

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

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

7

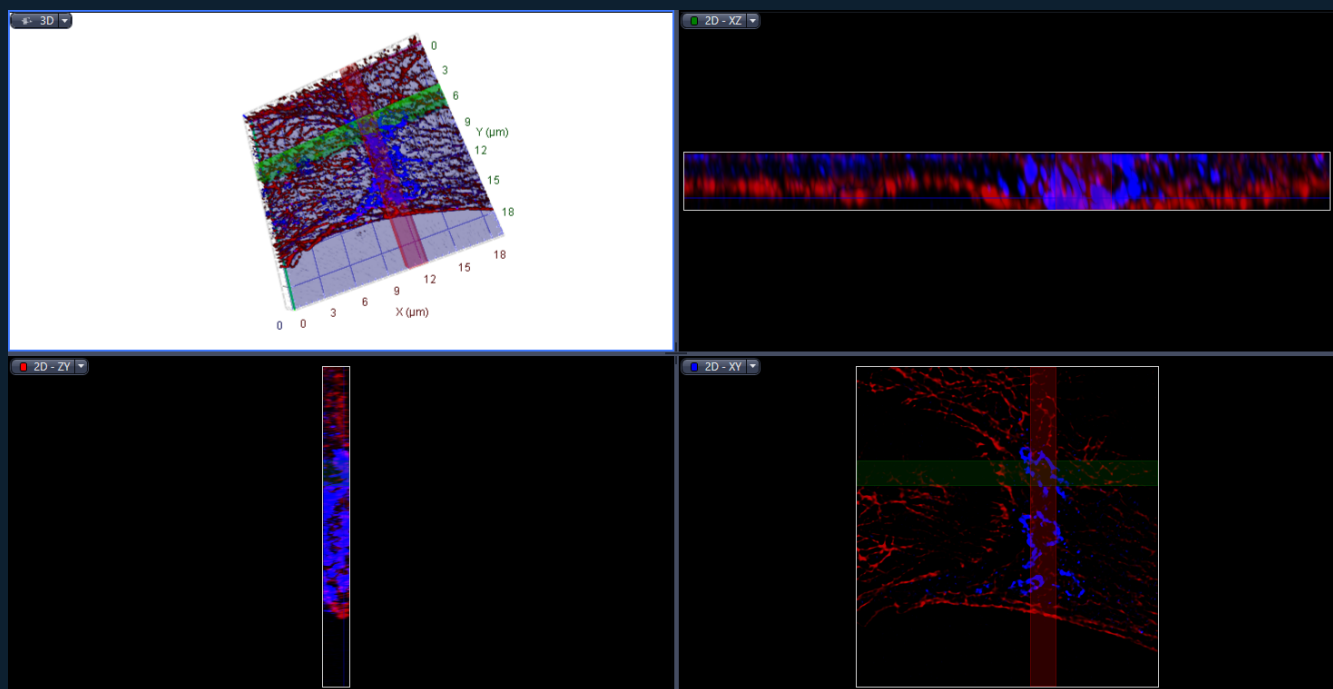
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000008 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

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

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L), and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Control

BENNETT ASSESSMENT

The preserved SA-000008 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved SA-000008 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 7 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	405/DAPI	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 555	2; AF405-49027; AF532-49014; 372 - 408; 515 - 545; 417 - 445; 555 - 595; 401
Widefield SIM — Apotome	405/DAPI; 555	2; AF405-49027; AF532-49014; 372 - 408; 515 - 545; 417 - 445; 555 - 595; 401
Confocal	405/DAPI; 488; 594	2; None; 405nm @0.3%; 561nm @0.3%; 401; 590; 422; 618
Airyscan	405/DAPI; 488; 680	2; None; 405nm @1.0%; 640nm @3.0%; 401; 679; 422; 702
SIM	405/DAPI; 488; 680	2; 405nm @3.50%; 642nm @3.50%; 405; 642; 515; 655; 405.000000
SIM ²	405/DAPI; 488; 680	2; 642nm @3.50%; 405nm @3.0%; 642; 405; 655; 515; 642.000000
Control	405/DAPI; 647	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 405/DAPI.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam705, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 400ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; X-Cite Xylis Lamp Intensity @8.0%. 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.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 78 Slices (7.7µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 1295 X 568; Image Size (Pixels): 1295 X 568; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 650ms; 120ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; X-Cite Xylis Lamp Intensity @6.0%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 150%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 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 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 44 Slices (2.58µm); Channels: 2; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.060µm; Image size (Pixels): 695 X 769; Image Size (Pixels): 695 X 769; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-PMT 3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.28µs; Frame Time: 2.64s. Illumination: Laser Wavelength: 405nm @0.3%; 561nm @0.3%. Optical/scan: Pinhole: 1.00AU / 41µm; 1.00AU / 58µm; Scan Zoom: 1.6; LSM Scan Speed: 11; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 150%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 7.5 (3D, Auto); Filter: 7.6 (3D, Auto). 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 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 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: 47 Slices (2.3µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 50.00nm; Image size (Pixels): 1365 X 1542; Image Size (Pixels): 1365 X 1542; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.88µs; Frame Time: 11.85s. Illumination: Laser Wavelength: 405nm @1.0%; 640nm @3.0%. Optical/scan: Pinhole: 5.00AU / 186µm; 5.00AU / 282µm; Scan Zoom: 1.2; LSM Scan Speed: 6; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 9.0 (3D, Auto). Structured source metadata values: 45.

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; 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 8

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: 25 Slices (1.44µm); Channels: 2; Scaling (Per Pixel): 31.30nm X 31.30nm X 60nm; Image size (Pixels): 1496 X 2011; Image Size (Pixels): 1496 X 2011; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 75ms; 100ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 405nm @3.50%; 642nm @3.50%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; 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: 58.

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; 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 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra 7. Objective: Plan-Apochromat 63X/1.46 oil Korr M27. Platform: Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 28 Slices (1.62µm); Channels: 2; Scaling (Per Pixel): 15.65nm X 15.65nm X 60.00nm; Image size (Pixels): 1209 X 1281; Image Size (Pixels): 1209 X 1281; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 75ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 642nm @3.50%; 405nm @3.0%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 8; Effective NA: 1.46. Processing: Background: Black. Structured source metadata values: 64.

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 8

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1; 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): 1385 X 1017; Image Size (Pixels): 1385 X 1017; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 650ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 29.

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 Imager.M1; ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000008 record contains Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². Missing modalities are not represented or inferred.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.29nm X 35.29nm X 50.00nm -> 0.03529 μ m X 0.03529 μ m X 0.05000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1365 X 1542; Image Size (Scaled)=48.17 μ m X 54.41 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 31.30nm X 31.30nm X 60nm -> 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)=1496 X 2011; Image Size (Scaled)=46.83 μ m X 62.95 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 15.65nm X 15.65nm X 60.00nm -> 0.01565 μ m X 0.01565 μ m X 0.06000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1209 X 1281; Image Size (Scaled)=18.92 μ m X 20.05 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

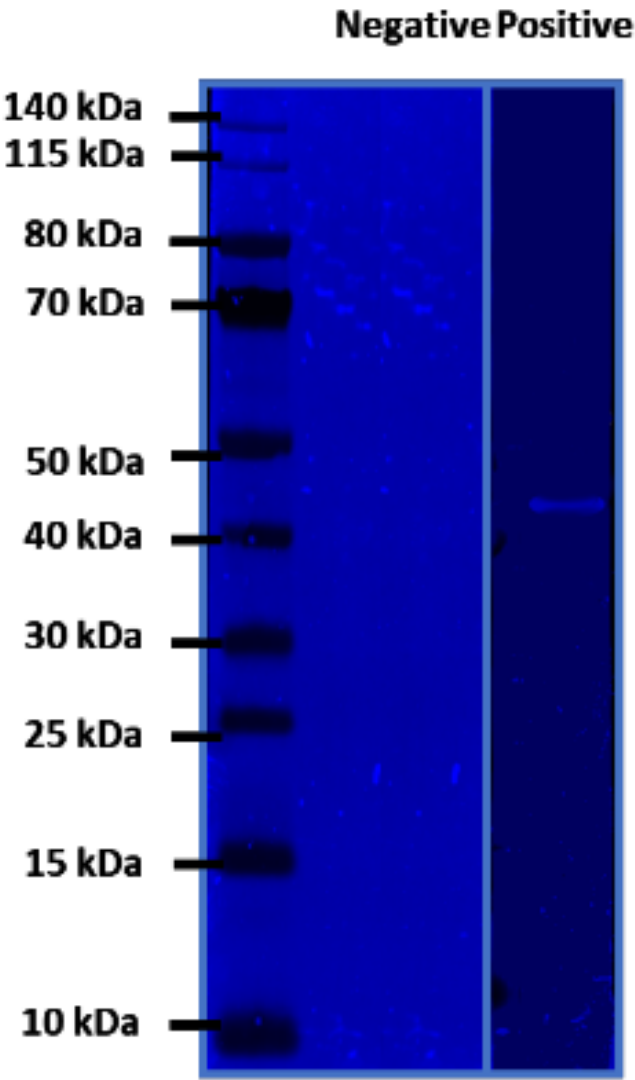
This dossier preserves the complete available evidence progression for SA-000008. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra 7	PRESERVED
7	SIM ²	ZEISS Elyra 7	PRESERVED

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	405/DAPI
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	375nm
Emission Filter	452±45nm
Detector	PMT
Intensity	10
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™ 405 Affinity Purified Goat Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000008

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™ 405 Affinity Purified Goat 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 = 375nm

Emission Filter = 452±45nm

Detector = PMT

Intensity = 10

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.

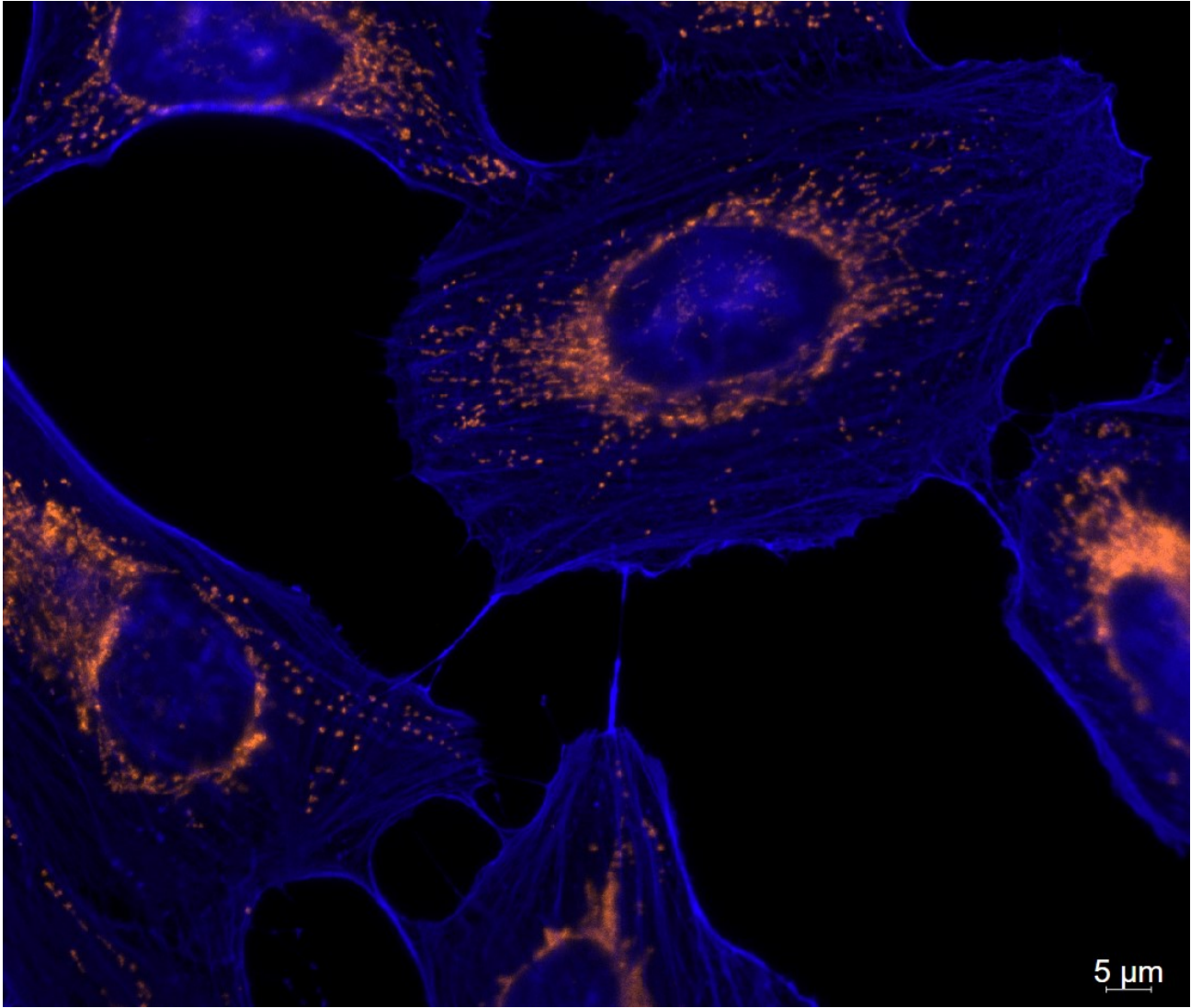
SOURCE PROVENANCE

Catalog	SA-000008
Stage	Western blot

SOURCE PROVENANCE

Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F195C8B69CE5E11F8F18B521D5C9CFD8BE68BCCBF4133EA8EBC23611B8C2E629
Method PDF SHA-256	AFD9E97F4AB7E07CAF34D68C08AA7B6B39E78AB8A527E7E593B86D46208ED6B2

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 555
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, Axiocam705, 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)	0.110µm X 0.110µm
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18µm X 112.59µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	AF405-49027 AF532-49014
Beam Splitter	412 550
Filter Excitation Wavelength	372 - 408 515 - 545
Filter Emission Wavelength	417 - 445 555 - 595
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	400ms 100ms
Excitation Wavelength	401 553
Emission Wavelength	422 568
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.65µm 0.88µm
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™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000008

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific

Cat ID# - SA 000034

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, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02)), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using. Prolong™ Diamond Antifade Mountant cat#36961.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam705, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18µm X 112.59µm

Bit Depth = 14 Bit

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

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

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10%
 Exposure Time = 400ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.65µm
 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 @8.0%
 Exposure Time = 100ms
 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

Post Processing

None

Image Correction

None

Image by J.K. Bennett

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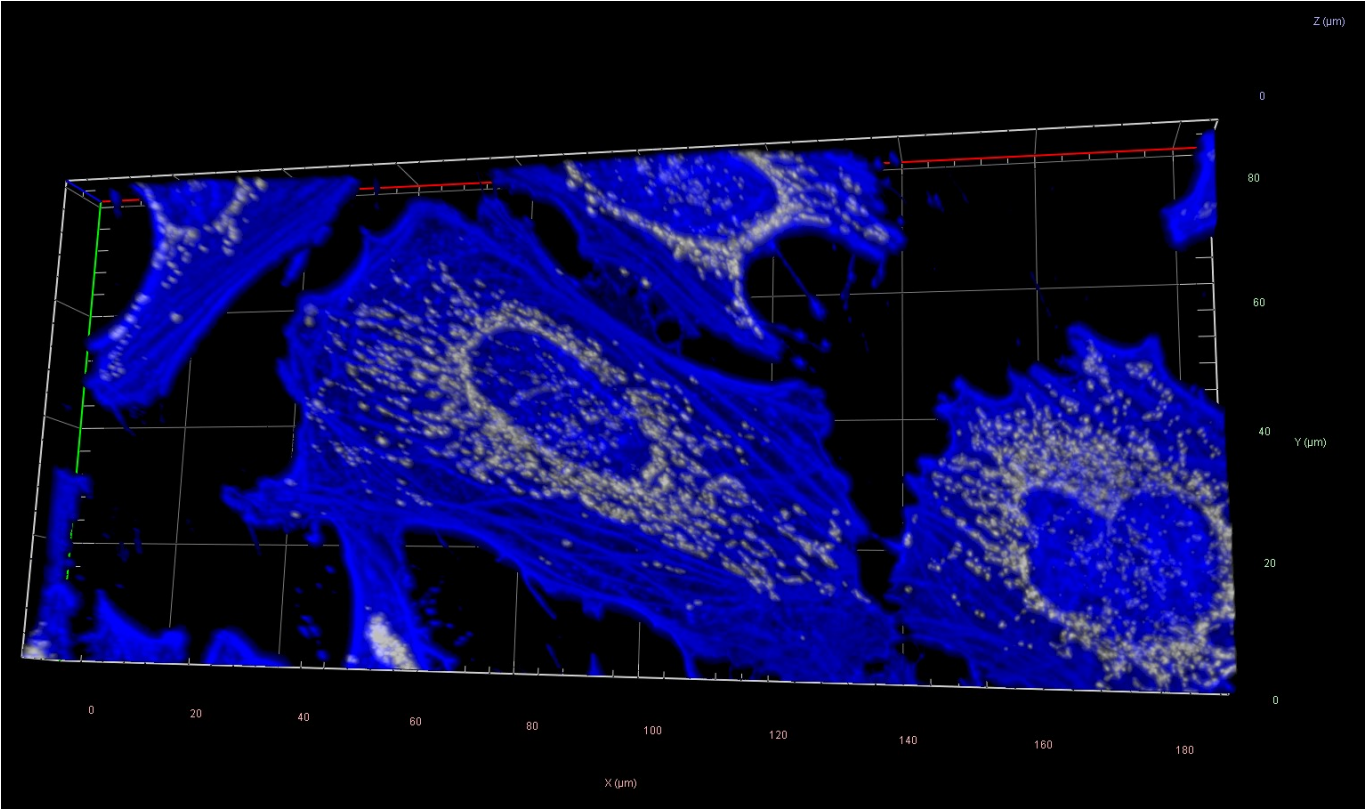
SOURCE PROVENANCE

Catalog	SA-000008
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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, AxioCam705, and X-Cite Xylys 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	241FC6F853E05F918EED8B31CED4C2CD3BFF5487CD18699F9130CE9B5D7FEF1B
Method PDF SHA-256	C2D7364E248BA4C2BF930670242BB4EF4F66F4FF03B3045FF4CF0E6C7BF4204A

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	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	78 Slices (7.7µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	1295 X 568
Image Size (Scaled)	185.00µm X 81.14µm
Bit Depth	14 Bit
Image Center Position	X: 94.55mm, Y: 55.57mm
Stage Position	X: 94.55mm, Y: 55.57mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF405-49027 AF532-49014
Beam Splitter	412 550
Filter Excitation Wavelength	372 - 408 515 - 545
Filter Emission Wavelength	417 - 445 555 - 595
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @10% X-Cite Xylis Lamp Intensity @6.0%
Exposure Time	650ms 120ms
Excitation Wavelength	401 553
Emission Wavelength	422 568
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	0.82µm 1.10µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 6% (555), Threshold 6% (405), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 150%, 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™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000008

Identifyn® SRM Alexa Fluor™ 555 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific

Cat ID# - SA 000034

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, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02)), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 555 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using. Prolong™ Diamond Antifade Mountant cat#36961.

Microscope

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

Z Stack - (3D)

Z-Stack = 78 Slices (7.7µm)

Channels = 2

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

Image Size (Pixels) = 1295 X 568

Image Size (Scaled) = 185.00µm X 81.14µm

Bit Depth = 14 Bit

Image Center Position = X: 94.55mm, Y: 55.57mm

Stage Position = X: 94.55mm, Y: 55.57mm

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

405 -

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10%
 Exposure Time = 650ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.82µm
 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 @6.0%
 Exposure Time = 120ms
 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

Post Processing - SIM Processing

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

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

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 6% (555), Threshold 6% (405), Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 150%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Identifyn® SRM Alexa Fluor™ 55 was pseudo-colored in Zen Software from orange, the default color, to dark grey to provide visual contrast between the 405/555 channels.

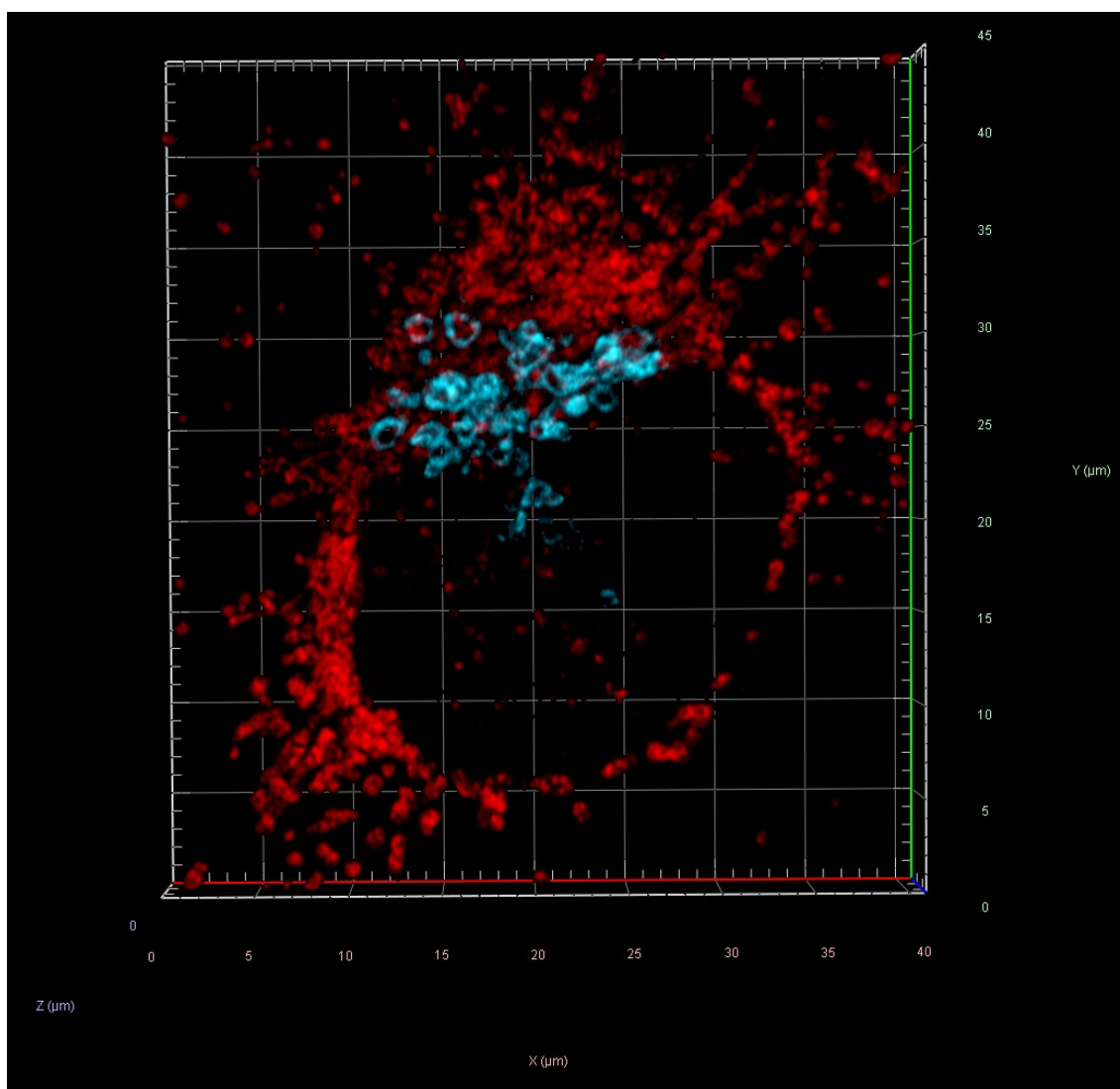
Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000008
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B589E3FE70B118CD544656FCB0E4C3A28C3F96FE0A74EC1C9E3873B3A737867B
Method PDF SHA-256	B396CB522F90BA9C16CEB48BFEAC1265667D274C19F1B0964AF73F48E271018D

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	44 Slices (2.58µm)
Channels	2
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.060µm
Image Size (Pixels)	695 X 769
Image Size (Scaled)	40.88µm X 45.24µm
Bit Depth	16 Bit
Image Center Position	X: 72.92mm, Y: 53.51mm
Stage Position	X: 72.92mm, Y: 53.51mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 41µm 1.00AU / 58µm
LMS MBS	Plate MBS 488/561 MBS 405/730
LMS SBS	Mirror
Laser Wavelength	405nm @0.3% 561nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.20
Pixel Time	0.28µs
Frame Time	2.64s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	401 590
Emission Wavelength	422 618
Effective NA	1.4
Detection Wavelength	395 - 461 585 - 640
Imaging Device	GaAsp-PMT 3
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.5 (3D, Auto) Filter: 7.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 1% (405), Threshold 1% (594), Ramp0% (405), Ramp 0% (594), Maximum 100% all channels.
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 150%, 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™ 405 Affinity Purified Goat Anti-Rabbit F(ab')₂ (H + L)

Cat ID# - SA 000008

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Donkey Anti-Mouse, IgG (H + L)

Cat ID# - SA 000048

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MTC02), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using Prolong™ Diamond Antifade Mountant cat#36961.

Microscope

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

Z Stack - (3D)

Z-Stack =44 Slices (2.58µm)

Channels = 2

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

Image Size (Pixels) = 695 X 769

Image Size (Scaled) = 40.88µm X 45.24µm

Bit Depth = 16 Bit

Image Center Position = X: 72.92mm, Y: 53.51mm

Stage Position =X: 72.92mm, Y: 53.51mm

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

405 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 41µm
 LMS MBS = Plate MBS 488/561 MBS 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28µs
 Frame Time = 2.64s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 395 - 461
 Imaging Device = GaAsp-PMT 3
 Detector Type = GaAsp-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.5 (3D, Auto)

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 58µm
 LMS MBS = Plate MBS 488/561 MBS 405/730
 LMS SBS = Mirror
 Laser Wavelength = 561nm @0.3%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.28µs
 Frame Time = 2.64s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 585 - 640
 Imaging Device = GaAsp-PMT 3
 Detector Type = GaAsp-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 7.6 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 1% (405), Threshold 1% (594), Ramp0% (405), Ramp 0% (594), Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 150%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from blue, the default color, to light blue to provide visual contrast between the 405/594 channels.

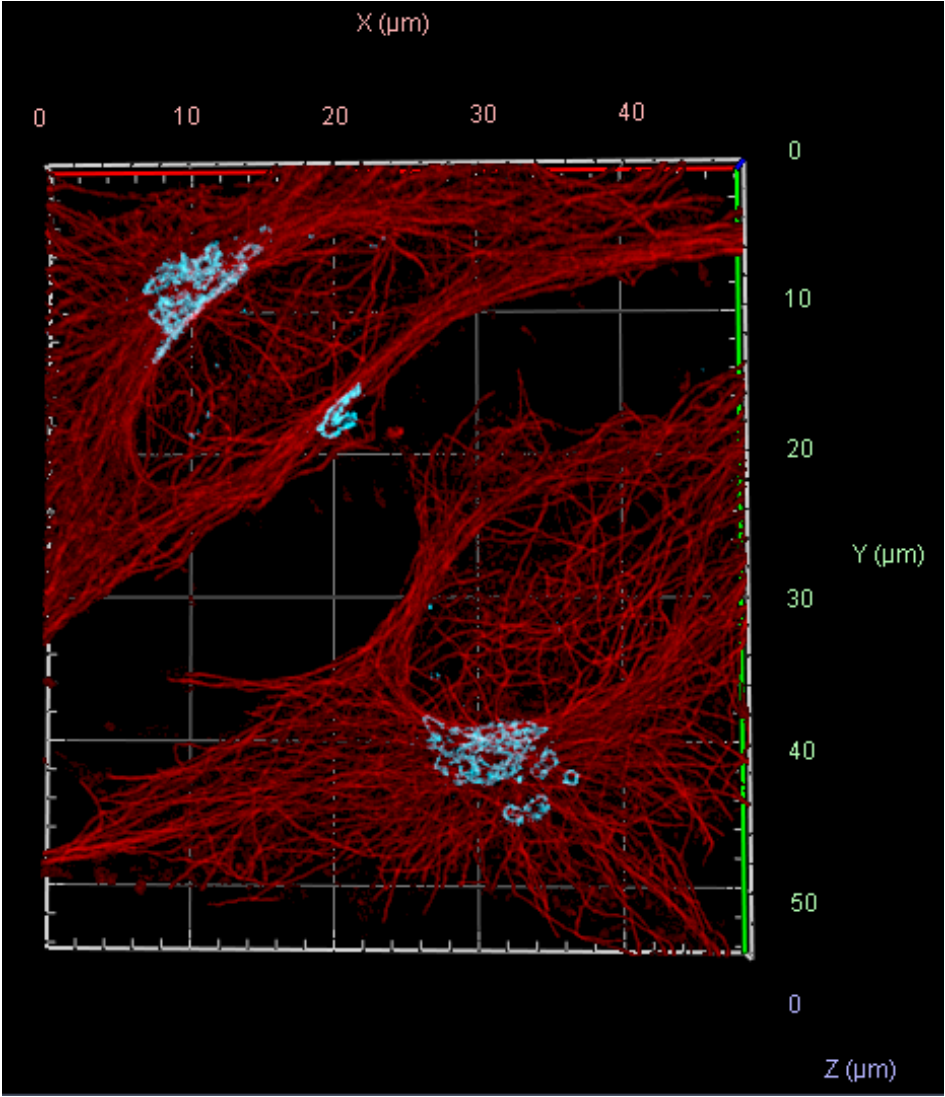
Image by J. K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000008
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	169B5E3731B1896533776F9643132B6463FDF413B646DF6FD5C6817D121D03AF
Method PDF SHA-256	65C208113F0E2CA1617FCE07148C41BEB47902C8FEFBA28F6B76649AAE5815EA

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	45

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	47 Slices (2.3µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.05000 µm
Image Size (Pixels)	1365 X 1542
Image Size (Scaled)	48.17µm X 54.41µm
Bit Depth	16 Bit
Image Center Position	X: 69.57mm, Y: 55.10mm
Stage Position	X: 69.57mm, Y: 55.10mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 186µm 5.00AU / 282µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	405nm @1.0% 640nm @3.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.2
Rotation	31.53°
Sampling	2.00
Pixel Time	0.88µs
Frame Time	11.85s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	401 679
Emission Wavelength	422 702
Effective NA	1.4
Detection Wavelength	400 - 485 650 - 700
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 9.0 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 8% (680), Threshold 5% (405), 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™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000008

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

Cat ID# - SA 000075

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 cat#36961.

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 = 47 Slices (2.3µm)

Channels = 2

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

Image Size (Pixels) = 1365 X 1542

Image Size (Scaled) = 48.17µm X 54.41µm

Bit Depth = 16 Bit

Image Center Position = X: 69.57mm, Y: 55.10mm

Stage Position = X: 69.57mm, Y: 55.10mm

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

405 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 186µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @1.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 31.53°
 Sampling = 2.00
 Pixel Time = 0.88µs
 Frame Time = 11.85s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 2
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.4
 Detection Wavelength = 400 - 485
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.0 (3D, Auto)

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00AU / 282µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @3.0%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.2
 Rotation = 31.53°
 Sampling = 2.00
 Pixel Time = 0.88µs
 Frame Time = 11.85s
 LSM Scan Speed = 6
 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 = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.0 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 8% (680), Threshold 5% (405), 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

Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from dark blue, the default color, to white to provide visual contrast between the 405/680 channels.

Image by P. Raut

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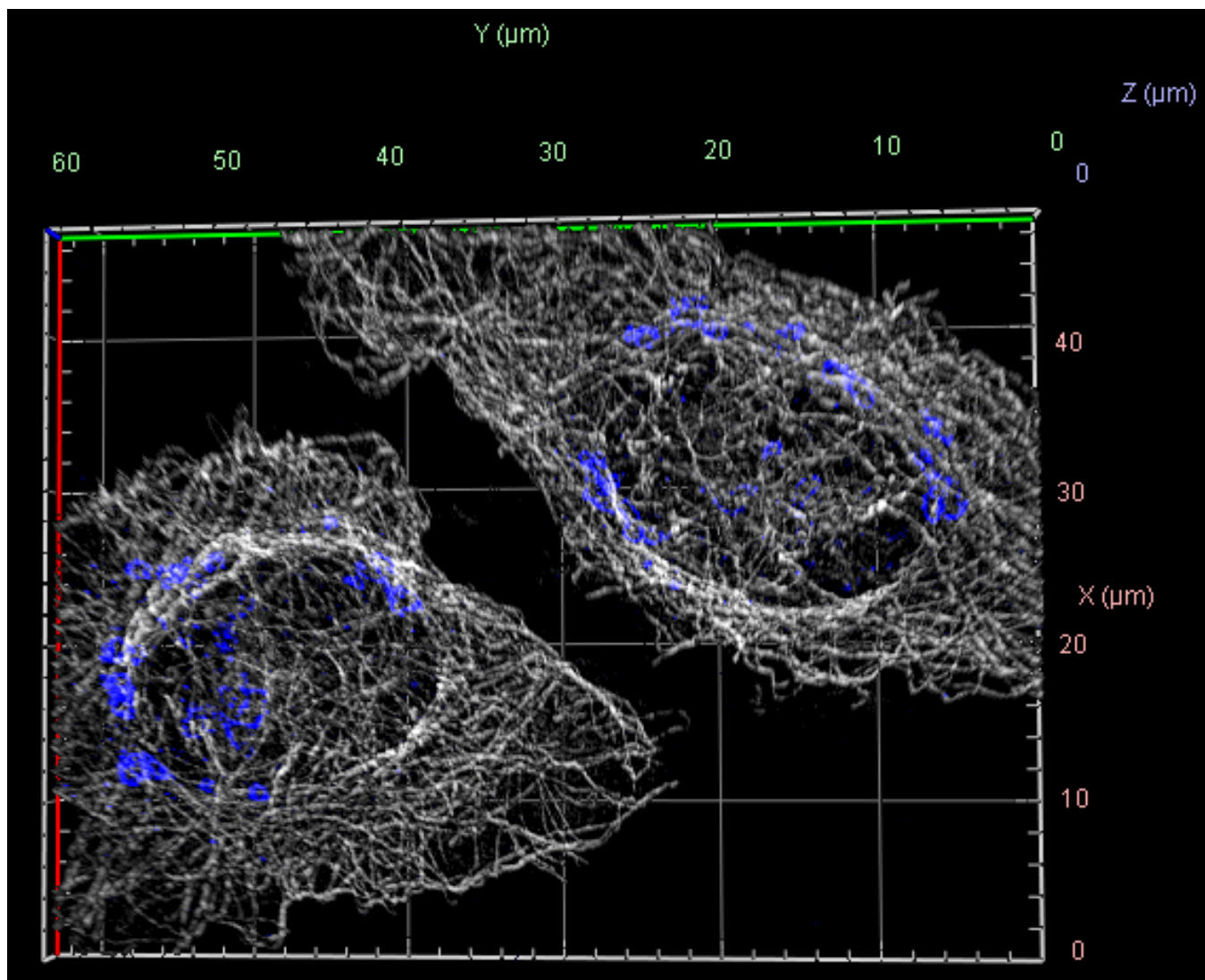
SOURCE PROVENANCE

Catalog	SA-000008
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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	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C6679B3DC7D67BB74CA7FA3D02E65E1C98C182810E38B386355323E7753233C9
Method PDF SHA-256	2D7E357A739F7070781C2AD907EFD65F06CA9933E59E37A276675B4FD79F7456

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	58

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 680	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 640 SBS LP 560
Beam Splitter 405	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Calibration Marker Positions	Marker 1: 0.00µm, 0.00µm, Marker 2: 0.00µm, 0.00µm, Marker 3 0.00µm, 0.00µm
Z-Stack	25 Slices (1.44µm)
Channels	2
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.06000 µm
Image Size (Pixels)	1496 X 2011
Image Size (Scaled)	46.83µm X 62.95µm
Bit Depth	16 Bit
Image Center Position	X: 5.98mm, Y: -552.40mm
Stage Position	X: 5.98mm, Y: -552.40mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	405nm @3.50% 642nm @3.50%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	405 642
Emission Wavelength	515 655
Effective NA	1.46
Detection Wavelength	495-550 655-1000
Imaging Device	Edge 4.2 SIM
Exposure Time	75ms 100ms
Depth of Focus	0.73µm 0.93µm
Binning Mode	1x1

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	Camera
Depth Offset	0
Detector Digital Gain	0.00
Channel	405.000000 642.000000
Grating Period (µm)	42.000000
Processing	3D
Auto Sharpness	Yes
Sharpness	9.522315 10.614963
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 1% (680), Threshold 1% (405) 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™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000008

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

Cat ID# - SA 000075

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, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody that followed this, the 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® 405 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 cat#36961.

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 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 = 25 Slices (1.44µm)

Channels = 2

Scaling (Per Pixel) = 31.30nm X 31.30nm X 60nm

Image Size (Pixels) = 1496 X 2011

Image Size (Scaled) = 46.83µm X 62.95µm

Bit Depth = 16 Bit

Image Center Position = X: 5.98mm, Y: -552.40mm

Stage Position = X: 5.98mm, Y: -552.40mm

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

405 -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @3.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 405
 Emission Wavelength = 515
 Effective NA = 1.46
 Detection Wavelength = 495-550
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 75ms
 Depth of Focus = 0.73µm
 Binning Mode = 1x1
 Detector Type = Camera
 Depth Offset = 0
 Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 405.000000
Grating Period (µm): 42.000000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 9.522315
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

680 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @3.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 642
 Emission Wavelength = 655
 Effective NA = 1.46
 Detection Wavelength = 655-1000
 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 - SIM Processing

Channel = 642.000000

Grating Period (µm): 42.000000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 10.614963
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise

Appearance = Threshold 1% (680), Threshold 1% (405) 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

Identifyn® SRM Alexa Fluor™ 680 was pseudo-colored in Zen Software from dark red, the default color, to white to provide visual contrast between the 405/680 channels.

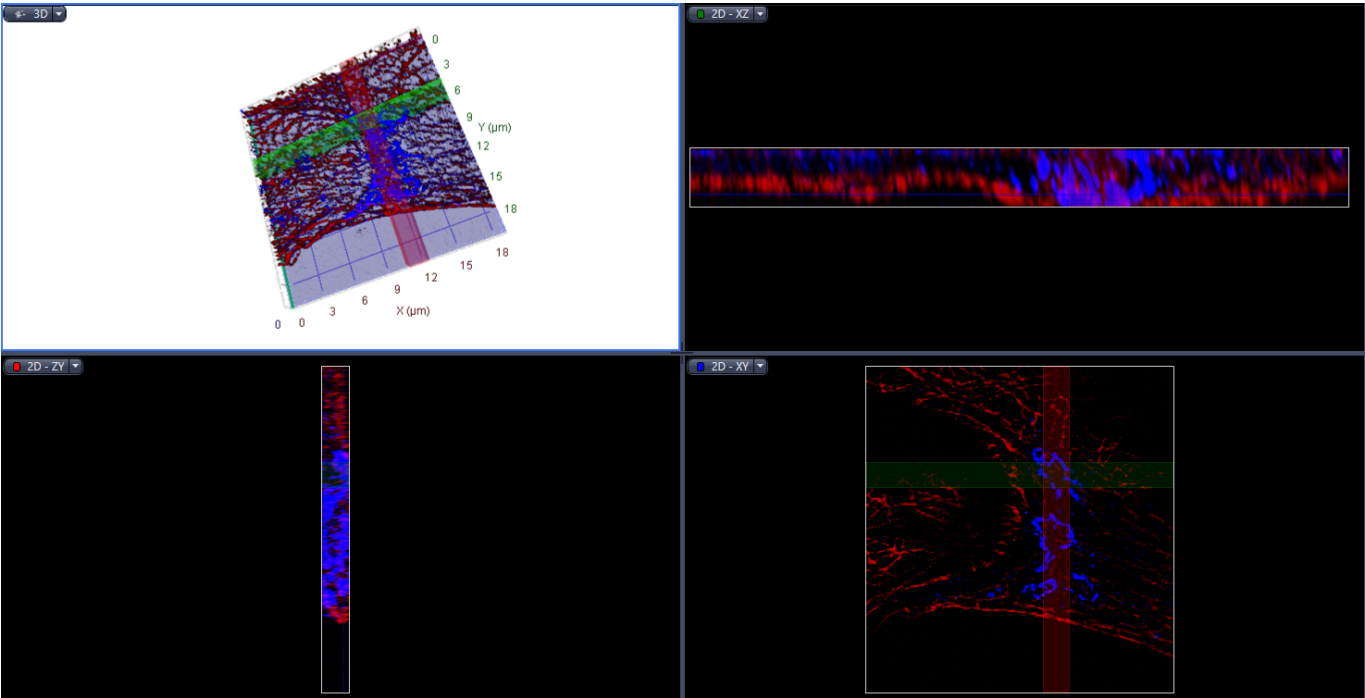
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000008
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	58
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4359741348E0275AD65E3B2E4ADA39F9DAA72D0D1A2787A0A08514911B79AA37
Method PDF SHA-256	66E3046DA9945B2408FCAE13C3448100593726D880B2A1C841F7F9D50663550F

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
METADATA VALUES	64

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 SBS LP 560
Beam Splitter 405	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Calibration Marker Positions	Marker 1: 0.00µm, 0.00µm, Marker 2: 0.00µm, 0.00µm, Marker 3 0.00µm, 0.00µm
Z-Stack	28 Slices (1.62µm)
Channels	2
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.06000 µm
Image Size (Pixels)	1209 X 1281
Image Size (Scaled)	18.92µm X 20.05µm
Bit Depth	16 Bit
Image Center Position	X: 1.31mm, Y: -175.00mm
Stage Position	X: 1.31mm, Y: -175.00mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	642nm @3.50% 405nm @3.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	8
Excitation Wavelength	642 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms 75ms
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)	42.000000 36.500000
Processing	3D
Input SNR	Medium
Iterations	16 5
Regularization Weight	0.0650000 0.065000
Processing Sampling	4
Output Sampling	4
Filter	Median
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 0% (680), Threshold 0% (405) Ramp 0% (680), Ramp 0% (405), Maximum 100% both channels
Background	Black
Light	Brightness 87%
Cut Lines	X 696 Y 376, Z 22
Line Width	X 106, Y 102, X 1
Cut Line Opacity	44

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

Cat ID# - SA 000008

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

Cat ID# - SA 000075

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, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 680 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody that followed this, the 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® 405 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 cat#36961.

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 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 = 28 Slices (1.62µm)

Channels = 2

Scaling (Per Pixel) = 15.65nm X 15.65nm X 60.00nm

Image Size (Pixels) = 1209 X 1281

Image Size (Scaled) = 18.92µm X 20.05µm

Bit Depth = 16 Bit

Image Center Position = X: 1.31mm, Y: -175.00mm

Stage Position = X: 1.31mm, Y: -175.00mm

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

680 -

Contrast Method = Fluorescence
 Laser Wavelength = 642nm @3.50%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 8
 Excitation Wavelength = 642
 Emission Wavelength = 655
 Effective NA = 1.46
 Imaging Device = Edge 4.2 SIM
 Exposure Time = 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): 42.000000

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

405 -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @3.0%
 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 = 75ms
 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): 36.500000

SIM2

Processing: 3D
 Input SNR: Medium
 Iterations: 5
 Regularization Weight: 0.065000
 Processing Sampling: 4
 Output Sampling: 4
 Filter: Median
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 0% (680), Threshold 0% (405) Ramp 0% (680), Ramp 0% (405), Maximum 100% both channels
 Background = Black
 Light = Brightness 87%

Tomo 3D Processing

Ortho Display

Cut Lines = X 696 Y 376, Z 22

Line Width = X 106, Y 102, X 1

Cut Line Opacity = 44

Image Corrections

None

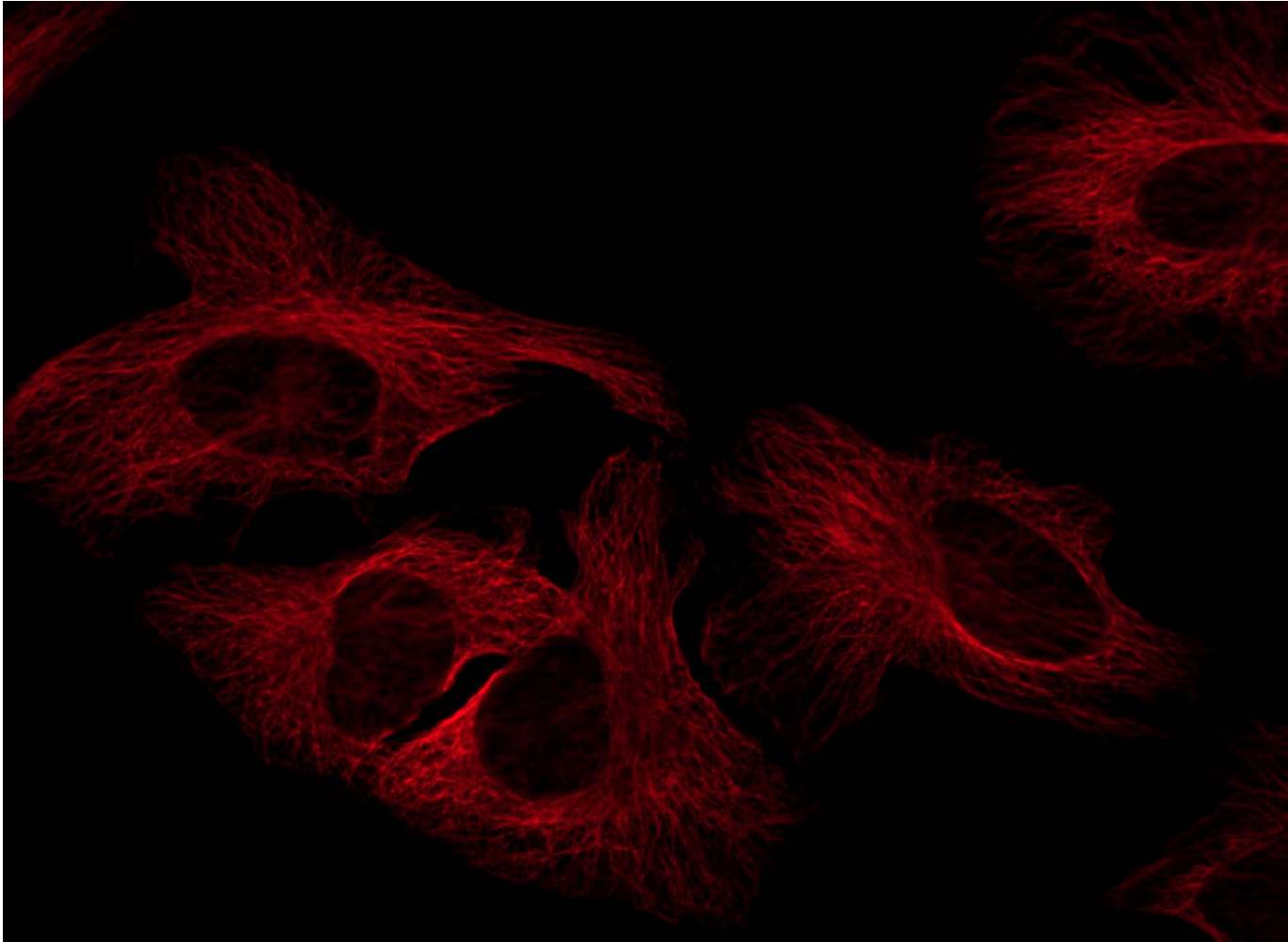
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000008
Stage	SIM ²
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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	62D1BE33058469F2C9A3B56CCAD7AAB62C1F8769CFDEA5D8D99DF6F49DBF1CBF
Method PDF SHA-256	11BB2082B2F8795246721C968CA83B2A72D3846353AE86C1BF99E1CFA04934EB

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	29

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)	1385 X 1017
Image Size (Scaled)	197.86µm X 145.29µm
Bit Depth	14 Bit
Image Center Position	X: 100.24mm, Y: 53.94mm
ROI Center Offset	X: 100.24µm, Y: 53.94µm
Reflector	AF405-49027 50 Cy5
Beam Splitter	412 660
Filter Excitation Wavelength	372 - 408 625 - 655
Filter Emission Wavelength	417 - 445 665 - 715
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25%
Exposure Time	650ms 200ms
Excitation Wavelength	401 653
Emission Wavelength	422 668
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	0.82µm 1.30µm
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™ 405 Affinity Purified Goat Anti-Rabbit, F(ab')₂ (H + L)

Cat ID# - SA 000008

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific

Cat ID# - SA 000056

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® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

To demonstrate the presence of cells in the sample, minus DAPI (which would overlap the 405), a second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using Prolong™ Diamond Antifade Mountant cat#36961.

Note that the lamp intensity and exposure times were set to the 647 control samples.

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) = 1385 X 1017

Image Size (Scaled) = 197.86µm X 145.29µm

Bit Depth = 14 Bit

Image Center Position = X: 100.24mm, Y: 53.94mm

ROI Center Offset = X: 100.24µm, Y: 53.94µm

405-

Reflector = AF405-49027
 Beam Splitter = 412
 Filter Excitation Wavelength = 372 - 408
 Filter Emission Wavelength = 417 - 445
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25%
 Exposure Time = 650ms
 Excitation Wavelength = 401
 Emission Wavelength = 422
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.82µm
 Binning Mode = 2,2

647 -

Reflector = 50 Cy5
 Beam Splitter = 660
 Filter Excitation Wavelength = 625 - 655
 Filter Emission Wavelength = 665 - 715
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25%
 Exposure Time = 200ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.25
 Imaging Device = Axiocam 807 mono
 Camera Adapter = 1X
 Depth of Focus = 1.30µm
 Binning Mode = 2,2

Post Processing -

None

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 Axio Imager.M1 Widefield. 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 data suggests the secondary antibody is highly specific and free of unconjugated (free) dye.

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000008
Stage	Control
Instrument	ZEISS Axio Imager.M1; 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	29
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	902ADA24E0283584C1C057BF5429DFAB04219711DB0F763C2893E1B34DE6F53B
Method PDF SHA-256	82745313B2165531C6B5B2B0679938973F4F454EA8076506EDD3B444FFFA993F