BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

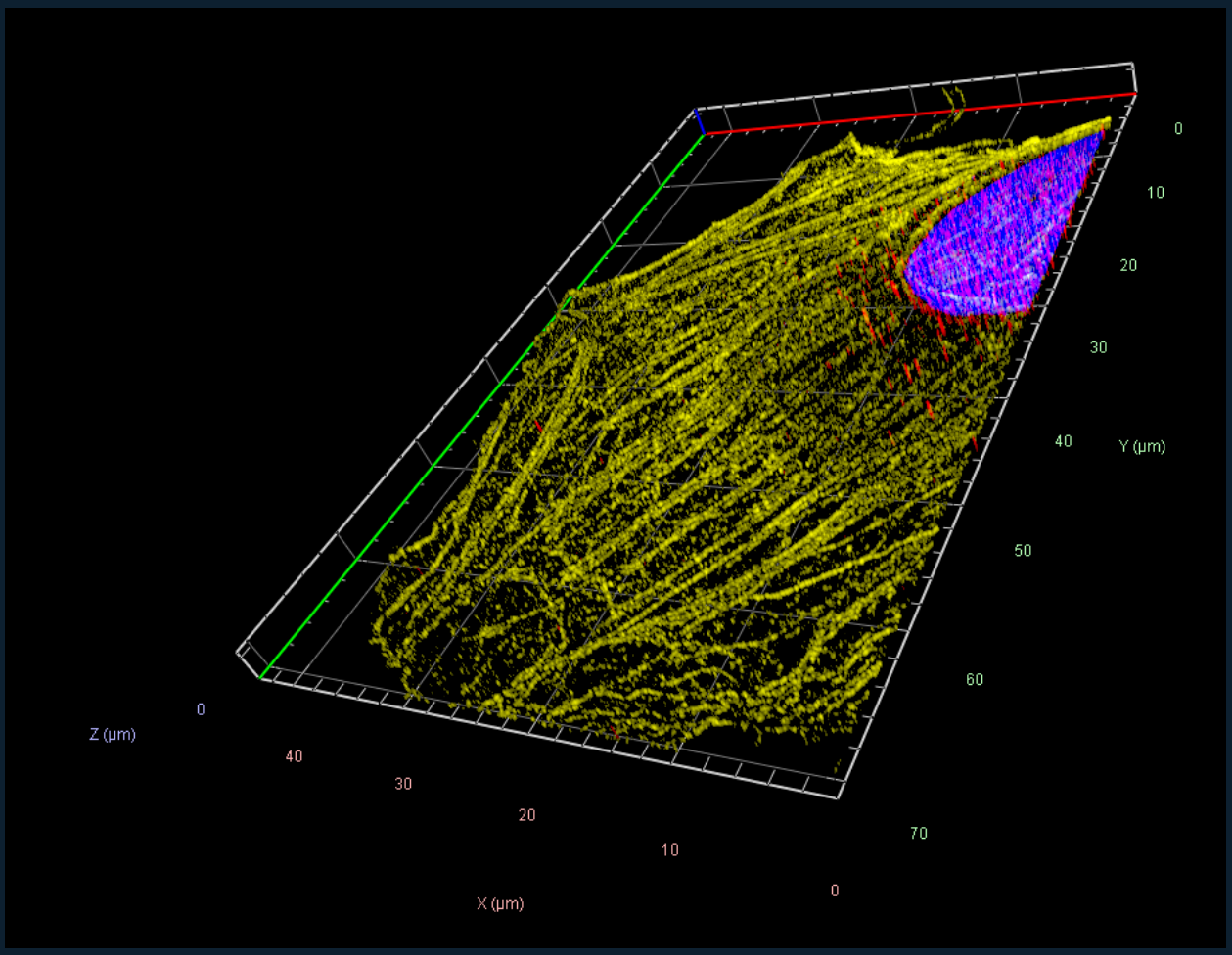
IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 532 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-000027 | 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™ 532 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-000027 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-000027 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	532	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 532; 555; 647	3; AF532-49014; 50 Cy5; 49 DAPI; 515-545; 625-655; 335-383; 555-595
Widefield SIM — Apotome	405/DAPI; 532; 555; 647	3; AF532-49014; 50 Cy5; 49 DAPI; 515 - 545; 625 - 655; 335-383; 555 - 595
Confocal	405/DAPI; 488; 532; 647; 750	3; None; 488nm @2.0%; 640nm @0.5%; 405nm @0.5%; 528; 653; 353
Airyscan	405/DAPI; 488; 532	2; None; 488nm @2.10%; 405nm @0.8%; 528; 353; 553; 465
SIM	405/DAPI; 488; 532; 647	3; 488nm @7.00%,; 642nm @2.00%,; 405nm @3.00%,; 488; 642; 405; 655
SIM²	405/DAPI; 488; 532; 647	3; 488nm @7.00%,; 642nm @2.00%,; 405nm @5.00%,; 488; 642; 405; 655
Control	405/DAPI; 532; 555	2; AF532-49014; 49 DAPI; 515 - 545; 335 - 383; 555 - 595; 420 - 470; 528

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 532.

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: 3; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 1388 X 1040; Image Size (Pixels): 1388 X 1040; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @50%; X-Cite Xylis Lamp Intensity @40%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 532; 555; 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

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: 52 Slices (15.3µm); Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.300µm; Image size (Pixels): 789 X 919; Image Size (Pixels): 789 X 919; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 50ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @60%. 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: 47.

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; 532; 555; 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 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: Channels: 3; Scaling (Per Pixel): 70.60nm X 70.60nm; Image size (Pixels): 2052 X 2052; Image Size (Pixels): 2052 X 2052; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsp-PMT; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.51µs; Frame Time: 40.59s. Illumination: Laser Wavelength: 488nm @2.0%; 640nm @0.5%. Optical/scan: Pinhole: 1.00AU / 45µm; 1.00AU / 57µm; Scan Zoom: 0.70; LSM Scan Speed: 7; Scan Direction: Bidirectional; Effective NA: 1.4. Structured source metadata values: 44.

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; 532; 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

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: 237 Slices (7.08µm); Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 30.00nm; Image size (Pixels): 2588 X 2588; Image Size (Pixels): 2588 X 2588; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.81µs; Frame Time: 12.92s. Illumination: Laser Wavelength: 488nm @2.10%; 405nm @0.8%. Optical/scan: Pinhole: 5.00 AU / 226µm; 5.00 AU / 181µm; Scan Zoom: 1.1; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.7 (3D, Auto); Super Resolution 8.4 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 532.

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: Channels: 3; Scaling (Per Pixel): 31.30nm X 31.30nm X 80.00nm; Image size (Pixels): 2560 X 2560; Image Size (Pixels): 2560 X 2560; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 40ms; Frame Time: 1.00s. Illumination: Laser Wavelength: 488nm @7.00%,; 642nm @2.00%,. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 16; 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: 63.

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; 532; 647.

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: Channels: 3; Scaling (Per Pixel): 15.65nm X 15.65nm X 80.00nm; Image size (Pixels): 2768 X 4539; Image Size (Pixels): 2768 X 4539; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 100ms; 40ms; Frame Time: 6.26s. Illumination: Laser Wavelength: 488nm @7.00%,; 642nm @2.00%,. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 16; Effective NA: 1.46. Processing: Projection: View Angle 45°, Scale Z 112%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 65.

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; 532; 647.

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): 1021 X 1037; Image Size (Pixels): 1021 X 1037; 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; 532; 555.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000027 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

Confocal / Scaling (Per Pixel): 70.60nm X 70.60nm -> 0.07060 µm X 0.07060 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)=2052 X 2052; Image Size (Scaled)=144.88µm X 144.88µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30nm X 35.30nm X 30.00nm -> 0.03530 µm X 0.03530 µm X 0.03000 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)=2588 X 2588; Image Size (Scaled)=91.35µm X 91.35µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 31.30nm X 31.30nm X 80.00nm -> 0.03130 µm X 0.03130 µm X 0.08000 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)=2560 X 2560; Image Size (Scaled)=80.14µm X 80.14µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 15.65nm X 15.65nm X 80.00nm -> 0.01565 µm X 0.01565 µm X 0.08000 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)=2768 X 4539; Image Size (Scaled)=43.32µm X 71.04µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

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

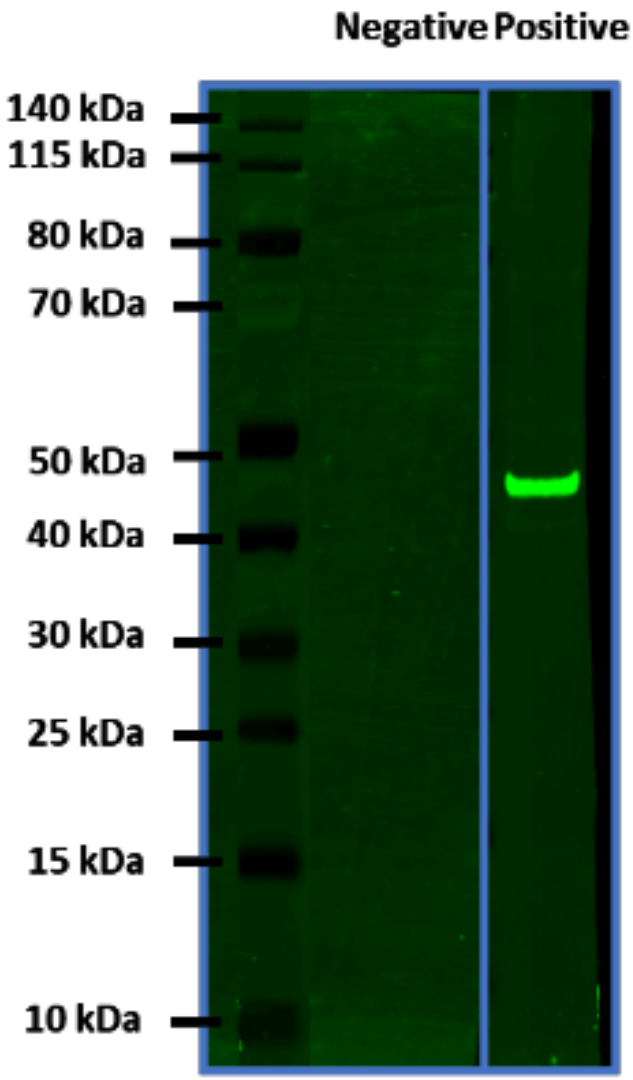
This dossier preserves the complete available evidence progression for SA-000027. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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	532
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	532nm
Emission Filter	572±28nm
Detector	Avalanche Photodiode
Intensity	2
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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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 Rabbit Recombinant Monoclonal antibody Actin Recombinant Monoclonal Antibody (JF47-01) (MA5-32530) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 532 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 = 532nm
Emission Filter = 572±28nm
Detector = Avalanche Photodiode
Intensity = 2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

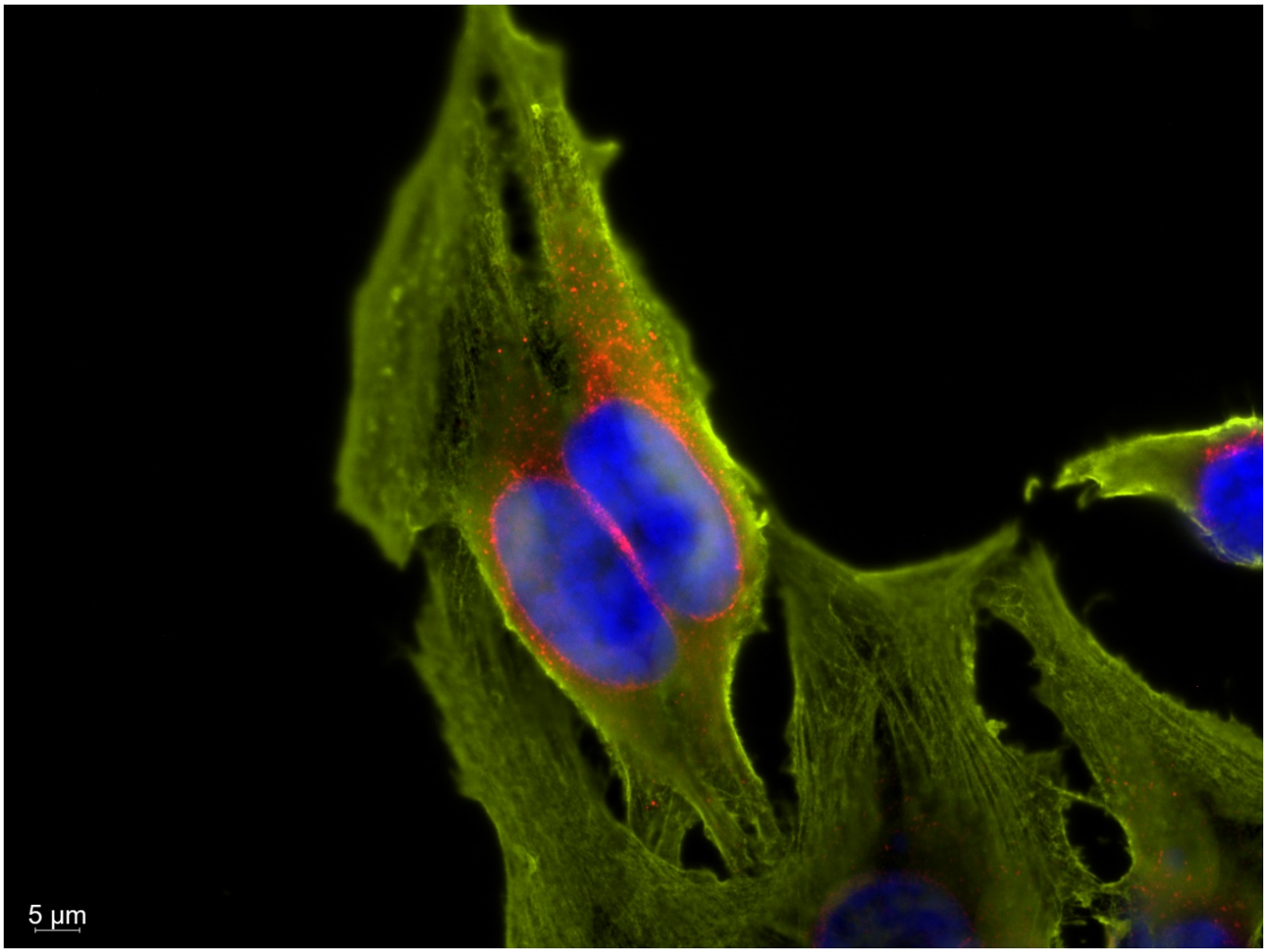
SOURCE PROVENANCE

Catalog	SA-000027
Stage	Western blot

SOURCE PROVENANCE

Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	1F35E8A90B0123812394FD5C89794495132FEA85734513B4D40DB7E43E91DE6D
Method PDF SHA-256	F58EDD2BE81B7787A1211E88BF9D2C8FDCB390361194424DB412D6BDE75EC829

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555; 647
METADATA VALUES	39

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 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	3
Scaling (Per Pixel)	0.102µm X 0.102µm
Image size (Pixels)	1388 X 1040
Image Size (Scaled)	142.10µm X 106.48µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	AF532-49014 | 50 Cy5 | 49 DAPI
Beam Splitter	550 | 660 | 395
Filter Excitation Wavelength	515-545 | 625-655 | 335-383
Filter Emission Wavelength	555-595 | 665-715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @50% | X-Cite Xylis Lamp Intensity @40% | X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms | 200ms | 80ms
Excitation Wavelength	528 | 653 | 353
Emission Wavelength	553 | 668 | 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.86µm | 1.03µm | 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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 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 = 3
Scaling (Per Pixel) = 0.102µm X 0.102µm
Image size (Pixels) = 1388 X 1040
Image Size (Scaled) = 142.10µm X 106.48µm
Bit Depth = 12 Bit
Image Center Position = X: 0.00µm, Y: 0.00µm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength =515-545
Filter Emission Wavelength = 555-595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @50%
Exposure Time = 150ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 0.86µm
Binning Mode = 1,1

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 @40%
Exposure Time = 200ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam MR R3
Camera Adapter = 1X
Depth of Focus = 1.03µm
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

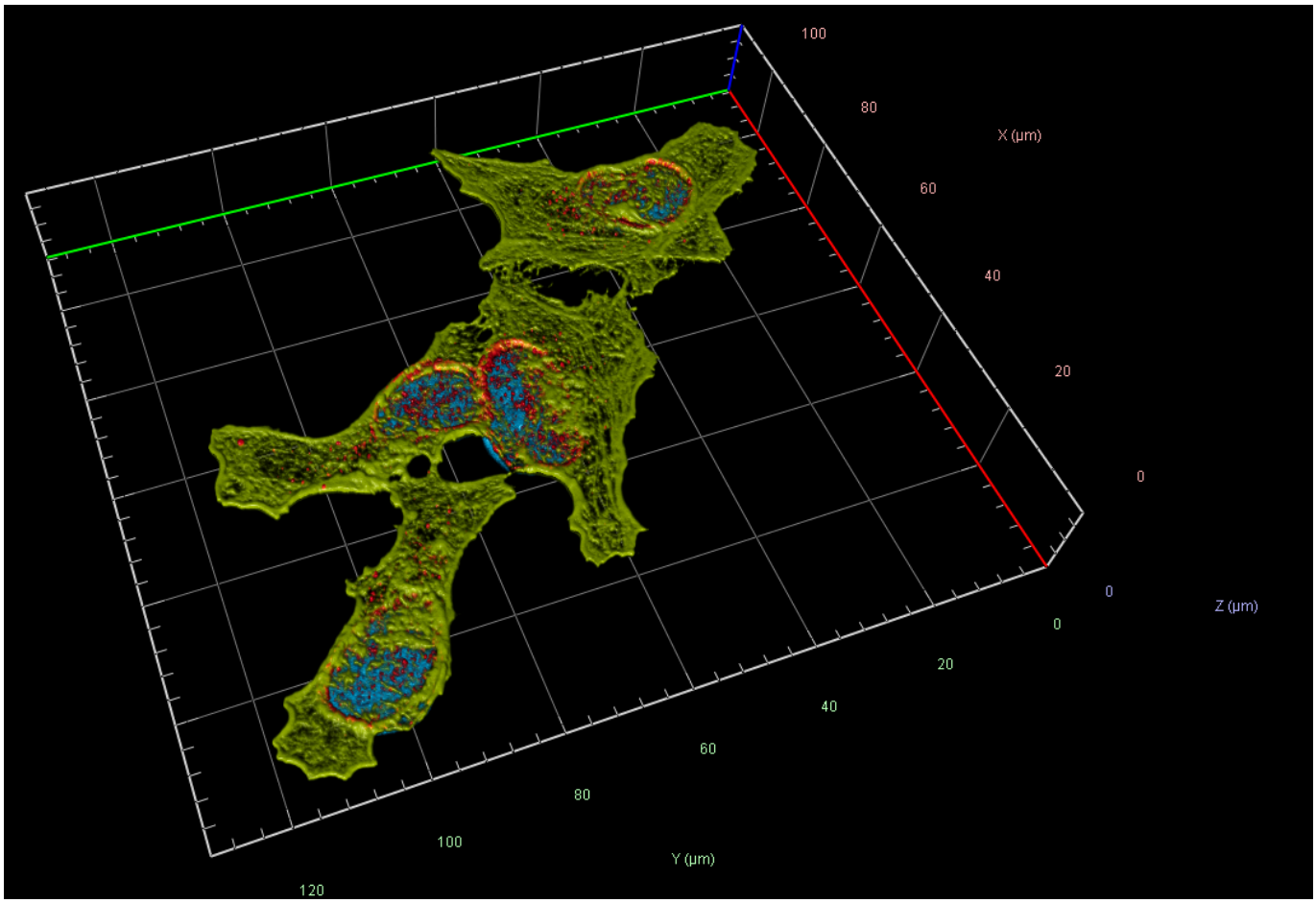
None

Image by E.F. Simon

SOURCE PROVENANCE

Catalog	SA-000027
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	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	2C4A90523570E8643C88646928D5B7E8D0C5889C92F87EB8845325417814DAC6
Method PDF SHA-256	30E8F45A7D12632B05587D35894D67A638FFDDF9C3318AC2CFCCE241D8B112F5

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; 532; 555; 647
METADATA VALUES	47

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	52 Slices (15.3µm)
Channels	3
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.300µm
Image Size (Pixels)	789 X 919
Image Size (Scaled)	112.71µm X 131.29µm
Bit Depth	14 Bit
Image Center Position	X: 90.80mm, Y: 48.35mm
Stage Position	X: 90.80mm, Y: 48.35mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	AF532-49014 | 50 Cy5 | 49 DAPI
Beam Splitter	550 | 660 | 395
Filter Excitation Wavelength	515 - 545 | 625 - 655 | 335-383
Filter Emission Wavelength	555 - 595 | 665 - 715 | 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @60% | X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	50ms | 200ms | 20ms
Excitation Wavelength	528 | 653 | 353
Emission Wavelength	553 | 668 | 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.07µm | 1.30µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode “Optical Sectioning” - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold11% (647), Threshold 11% (532), Threshold 3% (DAPI), Ramp 50% (647), Ramp 50% (532), Ramp 50% (DAPI) Maximum 100% all channels
Background	Black
Light	Brightness 100%, 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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 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 = 52 Slices (15.3µm)

Channels = 3
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.300µm
Image Size (Pixels) = 789 X 919
Image Size (Scaled) = 112.71µm X 131.29µm
Bit Depth = 14 Bit
Image Center Position = X: 90.80mm, Y: 48.35mm
Stage Position = X: 90.80mm, Y: 48.35mm
ROI Center Offset = X: 1.14, Y: 0.00µm

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 50ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.07µm
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 @60%
Exposure Time = 200ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 1.30µm
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 @8.0%
Exposure Time = 20ms
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 Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold11% (647), Threshold 11% (532), Threshold 3% (DAPI), Ramp 50% (647), Ramp 50% (532), Ramp 50% (DAPI) Maximum 100% all channels
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

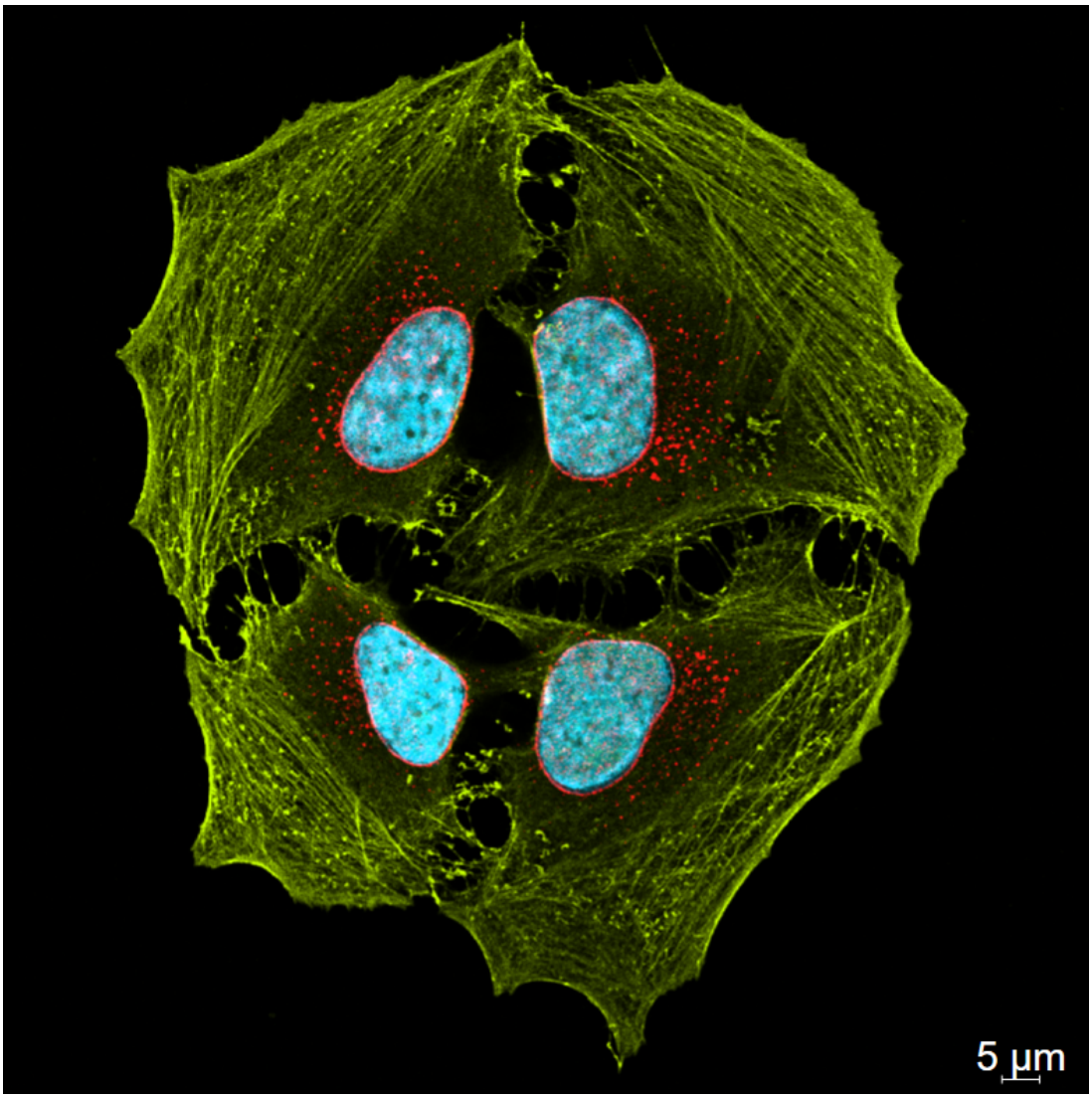
None

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000027
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	47
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	228627838F3C659CF907F7AC56BEFD36F30B531236176BB3EEFF1D5BA646505A
Method PDF SHA-256	D54B86A0067B107268CBF4CC0958B98B942FD04F675A982EB1CB9D1B8974C6C9

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647; 750
METADATA VALUES	44

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
Channels	3
Scaling (Per Pixel)	0.07060 µm X 0.07060 µm
Image Size (Pixels)	2052 X 2052
Image Size (Scaled)	144.88µm X 144.88µm
Bit Depth	16 Bit
Image Center Position	X: 72.04mm, Y: 50.83mm
Stage Position	X: 72.04mm, Y: 50.83mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 45µm | 1.00AU / 57µm | 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @2.0% | 640nm @0.5% | 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	0.70
Rotation	0°
Sampling	1.00
Pixel Time	0.51µs
Frame Time	40.59s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	528 | 653 | 353
Emission Wavelength	553 | 668 | 465
Effective NA	1.4
Detection Wavelength	500 - 577 | 656 - 700 | 410 - 470
Imaging Device	Airyscan | GaAsp-PMT
Detector Type	GaAsp-PMT
Detector Gain	750 V | 650 V
Detector Offset	0

FIELD	SOURCE-DOCUMENTED VALUE
Detector Digital Gain	1.0

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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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 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:

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 70.60nm X 70.60nm
Image Size (Pixels) = 2052 X 2052
Image Size (Scaled) = 144.88µm X 144.88µm
Bit Depth = 16 Bit
Image Center Position = X: 72.04mm, Y: 50.83mm
Stage Position = X: 72.04mm, Y: 50.83mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 45µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @2.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.70
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 40.59s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 16
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 500 - 577
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 57µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 640nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.70
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 40.59s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging - 16
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 656 - 700
Imaging Device = GaAsp-PMT
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 0.70
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.51µs
Frame Time = 40.59s
LSM Scan Speed = 7
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
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

Post Processing

None

Image Corrections

None

Image by J.K. Bennett

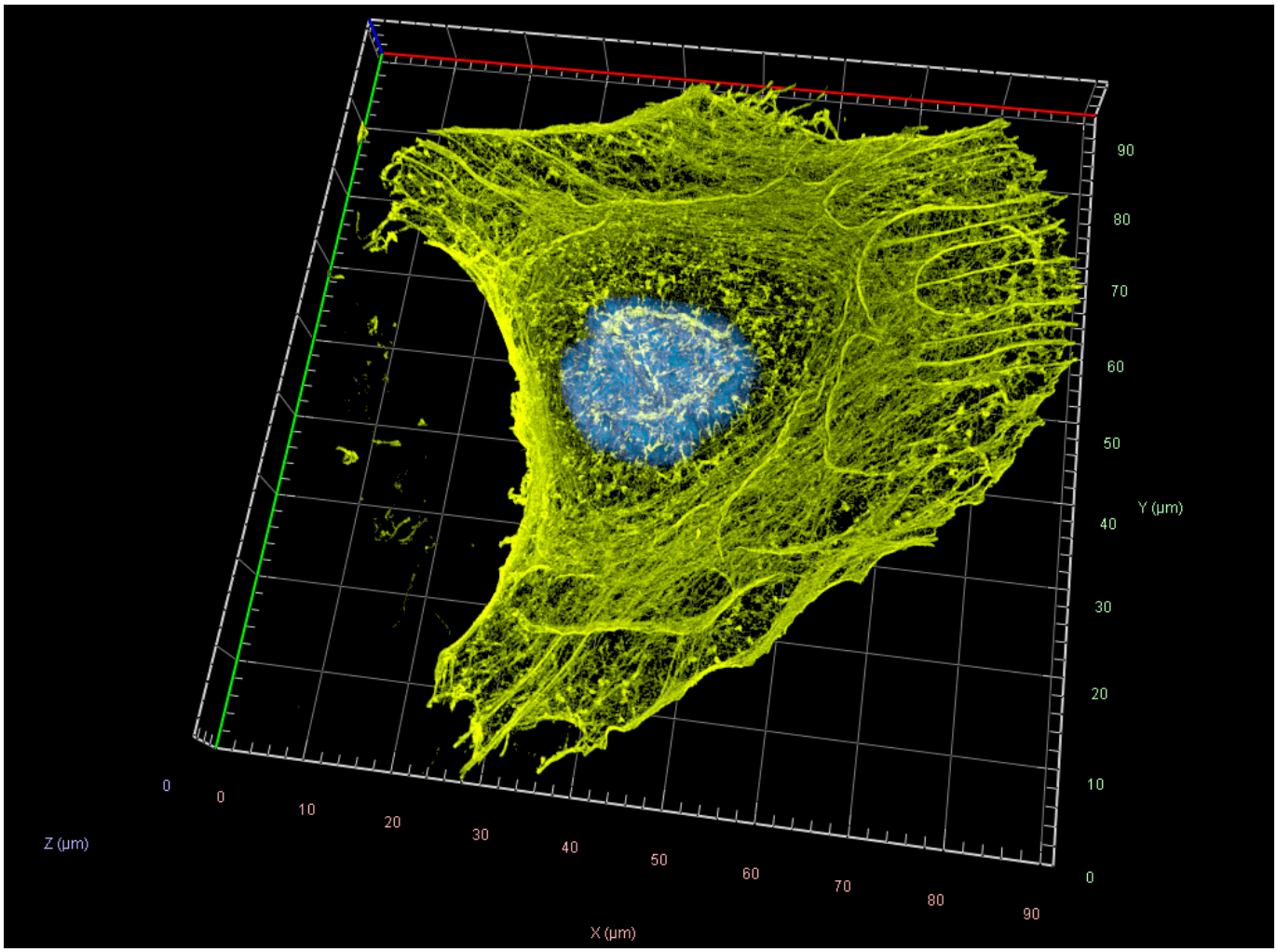
SOURCE PROVENANCE

Catalog	SA-000027
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:

SOURCE PROVENANCE

Structured source metadata values	44
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	FA67B970A357DFF3A78C574C2ED941B0AB4F7F7967061A45FE86EB21BCA4E9AB
Method PDF SHA-256	FD96F3F2CF70817BA681317A67AE78D5CC3C57486C3D7D672D42976E7C4098CD

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HCT116
DETECTED DYES	405/DAPI; 488; 532
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 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	237 Slices (7.08µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	2588 X 2588
Image Size (Scaled)	91.35µm X 91.35µm
Bit Depth	16 Bit
Image Center Position	X: 74.04mm, Y: 51.81mm
Stage Position	X: 74.04mm, Y: 51.81mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 226µm | 5.00 AU / 181µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @2.10% | 405nm @0.8%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.1
Rotation	0°
Sampling	2.00
Pixel Time	0.81µs
Frame Time	12.92s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	528 | 353
Emission Wavelength	553 | 465
Effective NA	1.4
Detection Wavelength	505-578 | 400-462
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.7 (3D, Auto) | Super Resolution 8.4 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 7% (532), Threshold 9% (DAPI), Ramp 0% (532), Ramp 0% (DAPI) Maximum 100% all channels
Background	Black
Light	Brightness 100%
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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

HCT116 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, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 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 = 237 Slices (7.08µm)

Channels = 2
Scaling (Per Pixel) = 35.30nm X 35.30nm X 30.00nm
Image Size (Pixels) = 2588 X 2588
Image Size (Scaled) = 91.35µm X 91.35µm
Bit Depth = 16 Bit
Image Center Position = X: 74.04mm, Y: 51.81mm
Stage Position = X: 74.04mm, Y: 51.81mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 226µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @2.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.81µs
Frame Time = 12.92s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.4
Detection Wavelength = 505-578
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 181µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.8%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.81µs
Frame Time = 12.92s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-462
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.4 (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 7% (532), Threshold 9% (DAPI), Ramp 0% (532), Ramp 0% (DAPI) Maximum 100% all channels
Background = Black
Light = Brightness 100%
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

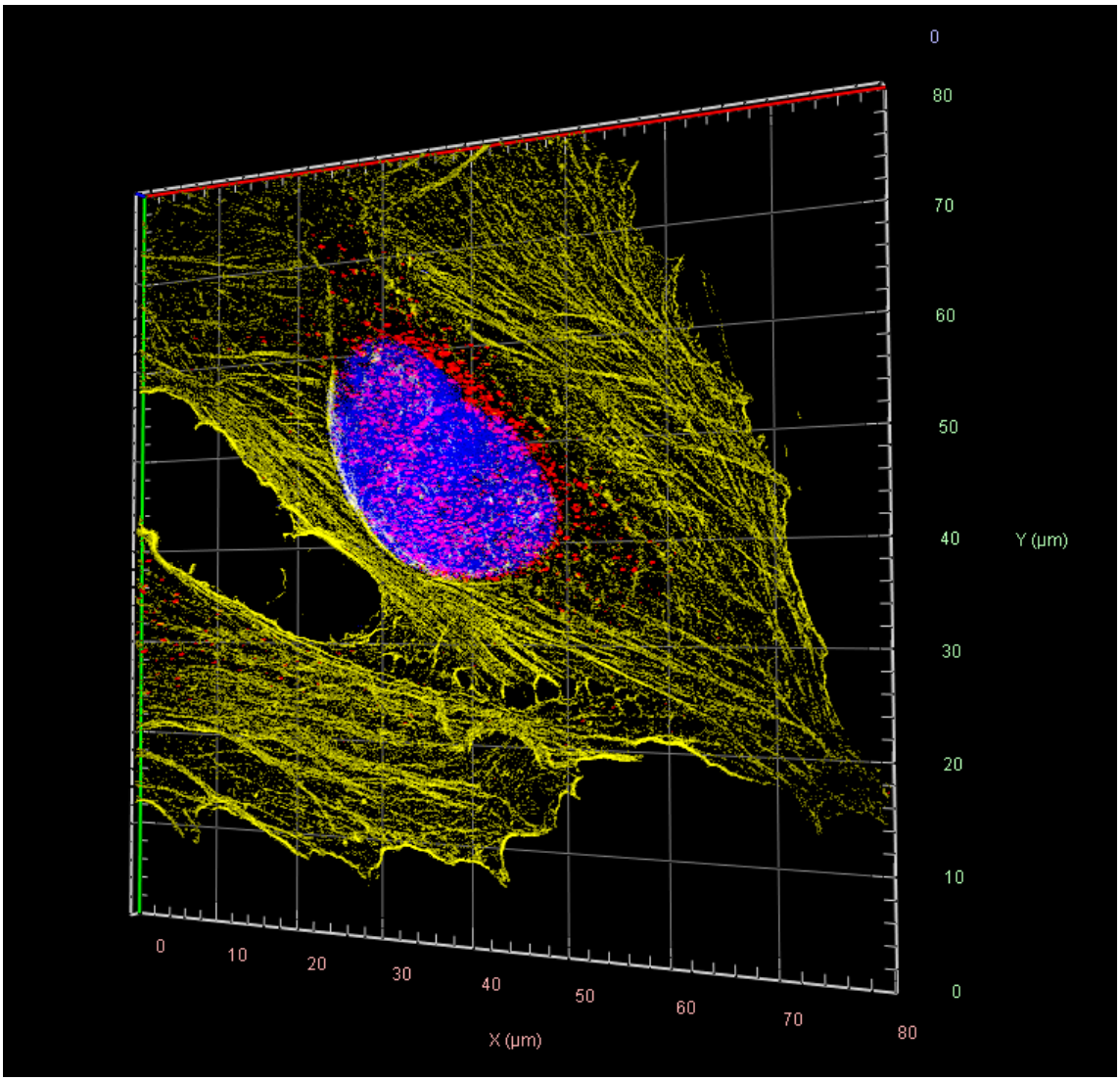
None

Image by J.K. Bennett

SOURCE PROVENANCE

Catalog	SA-000027
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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HCT116
Image SHA-256	C7E95A93366B780AD71F12729A1842423FF103347668DEC1373CDC0E6064784F
Method PDF SHA-256	F838887B5043475D81A8E4319F5428E37FA381DAFD43A39F68AAB39BA837E2DE

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	63

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 647	Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter	SBS LP 560
Beam Splitter532	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	21 Slices (1.60µm)
Channels	3
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.08000 µm
Image Size (Pixels)	2560 X 2560
Image Size (Scaled)	80.14µm X 80.14µm
Bit Depth	16 Bit
Image Center Position	X: -7.38mm, Y: 3.75mm
Stage Position	X: -7.38mm, Y: 3.75mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	488nm @7.00%, | 642nm @2.00%, | 405nm @3.00%,
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.00s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	16
Excitation Wavelength	488 | 642 | 405
Emission Wavelength	655 | 515
Effective NA	1.46
Detection Wavelength	655-1000 | 495-590
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms | 40ms | 50ms
Depth of Focus	0.93µm | 0.73µm

FIELD	SOURCE-DOCUMENTED VALUE
Binning Mode	1x1
Detector Type	Camera
Depth Offset	0
Detector Digital Gain	0.00
Channel	488.000000 | 642.000000 | 405.000000
Grating Period (µm)	27.500000
Processing	3D
Auto Sharpness	Yes
Sharpness	11.672929 | 10.121236 | 11.754867
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 8% (647), Threshold 4% (532), Threshold 8% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 180%, 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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 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 647 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

Beam Splitter532 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

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

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

Z Stack - (3D)

Z Stack = 21 Slices (1.60µm)

Channels = 3
Scaling (Per Pixel) = 31.30nm X 31.30nm X 80.00nm
Image Size (Pixels) = 2560 X 2560
Image Size (Scaled) = 80.14µm X 80.14µm
Bit Depth = 16 Bit
Image Center Position = X: -7.38mm, Y: 3.75mm
Stage Position = X: -7.38mm, Y: 3.75mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @7.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.00s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 488
Emission Wavelength = 655
Effective NA = 1.46
Detection Wavelength = 655-1000
Imaging Device = Edge 4.2 SIM
Exposure Time = 100ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 488.000000
Grating Period (µm): 27.500000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 11.672929
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

647 -

Contrast Method = Fluorescence
Laser Wavelength = 642nm @2.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.00s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 642
Emission Wavelength = 655
Effective NA = 1.46
Detection Wavelength = 655-1000
Imaging Device = Edge 4.2 SIM
Exposure Time = 40ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM Processing

Channel = 642.000000
Grating Period (µm): 27.500000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 10.121236
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
Laser Wavelength = 405nm @3.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 1.00s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 405
Emission Wavelength = 515
Effective NA = 1.46
Detection Wavelength = 495-590
Imaging Device = Edge 4.2 SIM
Exposure Time = 50ms
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): 27.500000

SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 11.754867
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF : No

3D Image Processing

3D Maximum Projection = Precise
Appearance = Threshold 8% (647), Threshold 4% (532), Threshold 8% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light =Brightness 180%, 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

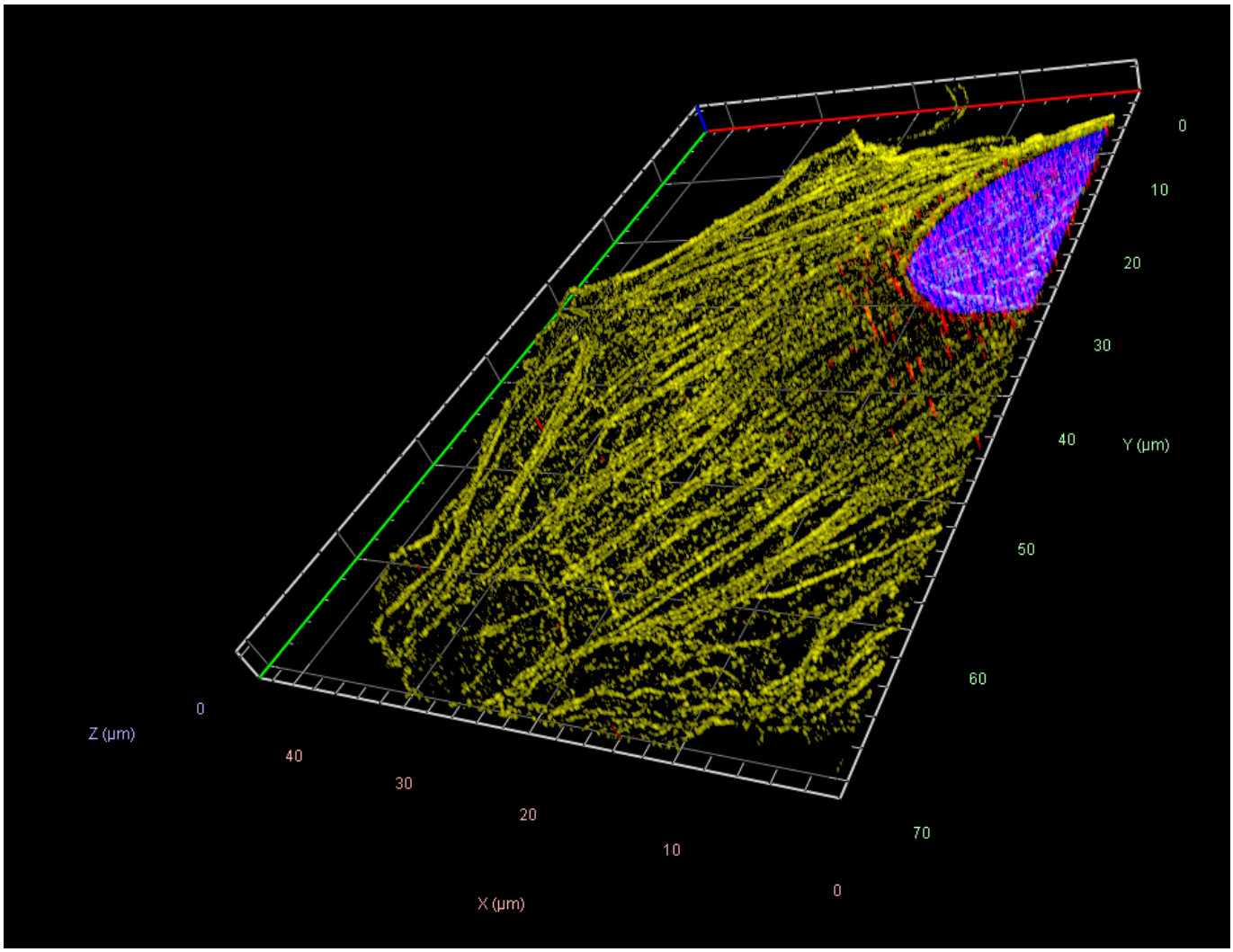
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000027
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	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	044BB42A03730AC82689C9A37987257D8A29EA5AC176DA98D14F93651B20A51E
Method PDF SHA-256	B9A57C549663AF0178E2B0C5DC73F6FF14ACC77A04D137FD78821A73D3FC05D4

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 532; 647
METADATA VALUES	65

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 647	Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter	SBS LP 560
Beam Splitter532	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	31 Slices (2.40µm)
Channels	3
Scaling (Per Pixel)	0.01565 µm X 0.01565 µm X 0.08000 µm
Image Size (Pixels)	2768 X 4539
Image Size (Scaled)	43.32µm X 71.04µm
Bit Depth	16 Bit
Image Center Position	X: -3.42mm, Y: 1.49mm
Stage Position	X: -3.42mm, Y: 1.49mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	488nm @7.00%, | 642nm @2.00%, | 405nm @5.00%,
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	6.26s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	16
Excitation Wavelength	488 | 642 | 405
Emission Wavelength	655 | 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	100ms | 40ms | 50ms
Depth of Focus	0.93µm | 0.73µm
Binning Mode	1x1

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	Camera
Depth Offset	0
Detector Digital Gain	0.00
Channel	488.000000 | 642.000000 | 405.000000
Grating Period (µm)	27.500000 | 36.500000
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
Detection Wavelength	655-1000
3D Maximum Projection	Precise
Appearance	Threshold 8% (647), Threshold 4% (532), Threshold 10% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 152%, Enabled Tone Mapping
Projection	View Angle 45°, Scale Z 112%, 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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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, Nuclear Pore Complex Monoclonal Antibody (39C7), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), 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 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 647 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

Beam Splitter532 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

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

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

Z Stack - (3D)

Z Stack = 31 Slices (2.40µm)

Channels = 3
Scaling (Per Pixel) = 15.65nm X 15.65nm X 80.00nm
Image Size (Pixels) = 2768 X 4539
Image Size (Scaled) = 43.32µm X 71.04µm
Bit Depth = 16 Bit
Image Center Position = X: -3.42mm, Y: 1.49mm
Stage Position = X: -3.42mm, Y: 1.49mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

532 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @7.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 6.26s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 488
Emission Wavelength = 655
Effective NA = 1.46
Imaging Device = Edge 4.2 SIM
Exposure Time = 100ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 488.000000
Grating Period (µm): 27.500000

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

647 -

Contrast Method = Fluorescence
Laser Wavelength = 642nm @2.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 6.26s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 642
Emission Wavelength = 655
Effective NA = 1.46
Detection Wavelength = 655-1000
Imaging Device = Edge 4.2 SIM
Exposure Time = 40ms
Depth of Focus = 0.93µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

Post Processing - SIM2 Processing

Channel = 642.000000
Grating Period (µm): 27.500000

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

DAPI -

Contrast Method = Fluorescence
Laser Wavelength = 405nm @5.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 6.26s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 405
Emission Wavelength = 515
Effective NA = 1.46
Imaging Device = Edge 4.2 SIM
Exposure Time = 50ms
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 Maximum Projection = Precise
Appearance = Threshold 8% (647), Threshold 4% (532), Threshold 10% (DAPI), Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light =Brightness 152%, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 112%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

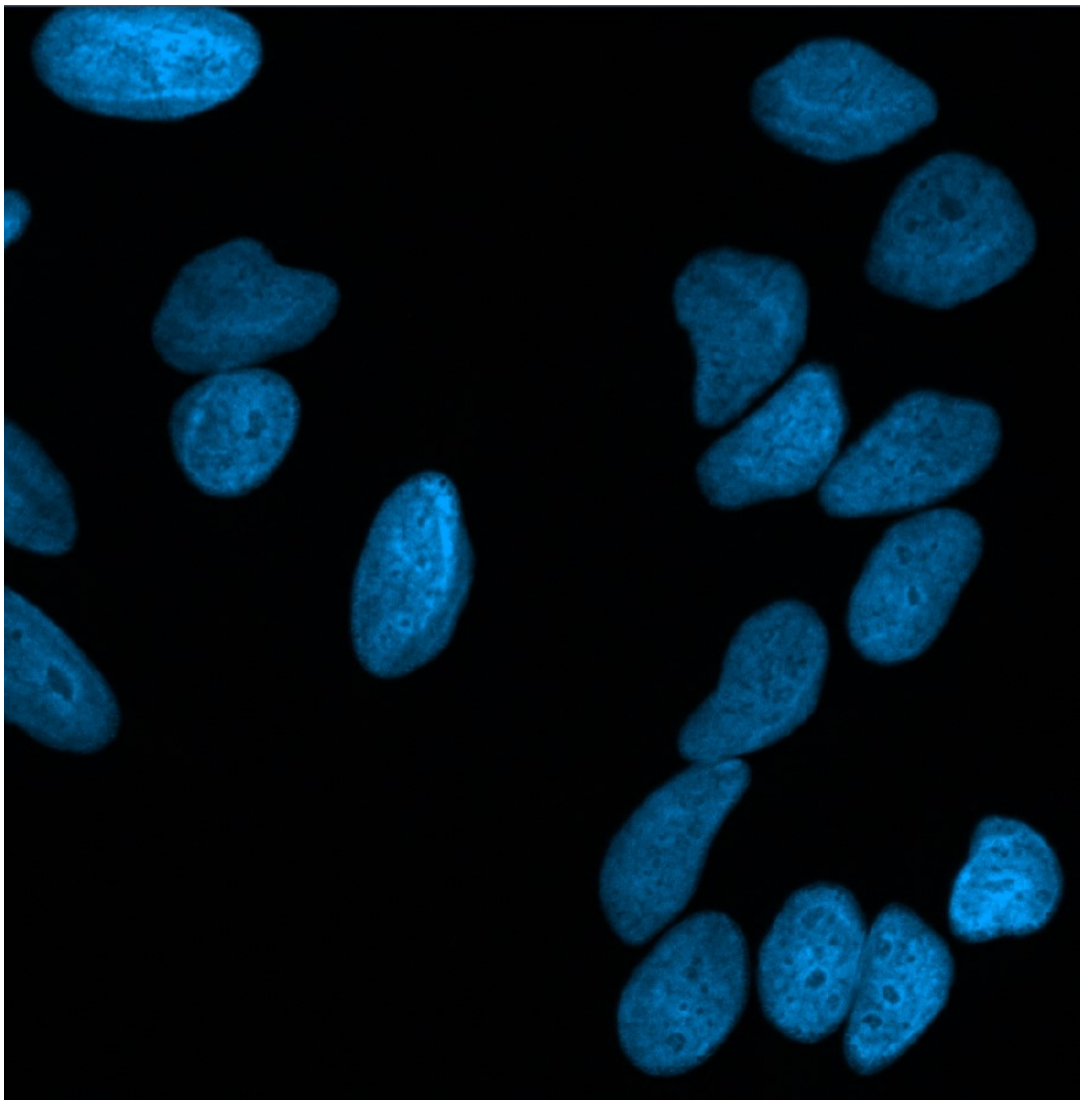
None

Image by P. Raut

SOURCE PROVENANCE

Catalog	SA-000027
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	65
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	376BFC9B2B4CE4D29624406DACDFB0BB287003BF993638A28826824FCBE9987F
Method PDF SHA-256	0BA933FA730CA0ACC35A388073EF731EF5A2F24E790FCAFD101F97EF28FF1C31

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 532; 555
METADATA VALUES	32

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1021 X 1037
Image Size (Scaled)	145.86µm X 148.14µm
Bit Depth	14 Bit
Image Center Position	X: 48.24mm, Y: 50.23mm
Stage Position	X: 48.24mm, Y: 50.23mm
ROI Center Offset	X: 1.14µm, Y: 0.00µm
Reflector	AF532-49014 | 49 DAPI
Beam Splitter	550 | 395
Filter Excitation Wavelength	515 - 545 | 335 - 383
Filter Emission Wavelength	555 - 595 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% | X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms | 30ms
Excitation Wavelength	528 | 353
Emission Wavelength	553 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.07µm | 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™ 532 Affinity Purified Donkey Anti-Rabbit IgG (H + L)
Cat ID# - SA 000027

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® 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 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) = 1021 X 1037
Image Size (Scaled) = 145.86µm X 148.14µm
Bit Depth = 14 Bit
Image Center Position = X: 48.24mm, Y: 50.23mm
Stage Position = X: 48.24mm, Y: 50.23mm
ROI Center Offset = X: 1.14µm, Y: 0.00µm

532 -

Reflector = AF532-49014
Beam Splitter = 550
Filter Excitation Wavelength = 515 - 545
Filter Emission Wavelength = 555 - 595
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @25%
Exposure Time = 200ms
Excitation Wavelength = 528
Emission Wavelength = 553
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.07µm
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-000027
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	750F24A3D09D8A77E9972DC2D73AEDD2EAE3F0620BC15978B966791F3FE35584
Method PDF SHA-256	F013EB0C2E8631B178CF0B56F192737FD631F0A887D95A822288128EFEA3EB1F