BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

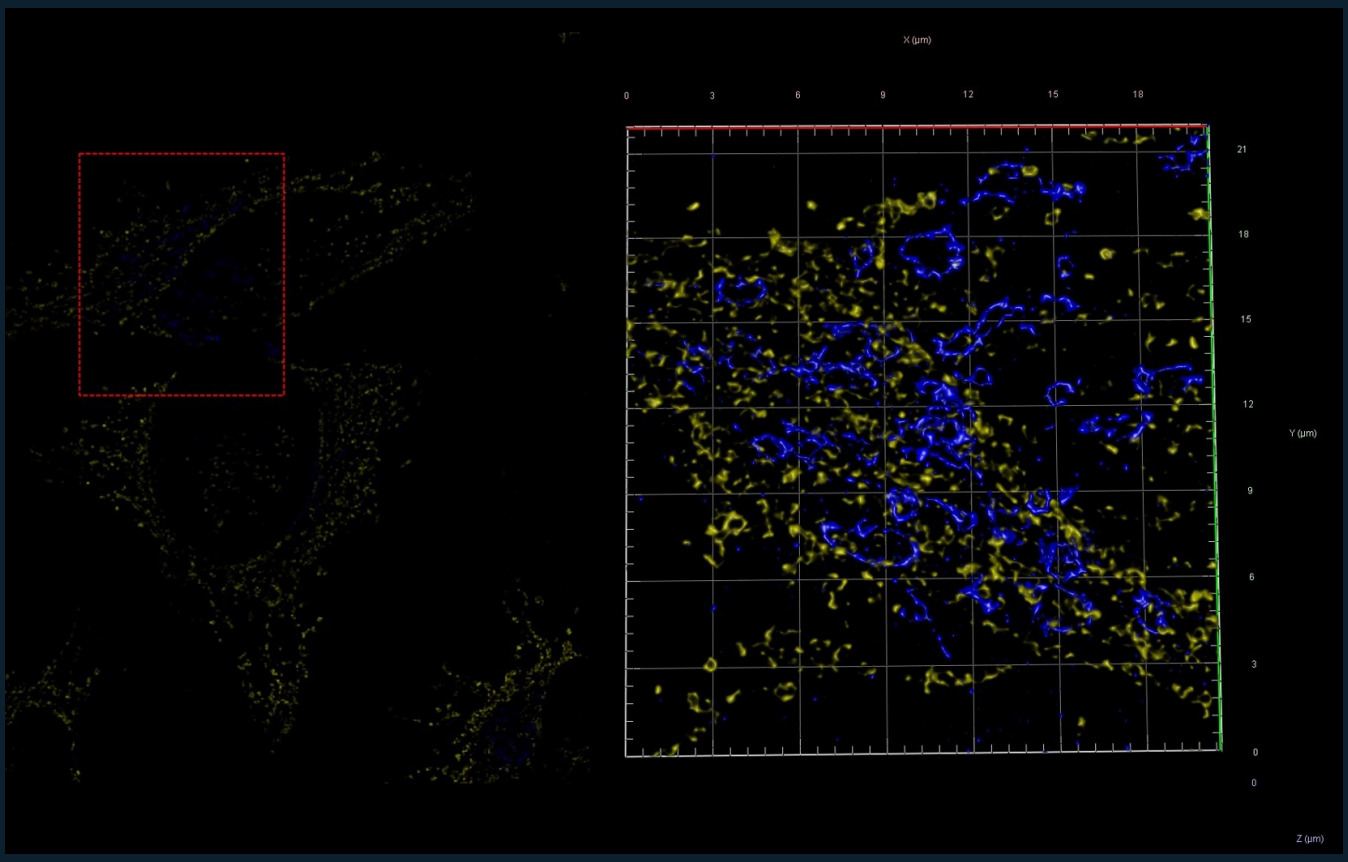
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

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

7	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SIM²

SA-000005 | 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 Donkey Anti-Rabbit, IgG (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-000005 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 / NIR-BOUNDED PARTIAL STEPDOWN

The preserved SA-000005 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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; 647; 750	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401
Widefield SIM — Apotome	405/DAPI; 647	2; AF405-49027; 50 Cy5; 372 - 408; 625 - 655; 417 - 445; 665 - 715; 401
Confocal	405/DAPI; 488; 594	2; None; 405nm @0.8%; 561nm @0.2%; 401; 590; 422; 618
Airyscan	405/DAPI; 488; 594	2; None; 405nm @0.3%; 561nm @0.3%; 401; 590; 422; 618
SIM	405/DAPI; 488; 532	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820#2-SR; T1-Axiocam 820 #2-SR; 405; 488
SIM²	405/DAPI; 488; 532	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T2-Axiocam 820#2-SR; T1-Axiocam 820 #2-SR; 405; 488
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, Axiocam 750, 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): 1211 X 929; Image Size (Pixels): 1211 X 929; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300ms; 120ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @10%; X-Cite Xylis Lamp Intensity @6.0%. 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; 647; 750.

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: 77 Slices (7.6µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.100µm; Image size (Pixels): 696 X 964; Image Size (Pixels): 696 X 964; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 650ms; 180ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @18%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. 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: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 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: 41 Slices (2.8µm); Channels: 2; Scaling (Per Pixel): 0.059µm X 0.059µm X 0.070µm; Image size (Pixels): 1045 X 1086; Image Size (Pixels): 1045 X 1086; 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 @0.8%; 561nm @0.2%. 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 23°, Scale Z 162%, 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: 7.9 (3D, Auto). Structured source metadata values: 51.

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 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: 55 Slices (2.16µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 40.00nm; Image size (Pixels): 1230 X 1254; Image Size (Pixels): 1230 X 1254; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.93µs; Frame Time: 11.04s. Illumination: Laser Wavelength: 405nm @0.3%; 561nm @0.3%. Optical/scan: Pinhole: 5.00AU / 215µm; 5.00AU / 309µm; Scan Zoom: 1.7; LSM Scan Speed: 6; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 257%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; LSM Plus Mode: Filter: 8.9 (3D, Auto); Filter: 9.7 (3D, Auto). Structured source metadata values: 47.

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 980, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

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. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 51 Slices (2.50µm); Channels: 2; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 2601 X 2596; Image Size (Pixels): 2601 X 2596; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820#2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 676.00ms; 1.59s. Illumination: Laser Wavelength: 405nm @4.0%; 488nm @8.0%; Illumination Wavelength: 405; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4. Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 48 Slices (2.35µm); Channels: 2; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 1215 X 1297; Image Size (Pixels): 1215 X 1297; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820#2. Timing: Exposure Time: 100ms; 120ms; Frame Time: 676.00ms; 1.59s. Illumination: Laser Wavelength: 488nm @4.0%; 488nm @8.0%; Illumination Wavelength: 405; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 11%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 532.

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): 1337 X 1014; Image Size (Pixels): 1337 X 1014; 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-000005 record contains the applicable NIR sequence: Western blot; Widefield; Widefield SIM — Apotome; Confocal; Airyscan; SIM; SIM². The historical boundary reflects the validated application available for the specific conjugate, deployed platform and workflow, not a universal scientific impossibility.

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 40.00nm -> 0.03529 µm X 0.03529 µm X 0.04000 µm. 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)=1230 X 1254; Image Size (Scaled)=43.41µm X 44.25µm; 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): 16.89nm X 16.89nm X 50.00nm -> 0.01689 µm X 0.01689 µm X 0.05000 µm. 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)=2601 X 2596; Image Size (Scaled)=43.93µm X 43.85µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689 µm X 0.01689 µm X 0.05000 µm. 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)=1215 X 1297; Image Size (Scaled)=20.52µm X 21.91µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

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®

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

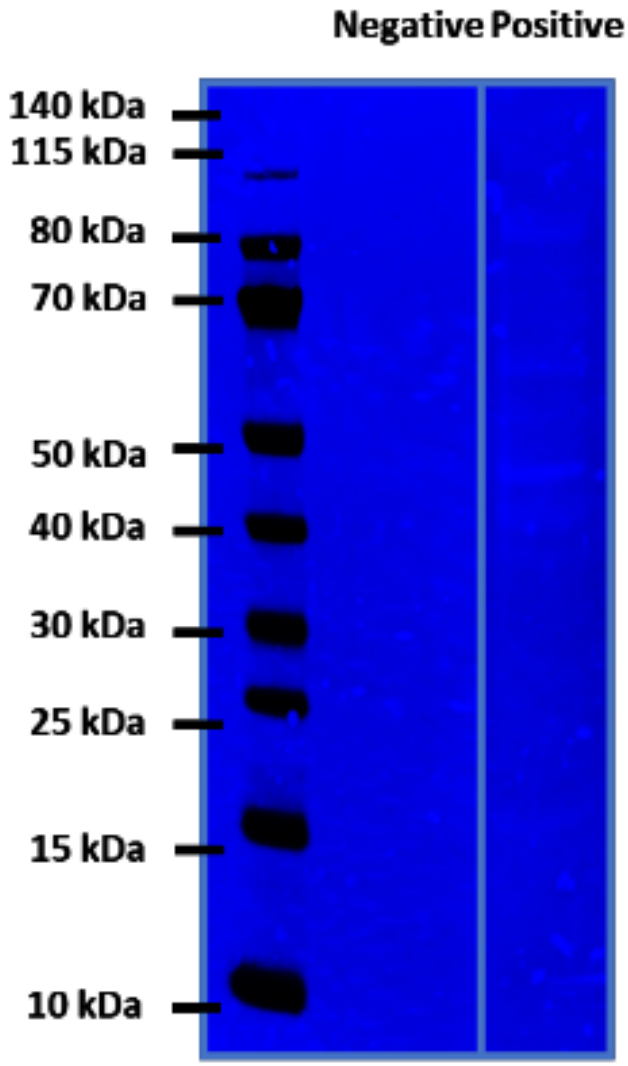
This dossier preserves the complete available evidence progression for SA-000005. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	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 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	ZEISS Elyra	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	8
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 Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000005

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 Donkey anti-Rabbit IgG was incubated for 1 hour, followed by washing 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 = 8
Pixel size = 100 µm
Scan Speed = Medium
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

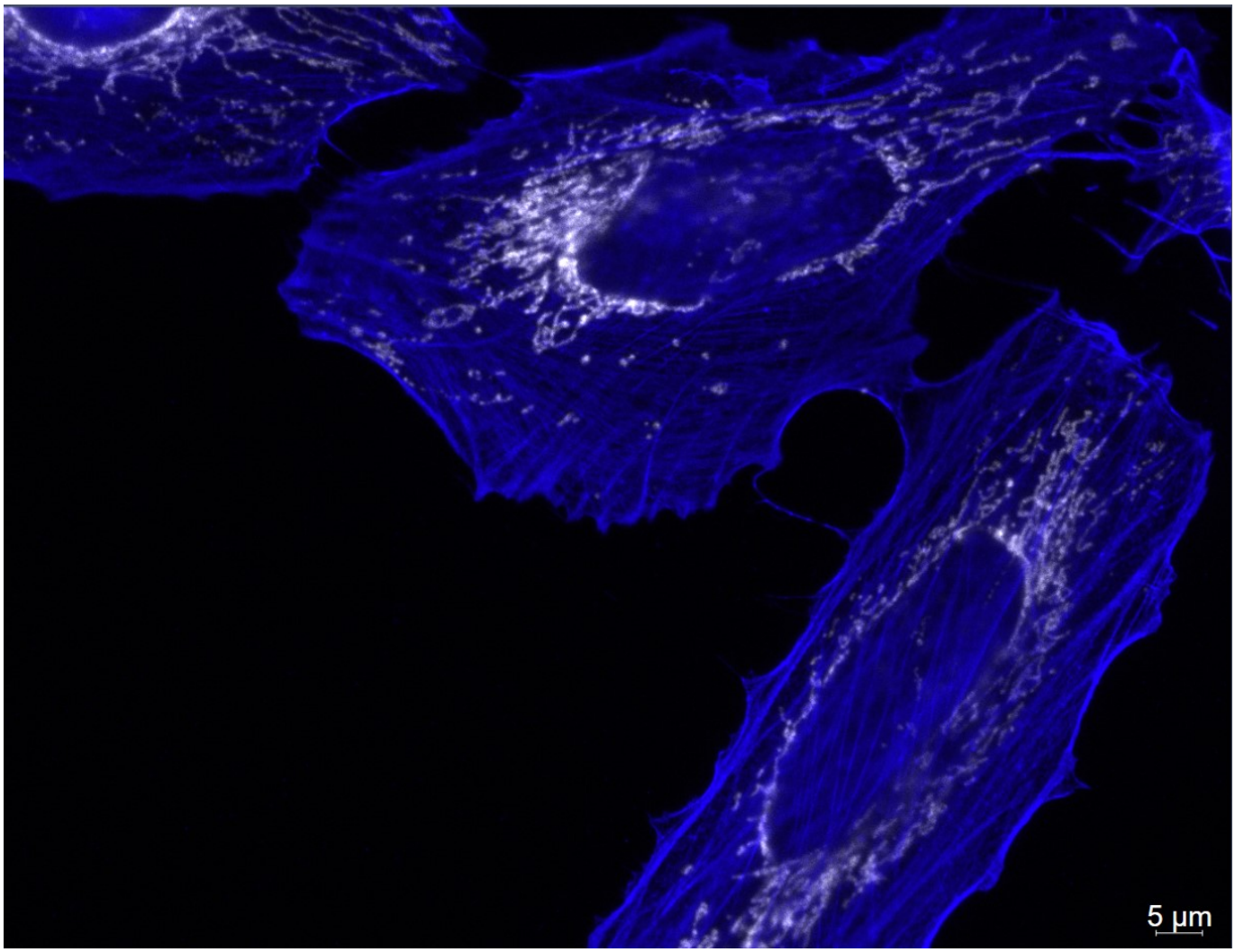
SOURCE PROVENANCE

Catalog	SA-000005
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	629355B5304EDC3A26D1A2F02D6475B312DB8A4D5E3C5104ACD900DF40D1469B
Method PDF SHA-256	9CC5DA16BD0A86A243101951402553AA141E3DBC91FB62A0CA0582D3FDADFE63

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647; 750
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 750, 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)	1211 X 929
Image Size (Scaled)	132.63µm X 101.75µm
Bit Depth	16 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
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 @10% | X-Cite Xylis Lamp Intensity @6.0%
Exposure Time	300ms | 120ms
Excitation Wavelength	401 | 653
Emission Wavelength	422 | 668
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	0.65µm | 1.03µm
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 Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000005

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. 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® 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.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, 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) = 1211 X 929
Image Size (Scaled) = 132.63µm X 101.75µm
Bit Depth = 16 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 = 300ms
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

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 @6.0%
Exposure Time = 120ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 1.03µm
Binning Mode = 2,2

Post Processing

None

Image Correction

Identifyn® SRM Alexa Fluor™ 647 was represented as white instead of its default red coloring to demonstrate contrast and show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405/647 dye products.

Image by J.K. Bennett

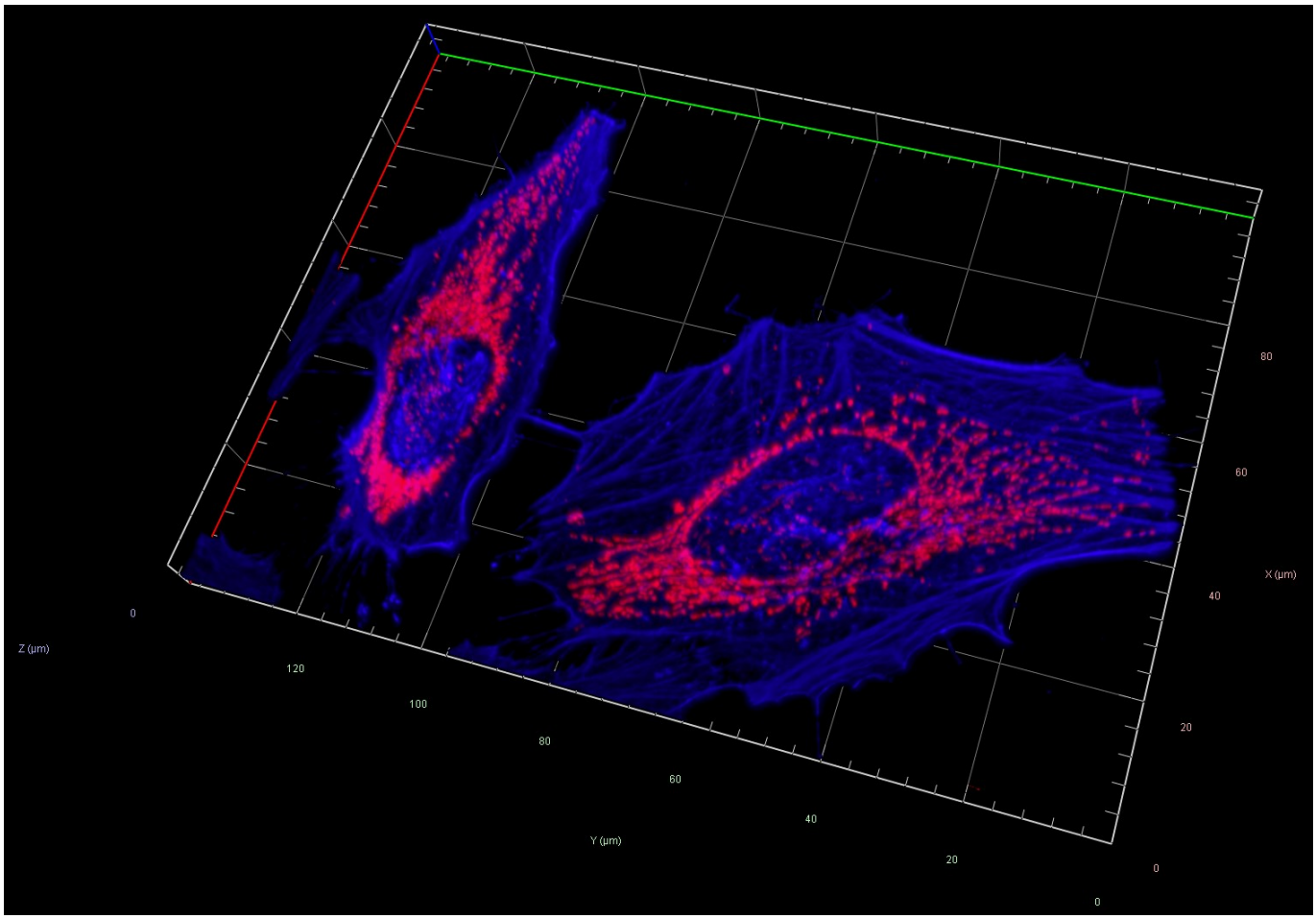
SOURCE PROVENANCE

Catalog	SA-000005

SOURCE PROVENANCE

Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 750, 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	505AC4BEBD39302DFE9C18037BB6131CB26C4433E6D8A78E003F702592F27D4F
Method PDF SHA-256	0F0CC5BB52E3F7F219F6C8313D09E98E2334EB62E07C8E44D43143E99D977753

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	77 Slices (7.6µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.100µm
Image Size (Pixels)	696 X 964
Image Size (Scaled)	99.43µm X 137.71µm
Bit Depth	14 Bit
Image Center Position	X: 95.28mm, Y: 52.21mm
Stage Position	X: 95.28mm, Y: 52.21mm
ROI Center Offset	X: 1.14mm, Y: 0.00mm
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 @18% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	650ms | 180ms
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
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 4% all channels, Ramp 0% all channels, Maximum 100%
Background	Black
Light	Brightness 7%, 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 Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000005

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. 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® 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.

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 = 77 Slices (7.6µm)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.100µm
Image Size (Pixels) = 696 X 964
Image Size (Scaled) = 99.43µm X 137.71µm
Bit Depth = 14 Bit
Image Center Position = X: 95.28mm, Y: 52.21mm
Stage Position = X: 95.28mm, Y: 52.21mm
ROI Center Offset = X: 1.14mm, Y: 0.00mm

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 @18%
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 @10%
Exposure Time = 180ms
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 - 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 4% all channels, Ramp 0% all channels, Maximum 100%
Background = Black
Light = Brightness 7%, 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)

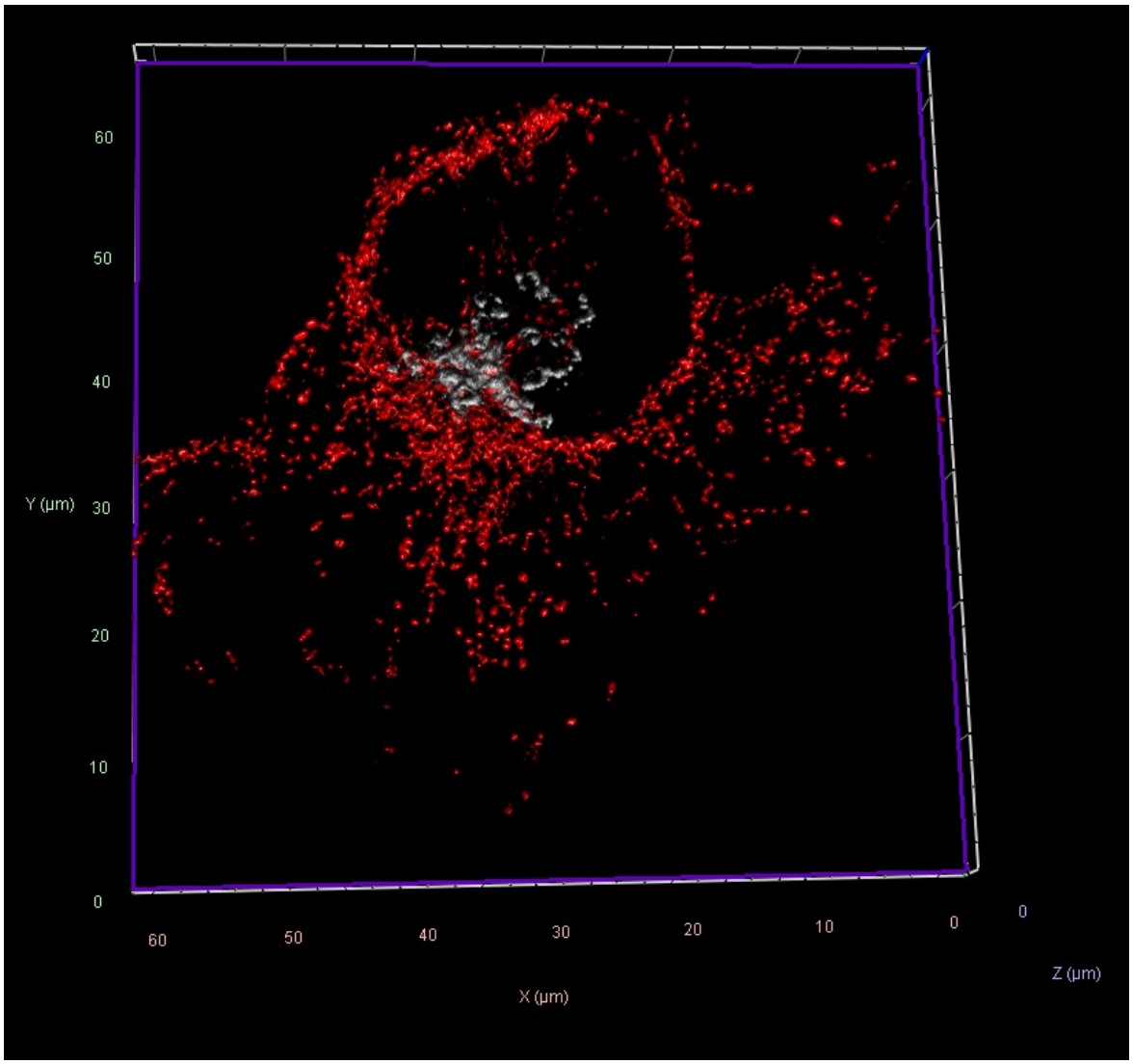
Image Corrections

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000005
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	C159F844C404D44B739E8F3A46E3232479A7303DC76193BE86A08BD89EFFF246
Method PDF SHA-256	DCE4606F2288DBB0BC7906F15270241A642347171B2C7B864DA17DFB01C8A060

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	51

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	41 Slices (2.8µm)
Channels	2
Scaling (Per Pixel)	0.059µm X 0.059µm X 0.070µm
Image Size (Pixels)	1045 X 1086
Image Size (Scaled)	61.47µm X 63.88µm
Bit Depth	16 Bit
Image Center Position	X: 66.69mm, Y: 50.47mm
Stage Position	X: 66.69mm, Y: 50.47mm
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 @0.8% | 561nm @0.2%
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: 7.9 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 1% (405), Threshold 5% (594), Ramp98% (405), Ramp 95% (594), Maximum 100% all channels
Background	Black
Light	Brightness 112%, Azimuth 48°, Elongation -80°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 23°, Scale Z 162%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	Clipping planes are introduced to pronounce regions of particular interest.
X - Y	Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z- Stack of the cell from the image, and a purple outline of the clipped perimeter was shown; Clip Back was Selected, and Clip Front was NOT selected. The cut plane was used to show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) and highlight the localization and specificity of the secondary antibody to the primary.
Position of Clip	-40%
Clip X	0°
Clip Y	0°

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 Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000005

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

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 =41 Slices (2.8µm)

Channels = 2
Scaling (Per Pixel) = 0.059µm X 0.059µm X 0.070µm
Image Size (Pixels) = 1045 X 1086
Image Size (Scaled) = 61.47µm X 63.88µm
Bit Depth = 16 Bit
Image Center Position = X: 66.69mm, Y: 50.47mm
Stage Position =X: 66.69mm, Y: 50.47mm
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 @0.8%
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 @0.2%
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: 7.9 (3D, Auto)

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 1% (405), Threshold 5% (594), Ramp98% (405), Ramp 95% (594), Maximum 100% all channels
Background = Black
Light = Brightness 112%, Azimuth 48°, Elongation -80°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 23°, Scale Z 162%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = Clipping planes are introduced to pronounce regions of particular interest.

X - Y = Active was selected, textured opaque was chosen to remove a horizontal portion of the upper Z- Stack of the cell from the image, and a purple outline of the clipped perimeter was shown; Clip Back was Selected, and Clip Front was NOT selected. The cut plane was used to show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) and highlight the localization and specificity of the secondary antibody to the primary.
Position of Clip = -40%
Clip X = 0°
Clip Y =0°

Image Corrections

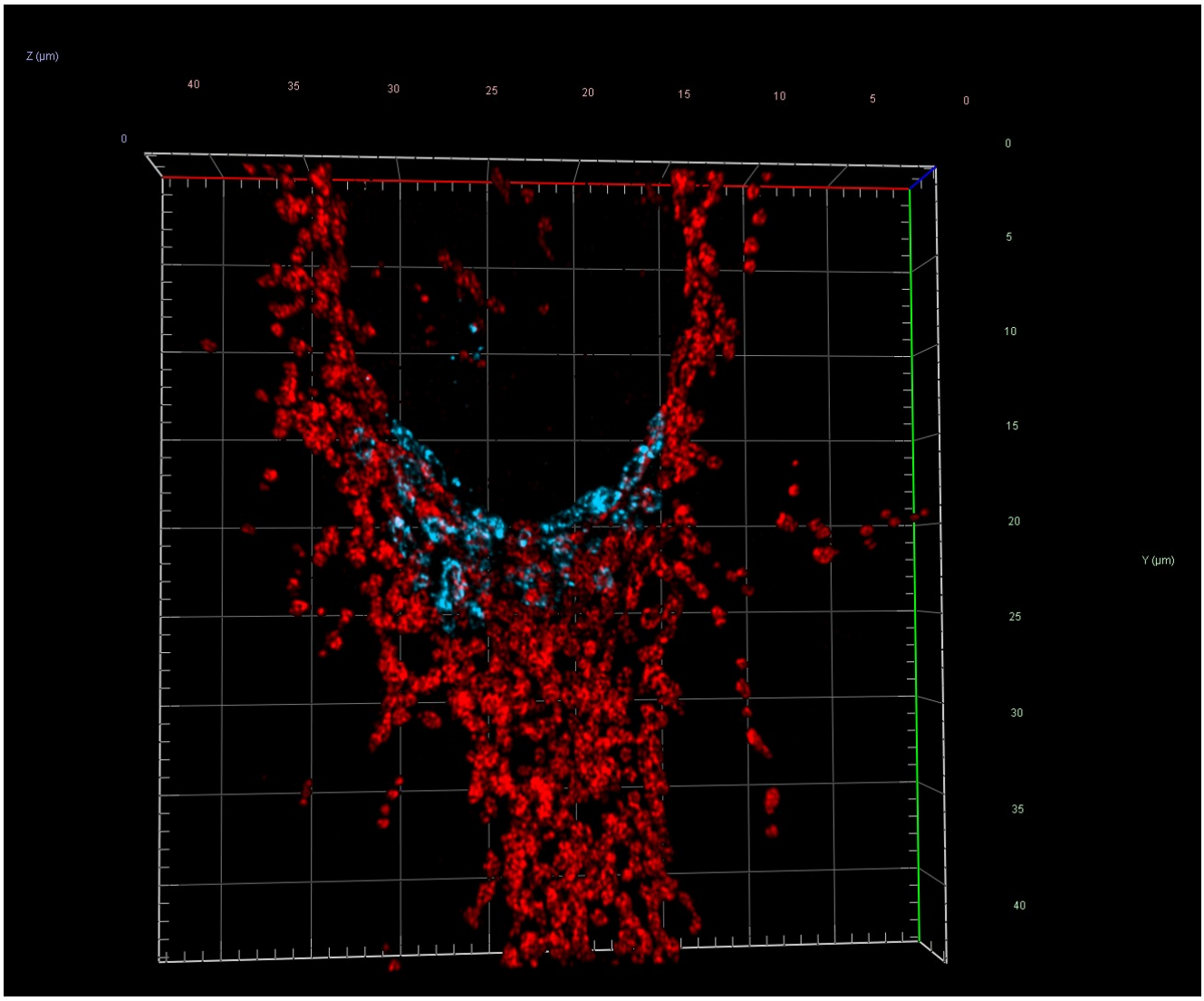
Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from blue, the default color, to white to provide visual contrast between the 405/594 channels.

Image by J. K. Bennett

SOURCE PROVENANCE

Catalog	SA-000005
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	51
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9DF3CD8E41CEF6C49F229CAA36700DFDA391C15B76FA50E4241A9AB62B685561
Method PDF SHA-256	EFB6E53D454D674A703A519439FFB2B604604BF3B44E1280F394754CD72086BA

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	47

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 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	55 Slices (2.16µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.04000 µm
Image Size (Pixels)	1230 X 1254
Image Size (Scaled)	43.41µm X 44.25µm
Bit Depth	16 Bit
Image Center Position	X: 66.04mm, Y: 48.77mm
Stage Position	X: 66.04mm, Y: 48.77mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 215µm | 5.00AU / 309µm
LMS MBS	Plate MBS 488/561 MBS 405/730
LMS SBS	SBS SP 505
Laser Wavelength	405nm @0.3% | 561nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	2.00
Pixel Time	0.93µs
Frame Time	11.04s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	401 | 590
Emission Wavelength	422 | 618
Effective NA	1.4
Detection Wavelength	350 - 499 | 527- 735
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.9 (3D, Auto) | Filter: 9.7 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 4% (405), Threshold 0% (594), Ramp0.0% (405), Ramp 0.0% (594), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Tone Mapping was NOT enabled.
Projection	View Angle 35°, Scale Z 257%, 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 Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000005

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000045

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 =55 Slices (2.16µm)

Channels = 2
Scaling (Per Pixel) = 35.29nm X 35.29nm X 40.00nm
Image Size (Pixels) = 1230 X 1254
Image Size (Scaled) = 43.41µm X 44.25µm
Bit Depth = 16 Bit
Image Center Position = X: 66.04mm, Y: 48.77mm
Stage Position =X: 66.04mm, Y: 48.77mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 215µm
LMS MBS = Plate MBS 488/561 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.93µs
Frame Time = 11.04s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 2
Excitation Wavelength = 401
Emission Wavelength = 422
Effective NA = 1.4
Detection Wavelength = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.9 (3D, Auto)

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 309µm
LMS MBS = Plate MBS 488/561 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 561nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.93µs
Frame Time = 11.04s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging - 2
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 527- 735
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 9.7 (3D, Auto)

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 4% (405), Threshold 0% (594), Ramp0.0% (405), Ramp 0.0% (594), Maximum 100% all channels
Background = Black
Light = Brightness 100%, Tone Mapping was NOT enabled.
Projection = View Angle 35°, Scale Z 257%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

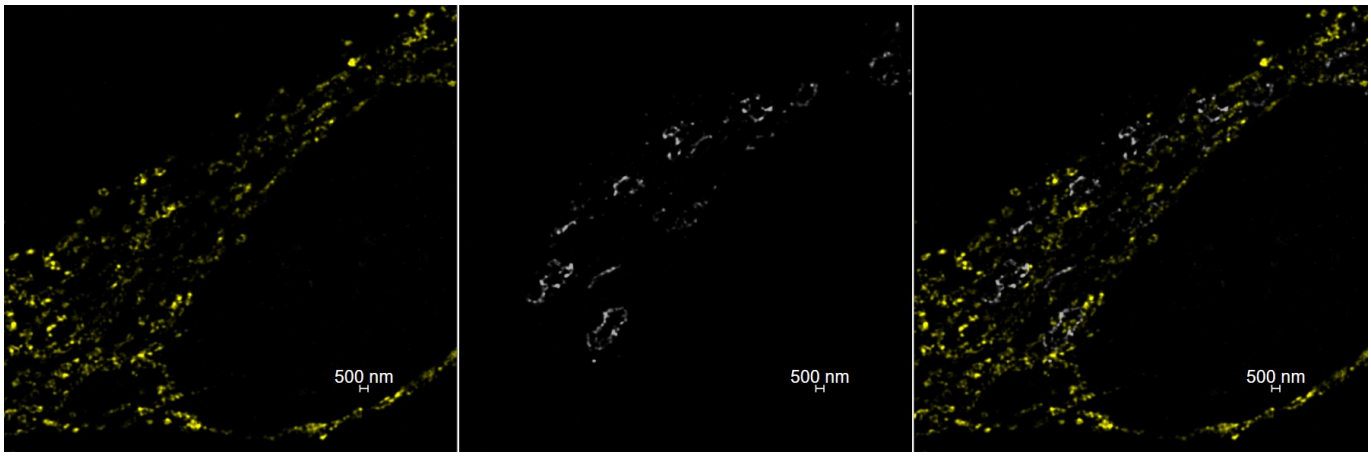
Identifyn® SRM Alexa Fluor™ 405 was pseudo-colored in Zen Software from blue, the default color, to light blue to provide visual contrast between the 405/594 channels.

Image by J. K. Bennett

SOURCE PROVENANCE

Catalog	SA-000005
Stage	Airyscan
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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D9B52B21EA3A1EFDD4A813AF4D0DEC3B6E7A34C1957A01E6C74EF03CE96EF24F
Method PDF SHA-256	CAF1B6E4B618BA187D659D150BE5FC3A6772A06ED8F05314207CEF24FBE3AC00

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	51 Slices (2.50µm)
Channels	2
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	2601 X 2596
Image Size (Scaled)	43.93µm X 43.85µm
Bit Depth	16 Bit
Image Center Position	X: 58.84mm, Y: 33.41mm
Stage Position	X: 58.84mm, Y: 33.41mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	405 | 488
Channel Name	T2-Axiocam 820#2-SR | T1-Axiocam 820 #2-SR
Excitation Wavelength	405 | 488
Emission Wavelength	445 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820#2
Camera Adapter	1X
Exposure Time	100ms | 120ms
Depth of Focus	0.69µm | 0.81µm
Binning Mode	2,2
Laser Wavelength	405nm @4.0% | 488nm @8.0%
Frame Time	676.00ms | 1.59s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	N/A
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.3046 (405), 9.5501 (532)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut

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 Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000005

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000023

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MTC02), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Golgi GOLPH2 Polyclonal Antibody, which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 51 Slices (2.50µm)

Channels = 2
Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm
Image Size (Pixels) = 2601 X 2596
Image Size (Scaled) = 43.93µm X 43.85µm
Bit Depth = 16 Bit
Image Center Position = X: 58.84mm, Y: 33.41mm
Stage Position = X: 58.84mm, Y: 33.41mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T2-Axiocam 820#2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @4.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 32.0µm grating

532 -
Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @8.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.3046 (405), 9.5501 (532)
Fitting = Fast Fit
Histogram = Scale to Min/Max
Baseline = Cut

Split View -

Left Panel panel shows Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific staining the mitochondria.

The Center Panel shows the Identifyn® SRM Alexa Fluor™ 405 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) staining the Golgi.

The right Panel is the merged image.

Image Corrections

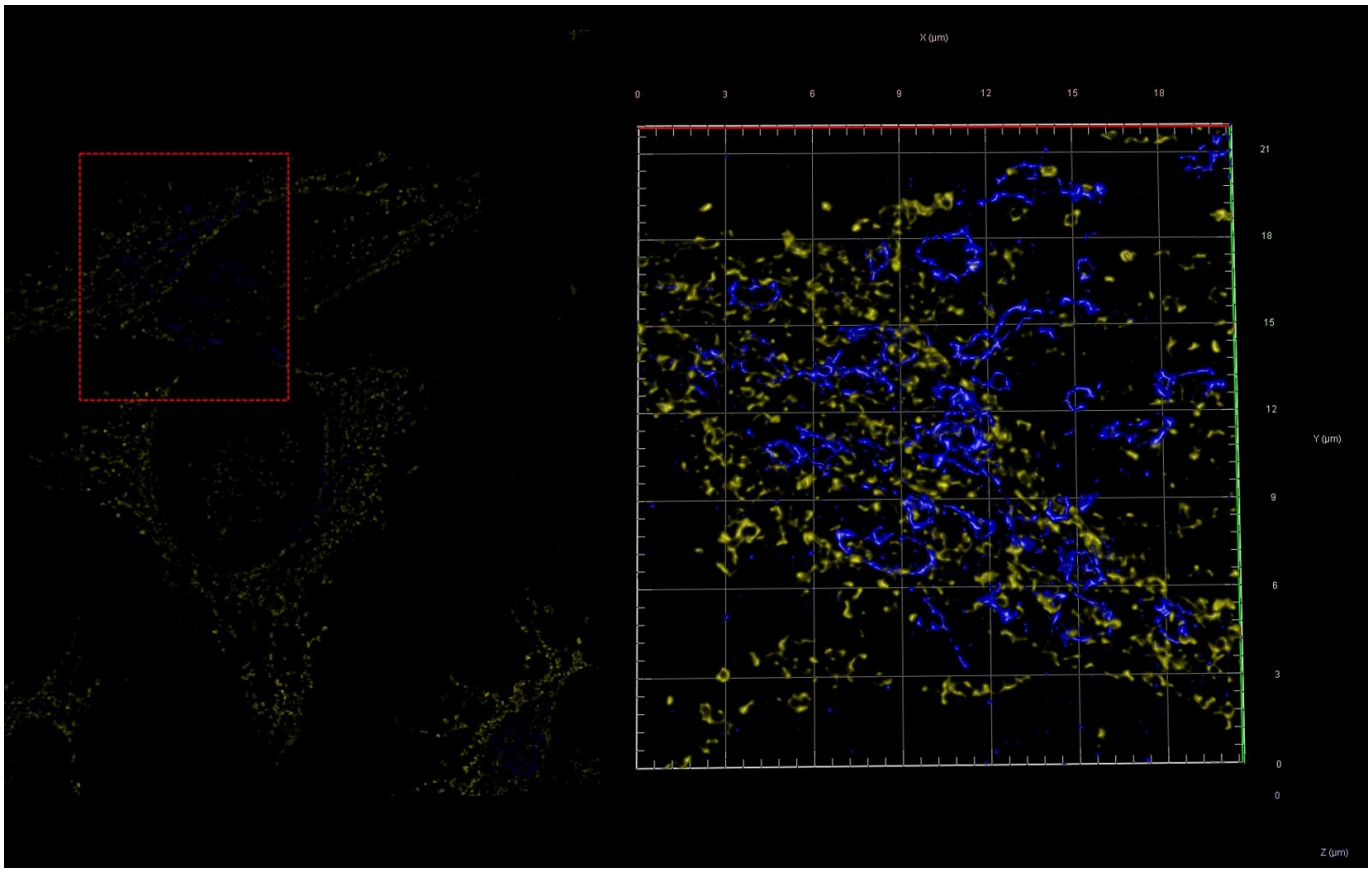
Identifyn® SRM Alexa Fluor™ 405 was represented as Light Grey instead of its default dark blue coloring to demonstrate contrast and show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 405/532 dye products.

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000005
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2EF1E129D2D54F35F1B3D5D4BFB4D8F7FAA6CDADD6C03BF3932DDBF1624F3F50
Method PDF SHA-256	6F3B16CE3B2FE729E47050B3D714C7BCF678FC8D80128F6C075226A813DBA960

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	53

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	48 Slices (2.35µm)
Channels	2
Scaling (Per Pixel)	0.01689 µm X 0.01689 µm X 0.05000 µm
Image Size (Pixels)	1215 X 1297
Image Size (Scaled)	20.52µm X 21.91µm
Bit Depth	16 Bit
Image Center Position	X: 59.69mm, Y: 32.68mm
Stage Position	X: 59.69mm, Y: 32.68mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	405 | 488
Channel Name	T2-Axiocam 820#2-SR | T1-Axiocam 820 #2-SR
Excitation Wavelength	405 | 488
Emission Wavelength	445 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820#2
Camera Adapter	1X
Exposure Time	100ms | 120ms
Depth of Focus	0.69µm | 0.81µm
Binning Mode	2,2
Laser Wavelength	488nm @4.0% | 488nm @8.0%
Frame Time	676.00ms | 1.59s
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4
Algorithm	SIM2

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.8839 (405), 8.8869 (532)
Order Combination	Weiner Filter
Regularization	None
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Histogram	Scale to Min/Max
3D Volume Projection	Precise
Appearance	Threshold 2% (405), Threshold 2% (532), Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness135%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 11%, 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 Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000005

Identifyn® SRM Alexa Fluor™ 532 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy Fragment Specific
Cat ID# - SA 000023

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MTC02), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 405 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Golgi GOLPH2 Polyclonal Antibody, which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 532 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D)

Z-Stack = 48 Slices (2.35µm)

Channels = 2
Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm
Image Size (Pixels) = 1215 X 1297
Image Size (Scaled) = 20.52µm X 21.91µm
Bit Depth = 16 Bit
Image Center Position = X: 59.69mm, Y: 32.68mm
Stage Position = X: 59.69mm, Y: 32.68mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

405 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T2-Axiocam 820#2-SR
Excitation Wavelength = 405
Emission Wavelength = 445
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 100ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 488nm @4.0%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 32.0µm grating

532 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T1-Axiocam 820 #2-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820#2
Camera Adapter = 1X
Exposure Time = 120ms
Depth of Focus = 0.81µm
Binning Mode = 2,2
Laser Wavelength = 488nm @8.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 8.8839 (405), 8.8869 (532)
Order Combination = Weiner Filter
Regularization = None
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Histogram = Scale to Min/Max

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 2% (405), Threshold 2% (532), Ramp 98% all channels, Maximum 100% all channels
Background = Black
Light = Brightness135%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping
Projection = View Angle 35°, Scale Z 11%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

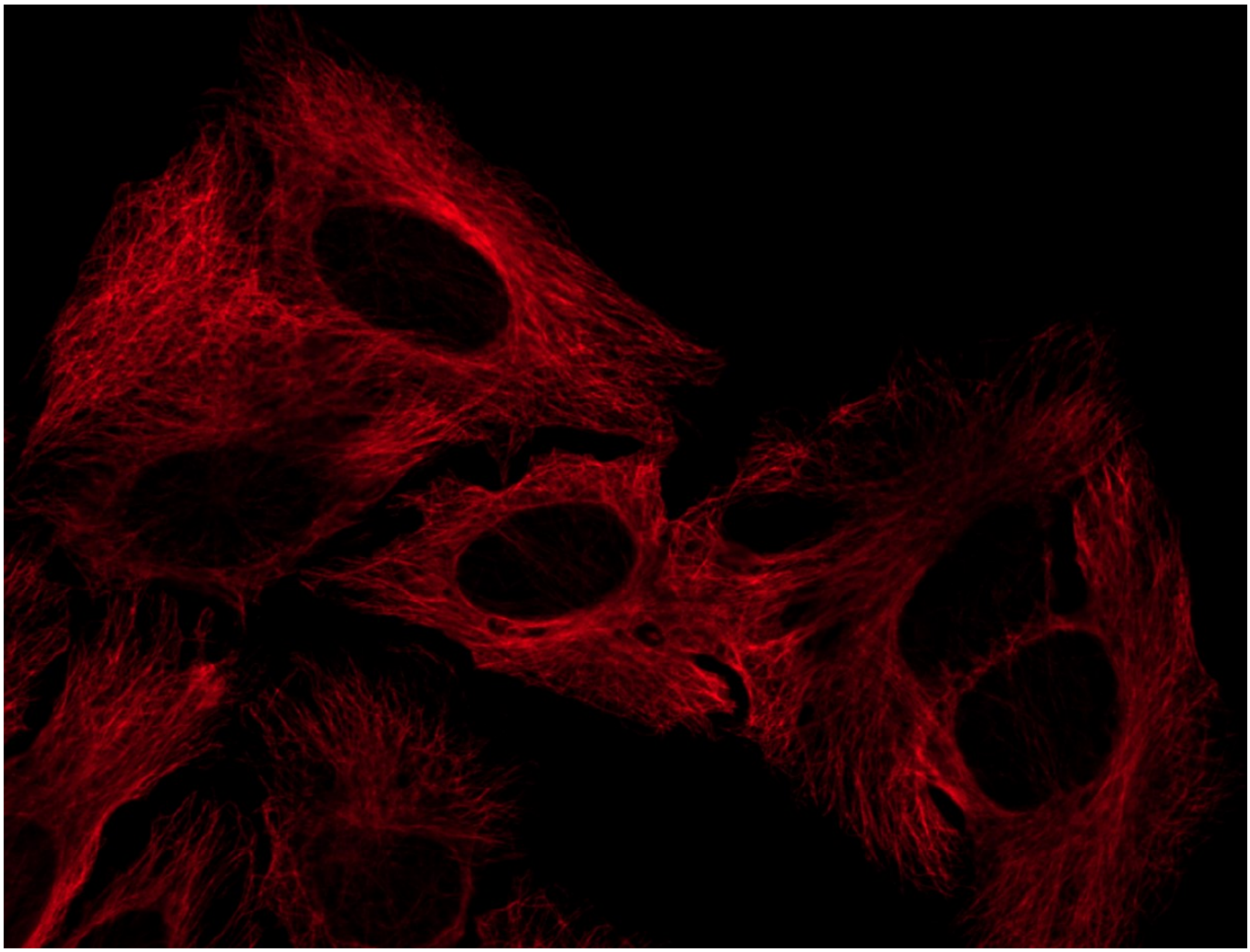
Left Panel 2 Dimensional view of the Region of Interest (ROI) Z-plane 25/48 used to create the 3-Dimensional Image, shown right.

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000005
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5D5DF75302856E7B602A641D567D9799502E88FF22270DD9BE46668B332AD5F5
Method PDF SHA-256	63B2395FE883295F05B2C91B8125136C8D740BAC3E243AB7C23833B8A6D9A6FD

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)	1337 X 1014
Image Size (Scaled)	191.00µm X 144.86µm
Bit Depth	14 Bit
Image Center Position	X: 98.52mm, Y: 54.24mm
ROI Center Offset	X: 98.52µm, Y: 54.24µm
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 Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000005

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) = 1337 X 1014
Image Size (Scaled) = 191.00µm X 144.86µm
Bit Depth = 14 Bit
Image Center Position = X: 98.52mm, Y: 54.24mm
ROI Center Offset = X: 98.52µm, Y: 54.24µm

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

SOURCE PROVENANCE

Catalog	SA-000005
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	40D02C696B47F3389D9A10ADE7CCD32DA2900F14ECCA816714BA53C667ECCC92
Method PDF SHA-256	535A608DB4875335C25DF10211C22DC4D7DEB1C42926205E255319787F8788AC