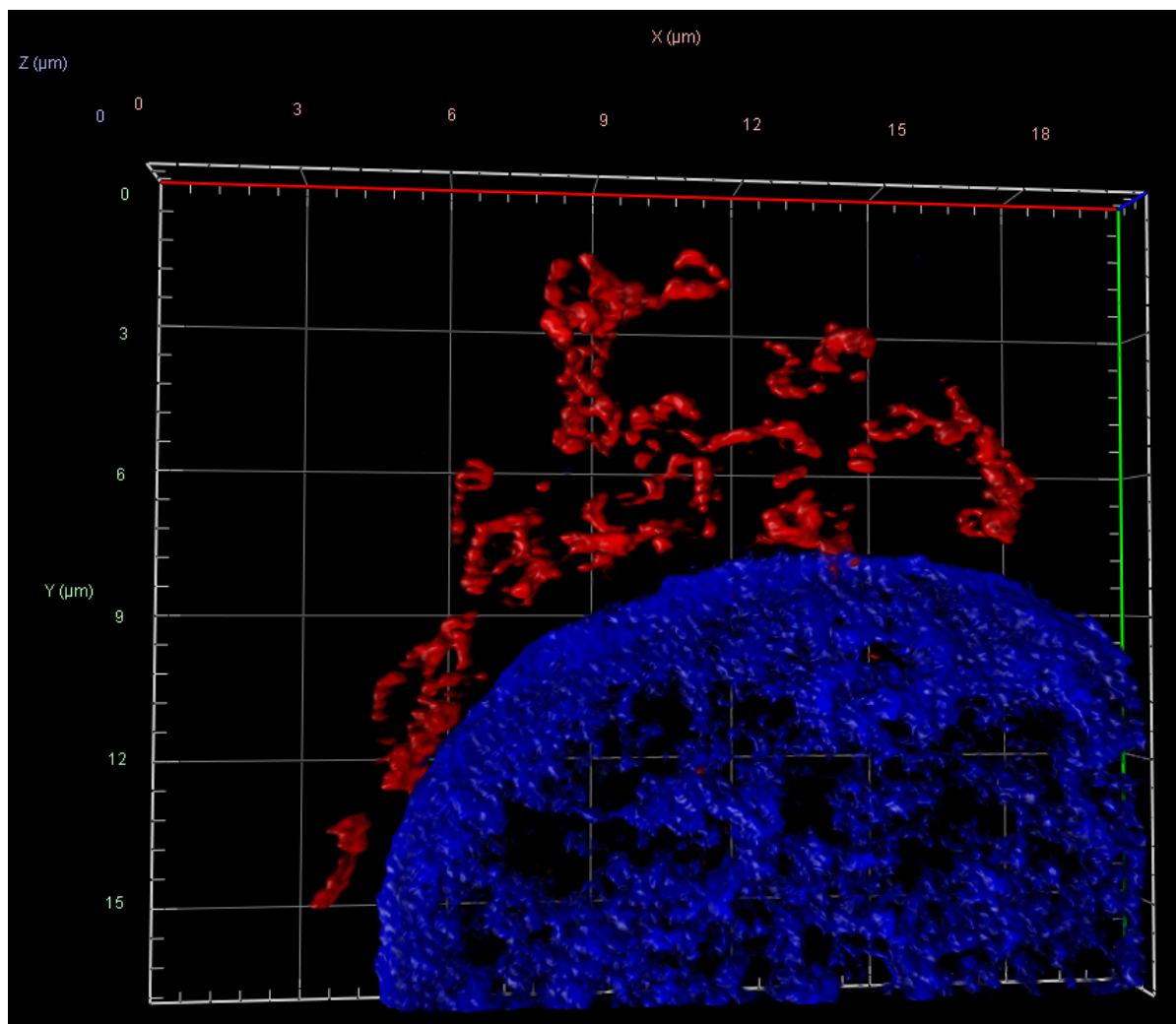
None

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000076
Stage	SIM
Instrument	ZEISS Elyra 7
Microscope configuration	Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A69436C7E00DA830D04A68356AA9269712EC05D03DFDA9B6F49EB6236F02D32A
Method PDF SHA-256	786443CA1B383E48C9890F74B1B75B5CED62FD867D3FE9E8F52343C696AA777D

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
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 680	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)	1321X 1080
Image Size (Scaled)	21.66µm X 23.73µm
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	642nm @8.0% 405nm @10%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	642 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms
Depth of Focus	0.93µm 0.73µm
Binning Mode	1x1
Detector Type	Camera

FIELD	SOURCE-DOCUMENTED VALUE
Depth Offset	0
Detector Digital Gain	0.00
Channel	642.000000 405.000000
Grating Period (µm)	32.000000
Processing	3D
Input SNR	Medium Low
Iterations	16 5
Regularization Weight	0.0650000 0.800000
Processing Sampling	4
Output Sampling	4
Filter	Median
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Volume Projection	Precise
Appearance	Threshold 10% (680), Threshold 4% (405) Ramp 10% (680), Ramp 96% (405), Maximum 100% both channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 55°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyfyn Standards for Optical Microscopy Methods™

Identifyfyn® SRM Alexa Fluor™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyfyn® 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 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 680 = 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) = 1321X 1080

Image Size (Scaled) = 21.66µm X 23.73µm

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

680 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @8.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 642
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 100ms
 Depth of Focus = 0.93µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 642.000000
Grating Period (µm): 32.000000

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%
 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): 32.000000

SIM2

Processing: 3D
 Input SNR: Low
 Iterations: 5
 Regularization Weight: 0.800000
 Processing Sampling: 4
 Output Sampling: 4
 Filter: Median
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 10% (680), Threshold 4% (405) Ramp 10% (680), Ramp 96% (405), Maximum 100% both channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 55°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

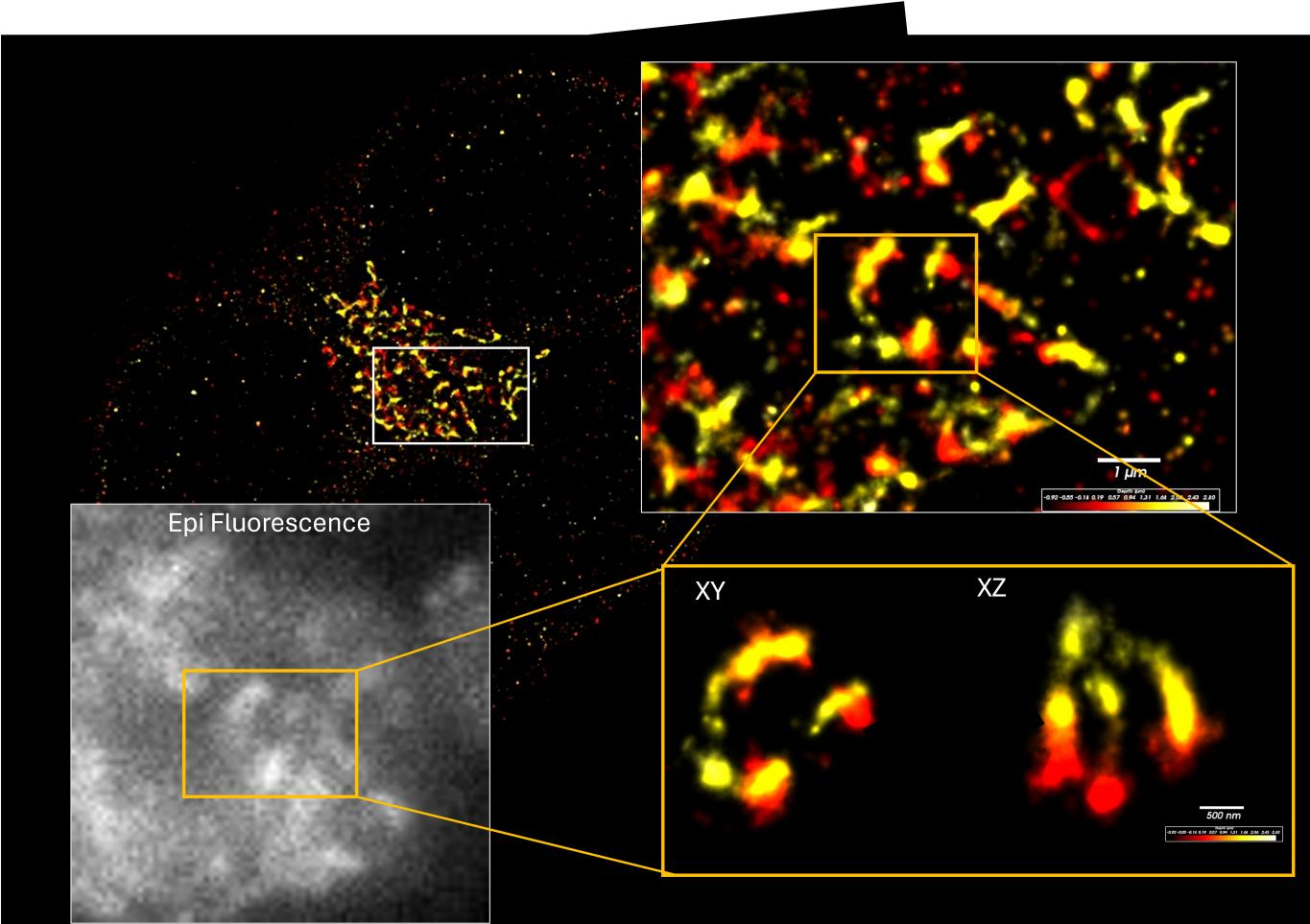
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000076
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	HeLa
Image SHA-256	B55E23A4DEA9F233B571FE71F886BE0011D97797015546D0B59085943245C668
Method PDF SHA-256	280DC74E2534736AF852C0FE297E6A6A18CF905F6833E9D8160CCE3788B9ADE8

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	680
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	159 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @1% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	200ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 158.1; End: 159.3
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	6 using Steps: 0.2µm (200nm)
Cycles	25
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	20%
Cutout Width	16 px
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: 15
Sizing Mode	Radial
Particle size	4nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.02
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	7 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	50nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	35
Axial precision	70

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

Cat ID# - SA 000076

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® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

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

Calibration -

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

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

Image Acquisition -

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

Configuration Tab - Capture Configuration

Number of Probes: 1

Number of Simultaneous Probes: 1

Number of Reference Probes: 1

Camera: Both Cameras

Color Channel: Both Channels

Probe Capture Mode: Interleaved

Z -Stack Capture Mode: Interleaved

Multiple Location Capture: Not selected

Post Record Reference: Not Selected

Fluidics: Not Selected

Autofocus Drift Correction: On

Microscope configuration

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

Camera Configuration

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

Probe Configuration

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

Experiment Configuration

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

Stage and Piezo Selection

Examination of Sample

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

Piezo Current: 159 µm

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

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

Advanced Tools Option

Widefield camera Exposure Time: 200ms
 Default values are maintained unless otherwise noted.

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

Autofocus - Calibration Values

Current Position" -0.979

Current Intensity: 4.5

Calibration Value: -5070 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: 158.1; End: 159.3

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

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input

Reference Probe 1 Laser control (640 nm (640 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: 200

Z Positions: 6 using Steps: 0.2µm (200nm)

Cycles: 25

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 20%

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16 px
 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: 15

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
 Sizing Mode: Radial
 Particle size: 4nm
 Color by: Depth
 Color Table: Single
 Opacity Mode: Depth; Opacity 1: 0.02
 Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
 Time Intervals: 7 Intervals
 Pixel Size: 50nm
 Smoothing: 8.00

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

PSF Z offset: -600 to +600

Radial precision: 35

Axial precision: 70

The other parameters were not applied.

Statistical analysis - NA

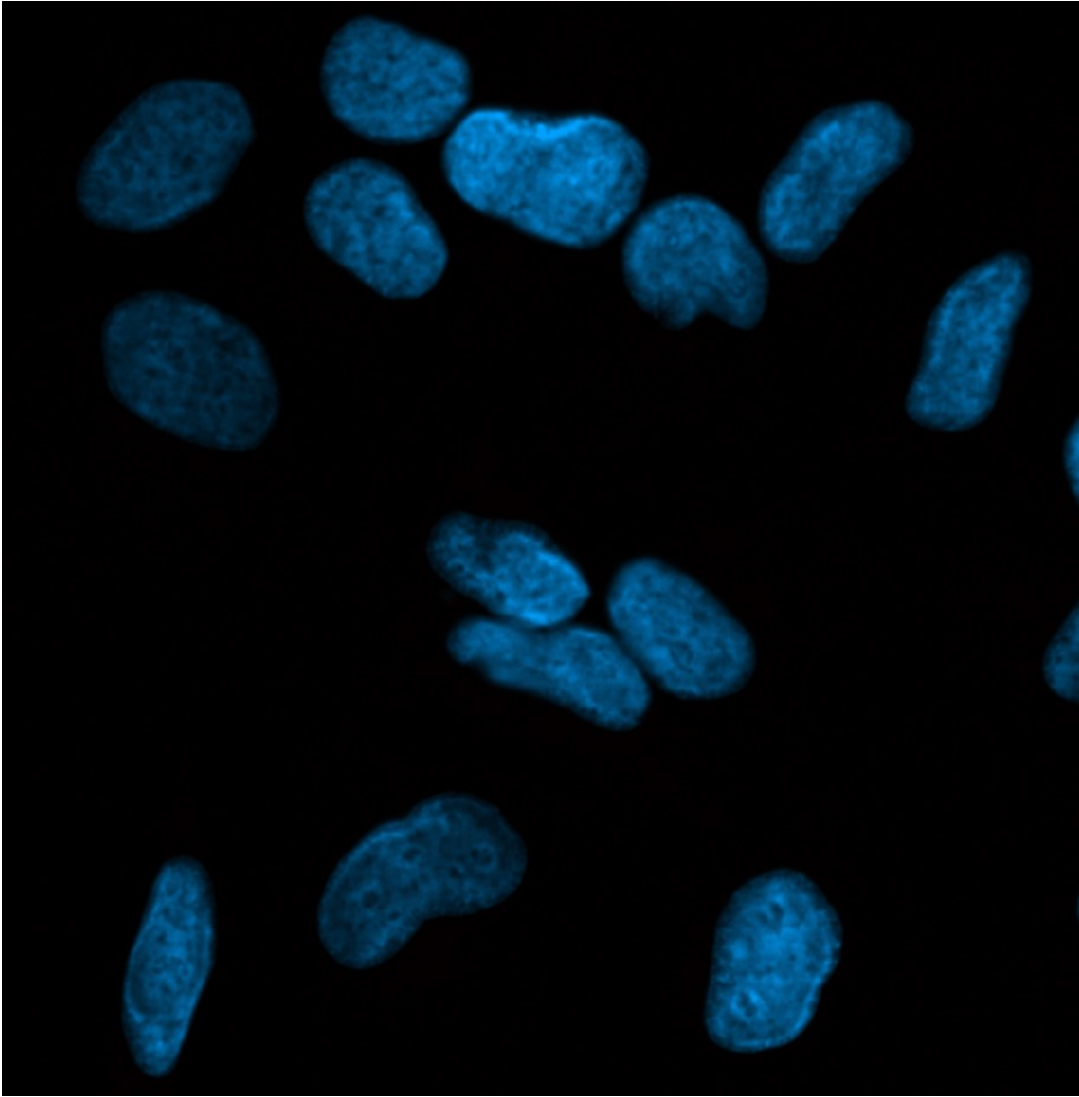
Image by S. Thakur

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SOURCE PROVENANCE

Catalog	SA-000076
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	F670F3E5D54EDCA6918265335C42210625AD274E45AD731A870FE09E4CF7CE35
Method PDF SHA-256	1EE996E120E826E426D5025EA7E96FC8DDF5372E694EF330213B4BE8E9F7F2DC

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 680
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)	984 X 997
Image Size (Scaled)	140.57µm X 142.43µm
Bit Depth	14 Bit
Image Center Position	X: 51.90mm, Y: 47.56mm
Stage Position	X: 51.90mm, Y: 47.56mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	50 Cy5 49 DAPI
Beam Splitter	660 395
Filter Excitation Wavelength	625 - 655 335-383
Filter Emission Wavelength	665 - 715 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	300ms 30ms
Excitation Wavelength	679 353
Emission Wavelength	702 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.36µm 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™ 680 Affinity Purified Donkey Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000076

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® 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 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) = 984 X 997

Image Size (Scaled) = 140.57µm X 142.43µm

Bit Depth = 14 Bit

Image Center Position = X: 51.90mm, Y: 47.56mm

Stage Position = X: 51.90mm, Y: 47.56mm

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

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 = 300ms

Excitation Wavelength = 679

Emission Wavelength = 702

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

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 @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-000076
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	C8D817A8B1DB970974EC4DD1C68716C7EE869E8945BF0742F4F27EDAFDEA23A7
Method PDF SHA-256	5D157B68585B91EA5C4BA48CC455DADAD7AD148D198071481502C13B7815885C