BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

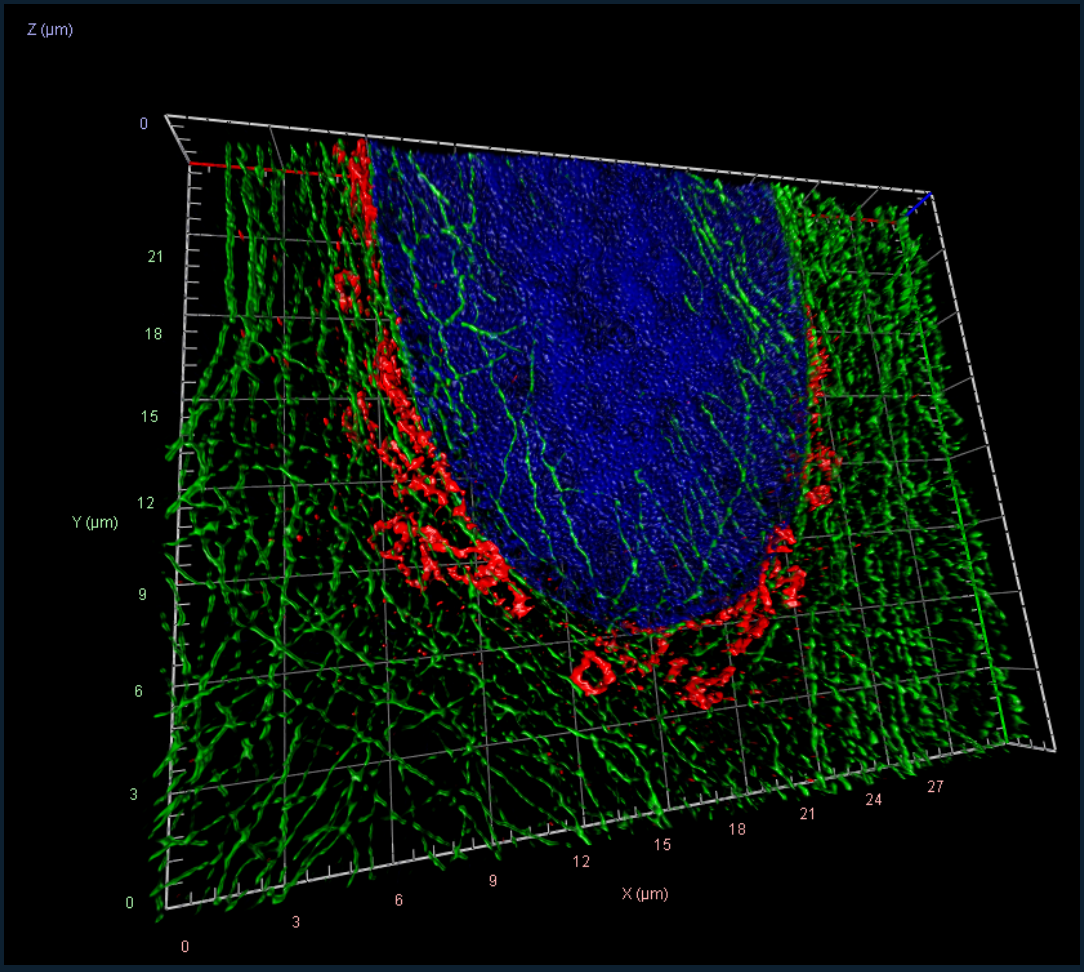
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit, F(ab')□ (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-000052 | 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™ 594 Affinity Purified Goat Anti-Rabbit, F(ab')□ (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-000052 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-000052 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	594	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 590
Widefield SIM — Apotome	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 590
Confocal	405/DAPI; 488; 594	2; None; 561nm @0.10%; 405nm @1.0%; 590; 353; 618; 465
Airyscan	405/DAPI; 488; 594	2; None; 561nm @0.5%; 405nm @1.20%; 590; 353; 618; 465
SIM	405/DAPI; 488; 594	3; 561nm @3.00%; 488nm @1.2%; 405nm @6.0%; 561; 488; 405; 515
SIM²	405/DAPI; 488; 594	3; 561nm @3.00%; 488nm @2.90%; 405nm @4.0%; 561; 488; 405; 515
Control	405/DAPI; 594	2; 45 Texas Red; 49 DAPI; 540 - 580; 335 - 383; 593 - 668; 420 - 470; 590

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 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

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 MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 687 X 642; Image Size (Pixels): 687 X 642; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms; 80ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @15%. 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; 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

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: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 346 X 339; Image Size (Pixels): 346 X 339; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 75ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%; X-Cite Xylis Lamp Intensity @16%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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 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: 35 Slices (1.65µm); Channels: 2; Scaling (Per Pixel): 0.071µm X 0.071µm X 0.050µm; Image size (Pixels): 447 X 531; Image Size (Pixels): 447 X 531; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsp-PMT 2; GaAsp-PMT 1; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.55µs; Frame Time: 9.48s. Illumination: Laser Wavelength: 561nm @0.10%; 405nm @1.0%. Optical/scan: Pinhole: 1.00AU / 54µm; 1.00 AU / 37µm; Scan Zoom: 1.5; LSM Scan Speed: 9; Scan Direction: Bidirectional; Averaging: 16; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 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 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 39 Slices (2.28µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 60.00nm; Image size (Pixels): 743 X 1201; Image Size (Pixels): 743 X 1201; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.59µs; Frame Time: 8.88s. Illumination: Laser Wavelength: 561nm @0.5%; 405nm @1.20%. Optical/scan: Pinhole: 5.00 AU / 272µm; 4.98 AU / 182µm; Scan Zoom: 1.6; 1.4; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; 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 9.1 (3D, Auto); Super Resolution 7.6 (3D, Auto). Structured source metadata values: 48.

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; 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 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: 46 Slices (2.25µm); Channels: 3; Scaling (Per Pixel): 31.30nm X 31.30nm X 50.00nm; Image size (Pixels): 1806 X 1522; Image Size (Pixels): 1806 X 1522; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 561nm @3.00%; 488nm @1.2%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 4; Effective NA: 1.46. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 76.

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; 594.

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: 45 Slices (2.2µm); Channels: 3; Scaling (Per Pixel): 15.65nm X 15.65nm X 50.00nm; Image size (Pixels): 1900 X 1519; Image Size (Pixels): 1900 X 1519; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 50ms; Frame Time: 1.0s. Illumination: Laser Wavelength: 561nm @3.00%; 488nm @2.90%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 4; Effective NA: 1.46. Processing: Projection: View Angle 80°, 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: 66.

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; 594.

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 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): 1074 X 951; Image Size (Pixels): 1074 X 951; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 594.

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-000052 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

SIM / Scaling (Per Pixel): 31.30nm X 31.30nm X 50.00nm -> 0.03130 µm X 0.03130 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)=1806 X 1522; Image Size (Scaled)=56.53µm X 47.64µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 15.65nm X 15.65nm X 50.00nm -> 0.01565 µm X 0.01565 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)=1900 X 1519; Image Size (Scaled)=29.74µm X 23.77µm; 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

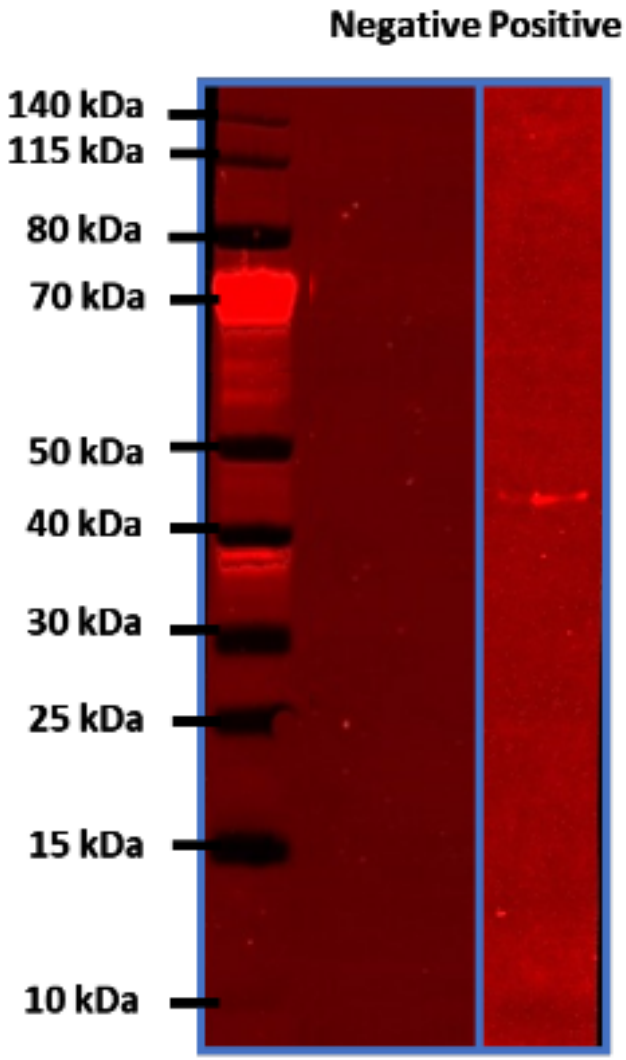
This dossier preserves the complete available evidence progression for SA-000052. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 900	PRESERVED
5	Airyscan	ZEISS LSM 900	PRESERVED
6	SIM	ZEISS Elyra 7	PRESERVED
7	SIM²	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	594
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	624±40nm
Detector	Avalanche Photodiode
Intensity	5
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Identifyn® LLC

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

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™ 594 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 = 532nm
Emission Filter = 624±40nm
Detector = Avalanche Photodiode
Intensity = 5
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

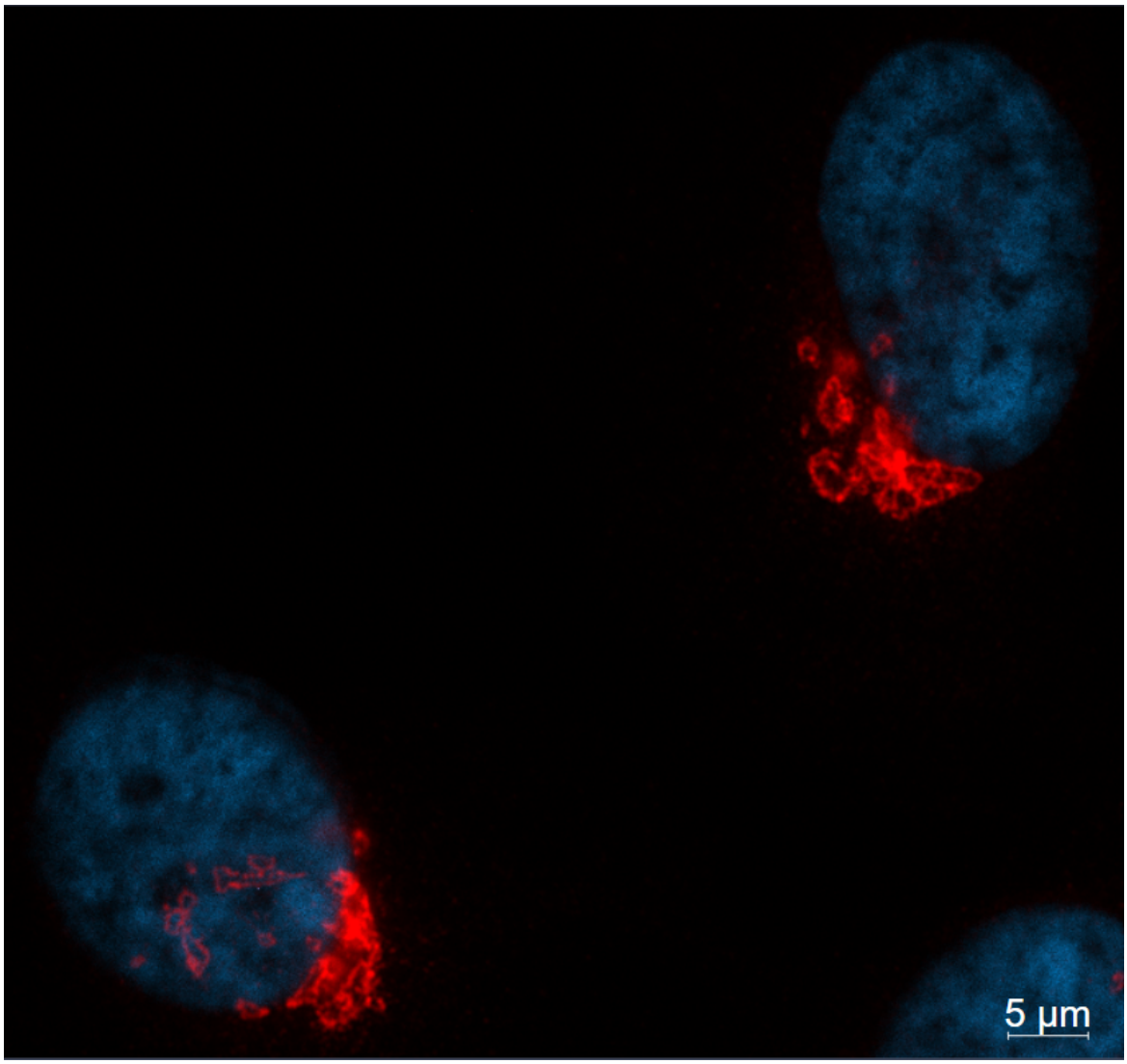
SOURCE PROVENANCE

Catalog	SA-000052
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	D2B117EFD323116956A09C1B3B8412A807B4C69F2D46C4B5863972844EFDD41D
Method PDF SHA-256	F5282213E4252BF82FC4A7A9B29B8C86C979F68A949AEF49463AE6E9FC9431D4

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
METADATA VALUES	30

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	2
Scaling (Per Pixel)	0.102µm X 0.102µm
Image size (Pixels)	687 X 642
Image Size (Scaled)	70.34µm X 65.73µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: -13.46µm, Y: -7.88µm
Reflector	45 Texas Red | 49 DAPI
Beam Splitter	585 | 395
Filter Excitation Wavelength	540 - 580 | 335 - 383
Filter Emission Wavelength	593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms | 80ms
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.96µm | 0.72µm
Binning Mode	1,1

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 with DAPI cat#P36962.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.102µm X 0.102µm
Image size (Pixels) = 687 X 642
Image Size (Scaled) = 70.34µm X 65.73µm
Bit Depth = 12 Bit
Image Center Position = X: 0.00µm, Y: 0.00µm
ROI Center Offset = X: -13.46µm, Y: -7.88µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 150ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.96µm
Binning Mode = 1,1

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 80ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 1,1

Post Processing

None

Image Correction

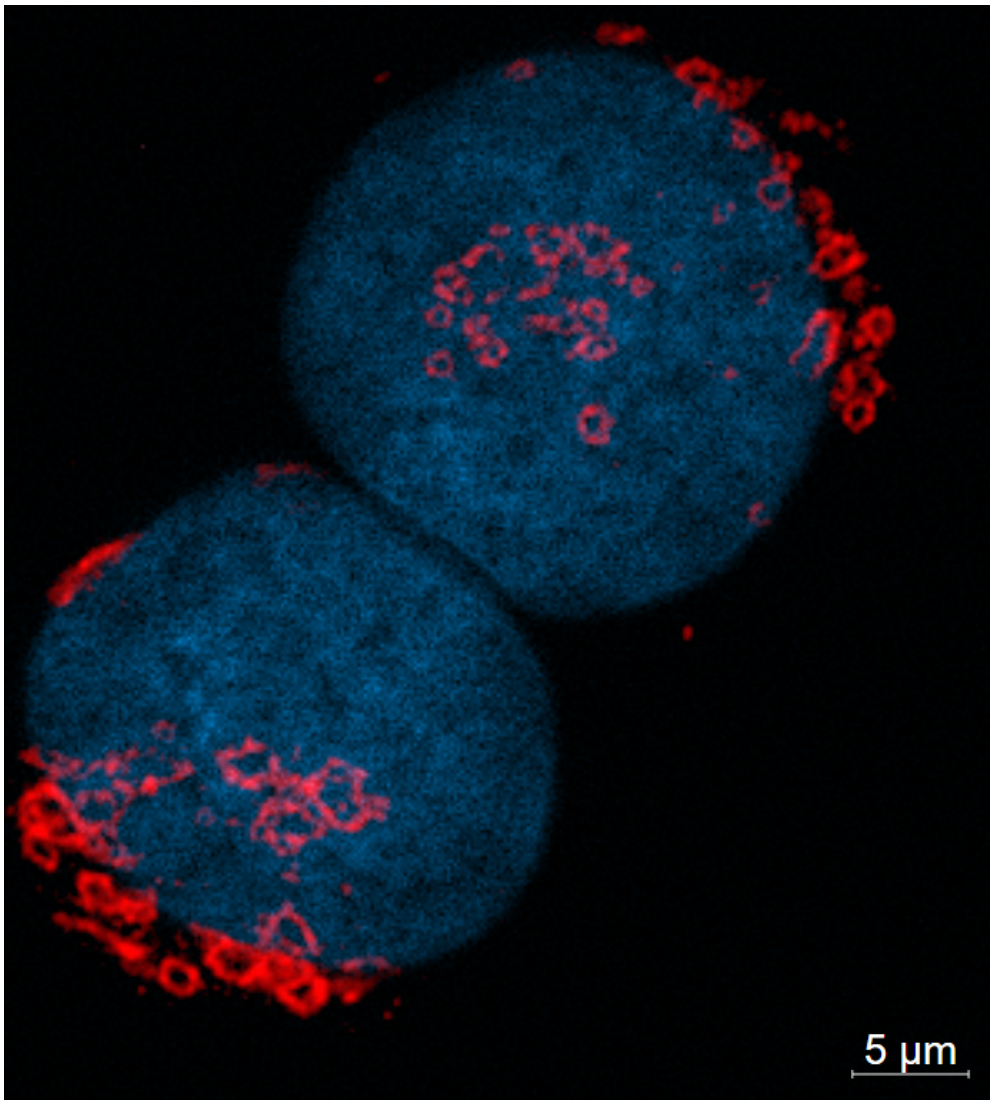
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	SA-000052
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2B212A90748E5FD0C537F70BD86E97653C70573FB3F125A3707528FC3C257EF9
Method PDF SHA-256	F317C3E8ECF1C6DEF140A8A7C262F4CFF5C3287A3E6D2A2F84AC97D7D8C182A1

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; 594
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	346 X 339
Image Size (Scaled)	49.43µm X 48.43µm
Bit Depth	14 Bit
Image Center Position	X: 52.63mm, Y:45.37mm
Stage Position	X: 52.63mm, Y:45.37mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	45 Texas Red | 49 DAPI
Beam Splitter	585 | 395
Filter Excitation Wavelength	540 - 580 | 335 - 383
Filter Emission Wavelength	593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15% | X-Cite Xylis Lamp Intensity @16%
Exposure Time	75ms | 30ms
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.20µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 346 X 339
Image Size (Scaled) = 49.43µm X 48.43µm
Bit Depth = 14 Bit
Image Center Position = X: 52.63mm, Y:45.37mm
Stage Position = X: 52.63mm, Y:45.37mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 75ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.20µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @16%
Exposure Time = 30ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

Image Corrections

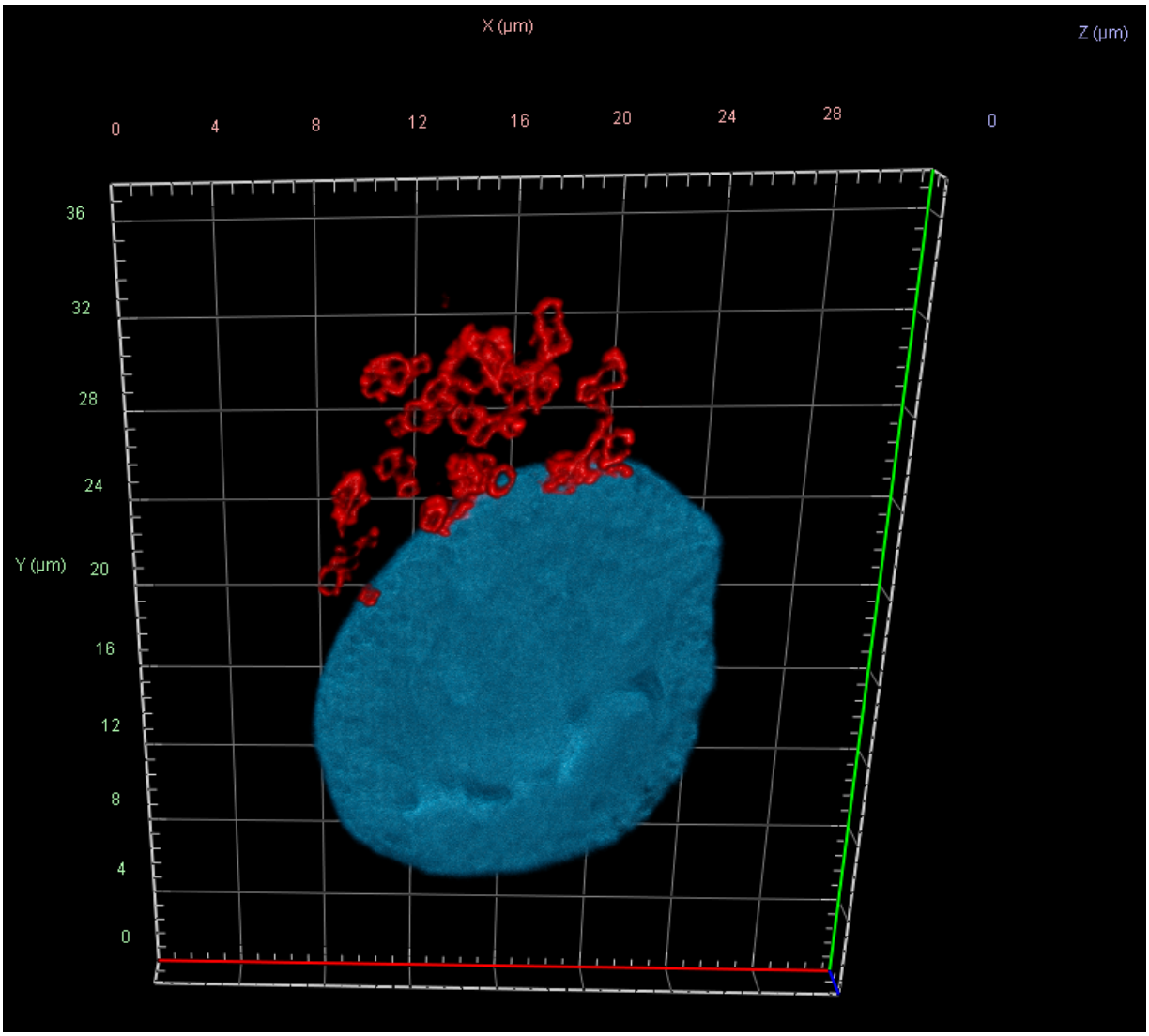
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	SA-000052
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	32
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	08A7F750CC552CC0D34DEF09C556DEA646936186D3EA4CF2C54664E392DA4AC9
Method PDF SHA-256	104A75D456589C73BA2AA1EC0CD38CD21BF968843DFF62F78E32AF6554B995D2

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	35 Slices (1.65µm)
Channels	2
Scaling (Per Pixel)	0.071µm X 0.071µm X 0.050µm
Image Size (Pixels)	447 X 531
Image Size (Scaled)	31.55µm X 37.47µm
Bit Depth	16 Bit
Image Center Position	X: 73.91mm, Y: 56.75mm
Stage Position	X: 73.91mm, Y: 56.75mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 54µm | 1.00 AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.10% | 405nm @1.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.5
Rotation	0°
Sampling	1.00
Pixel Time	0.55µs
Frame Time	9.48s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.4
Detection Wavelength	590 - 700 | 410-470
Imaging Device	GaAsp-PMT 2 | GaAsp-PMT 1
Detector Type	GaAsp-PMT
Detector Gain	650 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
Averaging	16
3D Volume Projection	Precise
Appearance	Threshold 8% (594), Threshold 5% (DAPI), Ramp92% (594), Ramp 095% (DAPI), Maximum 100% all channels
Background	Black
Light	Brightness 150%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack =35 Slices (1.65µm)

Channels = 2
Scaling (Per Pixel) = 0.071µm X 0.071µm X 0.050µm
Image Size (Pixels) = 447 X 531
Image Size (Scaled) = 31.55µm X 37.47µm
Bit Depth = 16 Bit
Image Center Position = X: 73.91mm, Y: 56.75mm
Stage Position = X: 73.91mm, Y: 56.75mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 54µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55µs
Frame Time = 9.48s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging - 16
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 590 - 700
Imaging Device = GaAsp-PMT 2
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.5
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.55µs
Frame Time = 9.48s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 16
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 410-470
Imaging Device = GaAsp-PMT 1
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 8% (594), Threshold 5% (DAPI), Ramp92% (594), Ramp 095% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness 150%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by P. Raut

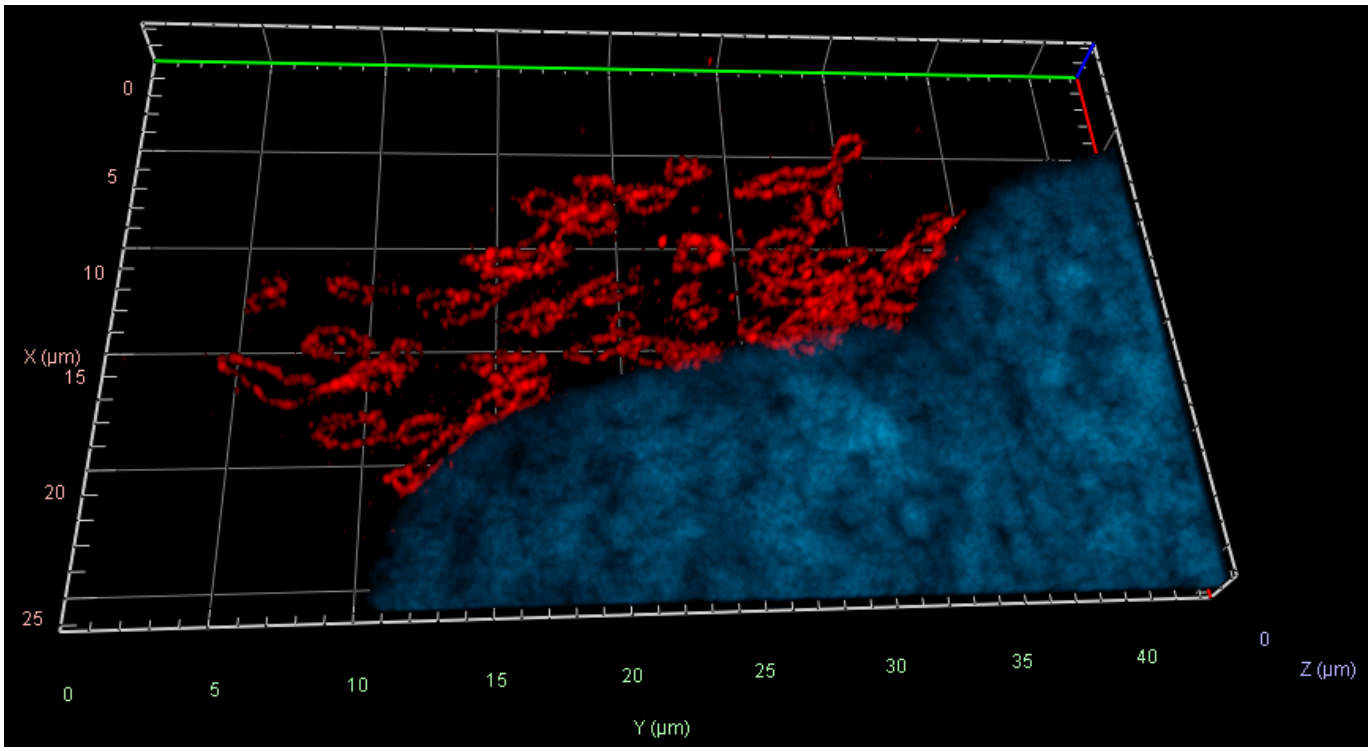
SOURCE PROVENANCE

Catalog	SA-000052

SOURCE PROVENANCE

Stage	Confocal
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8FEE89BFDC82952B0FBF8DC5185018D88D614308586AACE46C6E338025102F80
Method PDF SHA-256	0888046A89FD4ACC018BE94CA0F610CE07211479206B96FF2202C5A402FBA944

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	48

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	39 Slices (2.28µm)
Channels	2
Scaling (Per Pixel)	35.29nm X 35.29nm X 60.00nm
Image Size (Pixels)	743 X 1201
Image Size (Scaled)	26.22µm X 43.28µm
Bit Depth	16 Bit
Image Center Position	X: 66.12mm, Y: 47.52mm
Stage Position	X: 66.12mm, Y: 47.52mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 272µm | 4.98 AU / 182µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	561nm @0.5% | 405nm @1.20%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6 | 1.4
Rotation	0°
Sampling	2.00
Pixel Time	0.59µs
Frame Time	8.88s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.4
Detection Wavelength	598-700 | 400-464
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V | 850 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 9.1 (3D, Auto) | Super Resolution 7.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 5% (594), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 39 Slices (2.28µm)

Channels = 2
Scaling (Per Pixel) = 35.29nm X 35.29nm X 60.00nm
Image Size (Pixels) = 743 X 1201
Image Size (Scaled) = 26.22µm X 43.28µm
Bit Depth = 16 Bit
Image Center Position = X: 66.12mm, Y: 47.52mm
Stage Position = X: 66.12mm, Y: 47.52mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 272µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 561nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 8.88s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.4
Detection Wavelength = 598-700
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 9.1 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 4.98 AU / 182µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @1.20%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.4
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 8.88s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-464
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 7.6 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode “SR”
Super Resolution Auto Filter Selected
Process 3D Current Image
Created

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 5% (594), Threshold 2% (DAPI), Ramp 0% all channels, Maximum 100% all channels.
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

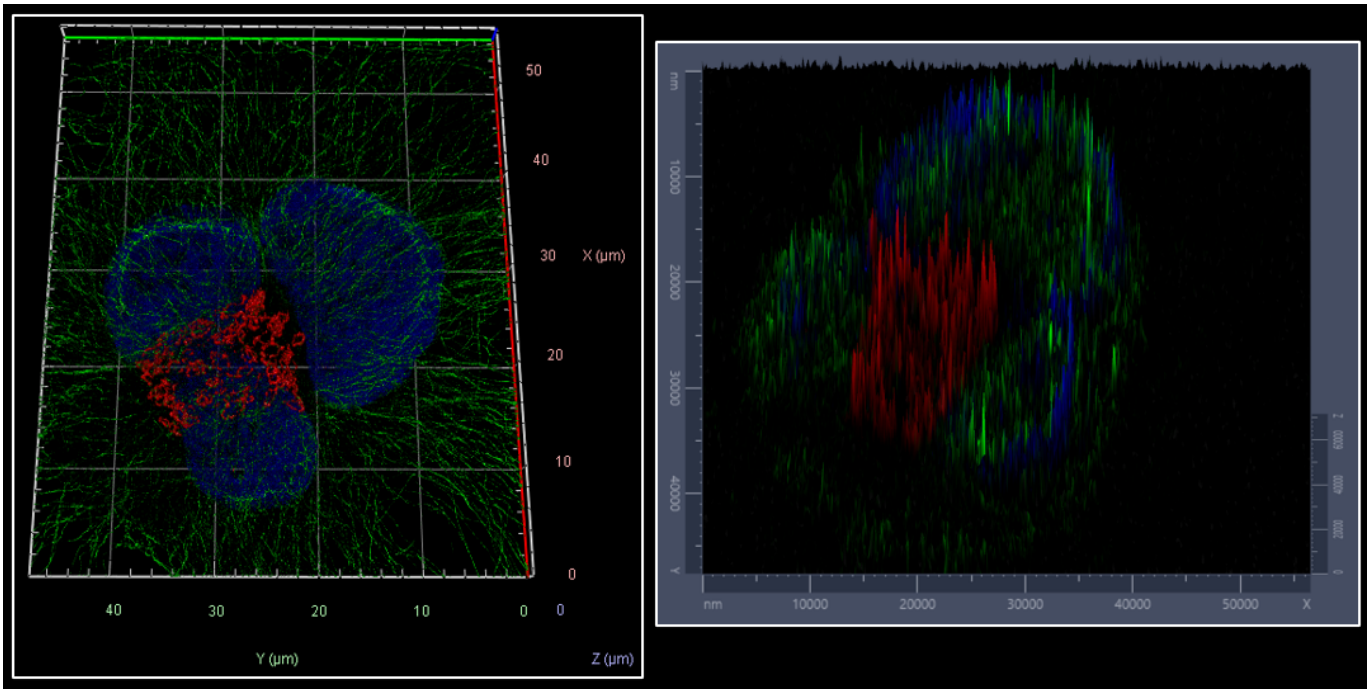
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000052
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	48
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	BE768597E40288B573F0EC2FD0ED144438DE47E0BCA6B621F494E7C2138EC6F9
Method PDF SHA-256	8C8F8720942F6E2B5A6952E1845F2965823CED70BA9BBDF89D7C5D525A96CE45

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	76

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 594	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 560 | SBS LP 640
Beam Splitter 488	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
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	46 Slices (2.25µm)
Channels	3
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.05000 µm
Image Size (Pixels)	1806 X 1522
Image Size (Scaled)	56.53µm X 47.64µm
Bit Depth	16 Bit
Image Center Position	X: 3.26mm, Y: -8.93mm
Stage Position	X: 3.26mm, Y: -8.93mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @3.00% | 488nm @1.2% | 405nm @6.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	4
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	515 | 655
Effective NA	1.46
Detection Wavelength	N/A
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms | 50ms | 55ms
Depth of Focus	0.73µm | 0.93µm

FIELD	SOURCE-DOCUMENTED VALUE
Binning Mode	1x1
Detector Type	Camera
Depth Offset	0
Detector Digital Gain	0.00
Channel	561.000000 (594) | 488.000000 | 405.000000
Grating Period (µm)	32.000000 | 27.500000 | 23.000000
Processing	3D
Auto Sharpness	Yes
Sharpness	10.202486 | 12.033171 | 11.580209
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Volume Projection	Precise
Appearance	Threshold 13% (594), Threshold 4% (488), Threshold 7% (DAPI), Ramp 87% (594), Ramp 96% (488), Ramp 93% (DAPI) Maximum 100% all channels
Background	Black
Light	Brightness100%, Azimuth 80°, elongation -130°, Enabled Directional Light and 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)
Render Mode	Surface
Grid distance	5
Angle X	19
Angle Y	-0
Z Scaling	1.6
Ambient	29
Specular	13
Shininess	22
Light Height	3.1
Partial Panorama	Start Angle 0.0º, Stop angle 80º
Number of Frames	8

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™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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

The second primary antibody that followed this, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 catalog number P36961.

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 594 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 560

Beam Splitter 488 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 640

Beam Splitter 405 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 640

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

Z Stack - (3D)

Z-Stack = 46 Slices (2.25µm)

Channels = 3
Scaling (Per Pixel) = 31.30nm X 31.30nm X 50.00nm
Image Size (Pixels) = 1806 X 1522
Image Size (Scaled) = 56.53µm X 47.64µm
Bit Depth = 16 Bit
Image Center Position = X: 3.26mm, Y: -8.93mm
Stage Position = X: 3.26mm, Y: -8.93mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Contrast Method = Fluorescence
Laser Wavelength = 561nm @3.00%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 561
Emission Wavelength = 515
Effective NA = 1.46
Detection Wavelength = N/A
Imaging Device = Edge 4.2 SIM
Exposure Time = 100ms
Depth of Focus = 0.73µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 561.000000 (594)
Grating Period (µm): 32.000000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 10.202486
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

488 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @1.2%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 488
Emission Wavelength = 655
Effective NA = 1.46
Detection Wavelength = N/A
Imaging Device = Edge 4.2 SIM
Exposure Time = 50ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 488.000000
Grating Period (µm): 27.500000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 12.033171
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
Laser Wavelength = 405nm @6.0%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 405
Emission Wavelength = 515
Effective NA = 1.46
Detection Wavelength = N/A
Imaging Device = Edge 4.2 SIM
Exposure Time = 55ms
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): 23.000000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 11.580209
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 13% (594), Threshold 4% (488), Threshold 7% (DAPI), Ramp 87% (594), Ramp 96% (488), Ramp 93% (DAPI) Maximum 100% all channels
Background = Black
Light = Brightness100%, Azimuth 80°, elongation -130°, Enabled Directional Light and 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)

2.5D Image Processing

Zeiss Zen Software converts a two-dimensional image into a height map called a 2.5D Image. The most significant extension in the Z-direction represents the intensity values, creating a pseudo-three-dimensional image representing fluorescent localization or colocalization of the collected signal and its intensity displayed in arbitrary units.

2.5D Display

Render Mode = Surface
Grid distance = 5
Pallete and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 19
Angle Y = -0
Z Scaling = 1.6
Ambient = 29
Specular = 13
Shininess = 22
Light Height = 3.1

2.5D Series

Partial Panorama = Start Angle 0.0º, Stop angle 80º
Number of Frames = 8

Image Corrections

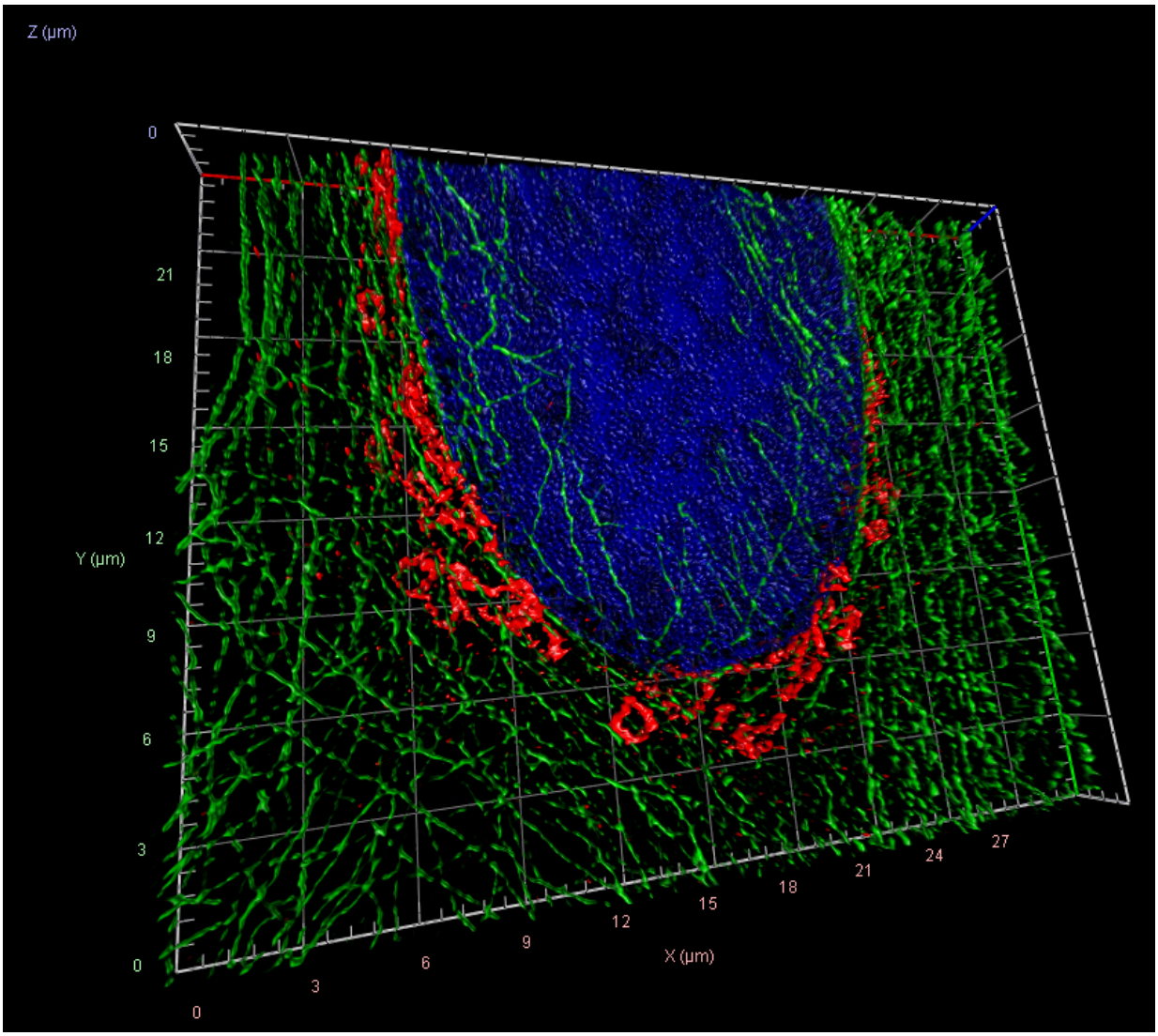
The 488 Channel was reduced by 10% using the Zen Software Histogram to provide enhanced visualization of the target 594 staining.

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000052
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	76
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6902B2A99ED1CD0D76BB70B22B98B1E90A501AEA9D05A065E3B30C9D3B71F7AA
Method PDF SHA-256	F33178EDEF6280945FCE44101E2CB56A7F0E83F599A644ECAF75878B8AC0046D

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	66

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 594	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter	SBS LP 640
Beam Splitter 488	Main Beam Splitter Non-Descanned: LBF 405/488/561/642
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	45 Slices (2.2µm)
Channels	3
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.05000 µm
Image Size (Pixels)	1900 X 1519
Image Size (Scaled)	29.74µm X 23.77µm
Bit Depth	16 Bit
Image Center Position	X: -153.80mm, Y: -8.30mm
Stage Position	X: -153.80mm, Y: -8.30mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	561nm @3.00% | 488nm @2.90% | 405nm @4.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	4
Excitation Wavelength	561 | 488 | 405
Emission Wavelength	515 | 655
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms | 50ms | 55ms
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	561.000000 (594) | 488.000000 | 405.000000
Grating Period (µm)	32.000000 | 27.500000 | 36.500000
Processing	3D
Input SNR	Medium
Iterations	16
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 Volume Projection	Precise
Appearance	Threshold 8% (594), Threshold 4% (488), Threshold 0% (DAPI), Ramp 92% (594), Ramp 96% (488), Ramp 98% (DAPI) Maximum 100% all channels
Background	Black
Light	Brightness147%, Azimuth 80°, elongation -130°, Enabled Directional Light and Tone Mapping
Projection	View Angle 80°, 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™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000019

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

The second primary antibody that followed this, Golgi GOLPH2 Polyclonal Antibody, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 catalog number P36961.

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 594 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 640

Beam Splitter 488 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 640

Beam Splitter 405 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642
Dual Camera Beam Splitter = SBS LP 640

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

Z Stack - (3D)

Z-Stack = 45 Slices (2.2µm)

Channels = 3
Scaling (Per Pixel) = 15.65nm X 15.65nm X 50.00nm
Image Size (Pixels) = 1900 X 1519
Image Size (Scaled) = 29.74µm X 23.77µm
Bit Depth = 16 Bit
Image Center Position = X: -153.80mm, Y: -8.30mm
Stage Position = X: -153.80mm, Y: -8.30mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

594 -

Contrast Method = Fluorescence
Laser Wavelength = 561nm @3.00%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 561
Emission Wavelength = 515
Effective NA = 1.46
Imaging Device = Edge 4.2 SIM
Exposure Time = 100ms
Depth of Focus = 0.73µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 561.000000 (594)
Grating Period (µm): 32.000000

SIM2

Processing: 3D
Input SNR: Medium
Iterations: 16
Regularization Weight: 0.0650000
Processing Sampling: 4
Output Sampling: 4
Filter: Median
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

488 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @2.90%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 488
Emission Wavelength = 655
Effective NA = 1.46
Imaging Device = Edge 4.2 SIM
Exposure Time = 50ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 488.000000
Grating Period (µm): 27.500000

SIM2

Processing: 3D
Input SNR: Medium
Iterations: 16
Regularization Weight: 0.0650000
Processing Sampling: 4
Output Sampling: 4
Filter: Median
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
Laser Wavelength = 405nm @4.0%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.0s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 4
Excitation Wavelength = 405
Emission Wavelength = 515
Effective NA = 1.46
Imaging Device = Edge 4.2 SIM
Exposure Time = 55ms
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: 16
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 Volume Projection = Precise
Appearance = Threshold 8% (594), Threshold 4% (488), Threshold 0% (DAPI), Ramp 92% (594), Ramp 96% (488), Ramp 98% (DAPI) Maximum 100% all channels
Background = Black
Light = Brightness147%, Azimuth 80°, elongation -130°, Enabled Directional Light and Tone Mapping
Projection = View Angle 80°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

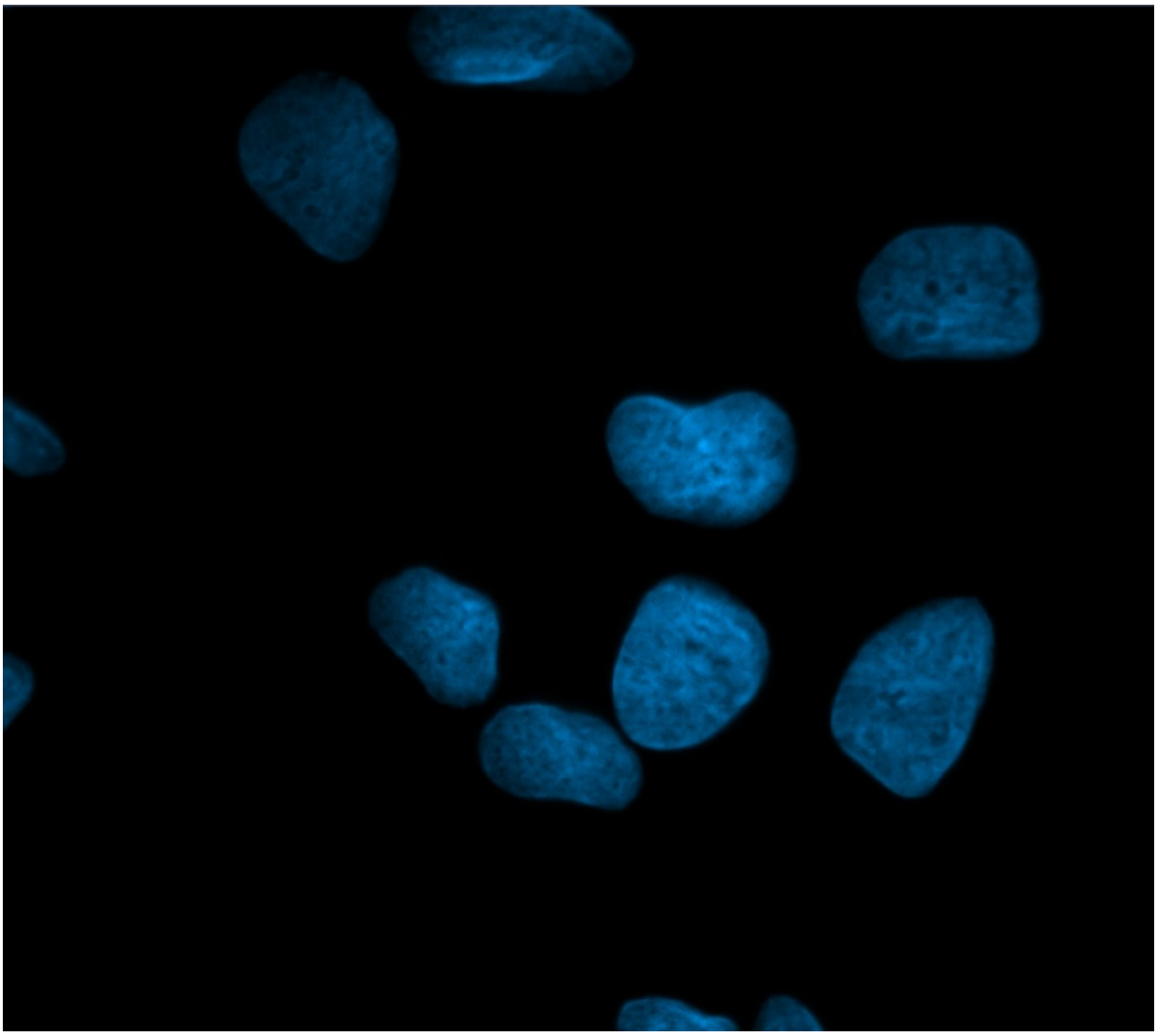
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000052
Stage	SIM²
Instrument	ZEISS Elyra 7
Microscope configuration	Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Structured source metadata values	66
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5FE1A930F2419B245F0E0E2A8084EE454A36340F57A895457A655EBFF568B0C3
Method PDF SHA-256	F43465EB4E6AE6A7E8C9E026C64900CECEC22EC12F3F7EC12981552EC19EFF04

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 594
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1074 X 951
Image Size (Scaled)	153.43µm X 135.86µm
Bit Depth	14 Bit
Image Center Position	X: 49.73mm, Y: 51.74mm
Stage Position	X: 49.73mm, Y: 51.74mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	45 Texas Red | 49 DAPI
Beam Splitter	585 | 395
Filter Excitation Wavelength	540 - 580 | 335 - 383
Filter Emission Wavelength	593 - 668 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms | 30ms
Excitation Wavelength	590 | 353
Emission Wavelength	618 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.20µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Rabbit F(ab')□ (H + L)
Cat ID# - SA 000052

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® 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 with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm
Image Size (Pixels) = 1074 X 951
Image Size (Scaled) = 153.43µm X 135.86µm
Bit Depth = 14 Bit
Image Center Position = X: 49.73mm, Y: 51.74mm
Stage Position = X: 49.73mm, Y: 51.74mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 200ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.20µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10%
Exposure Time = 30ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

Image Corrections

None

Control Summary -

In the absence of a primary antibody, Identifyn Standards for Optical Microscopy Methods™ were applied using standard staining methodology and image acquisition settings using the Zeiss Apotome SIM. With no image correction, the image demonstrates that the secondary antibody, minus a primary target, had no off-target binding and no significant presence of free dye within the sample.

The control data suggests the secondary antibody is highly specific and free of unconjugated (free) dye post-conjugation.

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000052
Stage	Control
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	32

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5CBBE1F838B4B759B279CC81E2B69F5F80B9D91BBDE74D303D56BCF2773CC9C0
Method PDF SHA-256	0F55DEA3BEFE1FBA6D55695E58DDC7C0DCAE99C85BEC602F7BF7B972476448F3