

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

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

8

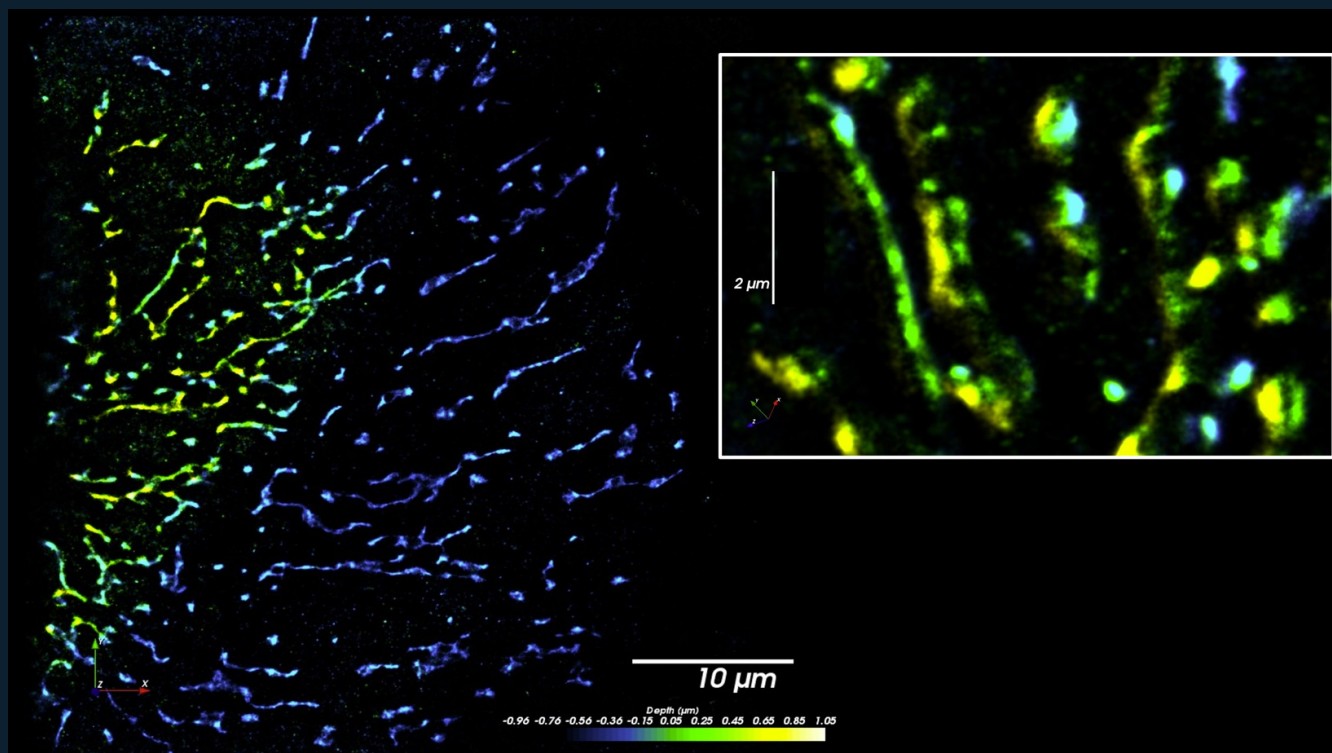
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000022 | 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
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 488 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 -> Single-molecule STORM
-> Control

BENNETT ASSESSMENT

The preserved SA-000022 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

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

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved SA-000022 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 594	3; 38 HE eGFP; 45 Texas Red; 49 DAPI; 450 - 490; 540 - 580; 335 - 383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 594	3; 38 HE eGFP; 45 Texas Red; 49 DAPI; 450 - 490; 540 - 580; 335-383; 500 - 550
Confocal	405/DAPI; 488; 594	3; None; 488nm @0.9%; 561nm @0.6%; 405nm @0.5%; 493; 590; 353
Airyscan	405/DAPI; 488; 594	3; None; 488nm @.02%; 561nm @1.5%; 405nm @0.5%; 493; 590; 353
SIM	405/DAPI; 488; 594	3; 488nm @2.0%; 561nm @9.0%; 405nm @10.0%; 488; 561; 405; 655
SIM ²	405/DAPI; 488; 594; 647	2; 488nm @2.0%; 405nm @10.0%; 488; 405; 655; 515; 488.000000
Single-molecule STORM	488	Both Channels
Control	405/DAPI; 488	2; 38 HE eGFP; 49 DAPI; 450 - 490; 335-383; 500 -550; 420-470; 493

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 488.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 1002 X 917; Image Size (Pixels): 1002 X 917; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 160ms; 50ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @40%; X-Cite Xylis Lamp Intensity @15%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

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; 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

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylys Lamp was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 827 X 719; Image Size (Pixels): 827 X 719; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 150ms; 200ms. Illumination: Light source: X-Cite Xylys Lamp Intensity @40%; X-Cite Xylys Lamp Intensity @55%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 41.

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; 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

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 59 Slices (4.06µm); Channels: 3; Scaling (Per Pixel): 70.58nm X 70.58nm X 70.00nm; Image size (Pixels): 667 X 741; Image Size (Pixels): 667 X 741; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-PMT 1; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.59µs; Frame Time: 4.44s. Illumination: Laser Wavelength: 488nm @0.9%; 561nm @0.6%. Optical/scan: Pinhole: 1.00AU / 45µm; 1.00AU / 54µm; Scan Zoom: 1.6; LSM Scan Speed: 9; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

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 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 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: 82Slices (3.24µm); Channels: 3; Scaling (Per Pixel): 35.30nm X 35.30nm X 40nm; Image size (Pixels): 1197 X 758; Image Size (Pixels): 1197 X 758; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.51µs; Frame Time: 5.07s. Illumination: Laser Wavelength: 488nm @.02%; 561nm @1.5%. Optical/scan: Pinhole: 5.31 AU / 236µm; 5.00 AU / 272µm; Scan Zoom: 1.4; LSM Scan Speed: 7; 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 9.6 (3D, Auto); Super Resolution 9.0 (3D, Auto). Structured source metadata values: 53.

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 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra 7. Objective: Plan-Apochromat 63X/1.46 oil Korr M27. Platform: Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 36Slices (2.1µm); Channels: 3; Scaling (Per Pixel): 31.30nm X 31.30nm X 60nm; Image size (Pixels): 2555 X 1716; Image Size (Pixels): 2555 X 1716; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 50ms; 100ms; Frame Time: 1.00s; 1.0s. Illumination: Laser Wavelength: 488nm @2.0%; 561nm @9.0%. 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: 62.

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 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra 7. Objective: Plan-Apochromat 63X/1.46 oil Korr M27. Platform: Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye: Acquisition: Z-Stack: 36Slices (2.1µm); Channels: 2; Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm; Image size (Pixels): 2555 X 1716; Image Size (Pixels): 2555 X 1716; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 50ms; Frame Time: 1.00s. Illumination: Laser Wavelength: 488nm @2.0%; 405nm @10.0%. 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: 56.

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; 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 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected.

Structured source metadata values: 71.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 488.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1171 X 968; Image Size (Pixels): 1171 X 968; 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: 30.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Control, documents platform context as ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488.

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-000022 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30nm X 35.30nm X 40nm -> 0.03530 μm X 0.03530 μm X 0.04000 μm . Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1197 X 758; Image Size (Scaled)=42.26 μm X 26.76 μm ; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 31.30nm X 31.30nm X 60nm -> 0.03130 μm X 0.03130 μm X 0.06000 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)=2555 X 1716; Image Size (Scaled)=79.98 μm X 53.72 μm ; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm -> 0.03130 μm X 0.03130 μm X 0.06000 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)=2555 X 1716; Image Size (Scaled)=79.98 μm X 53.72 μm ; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

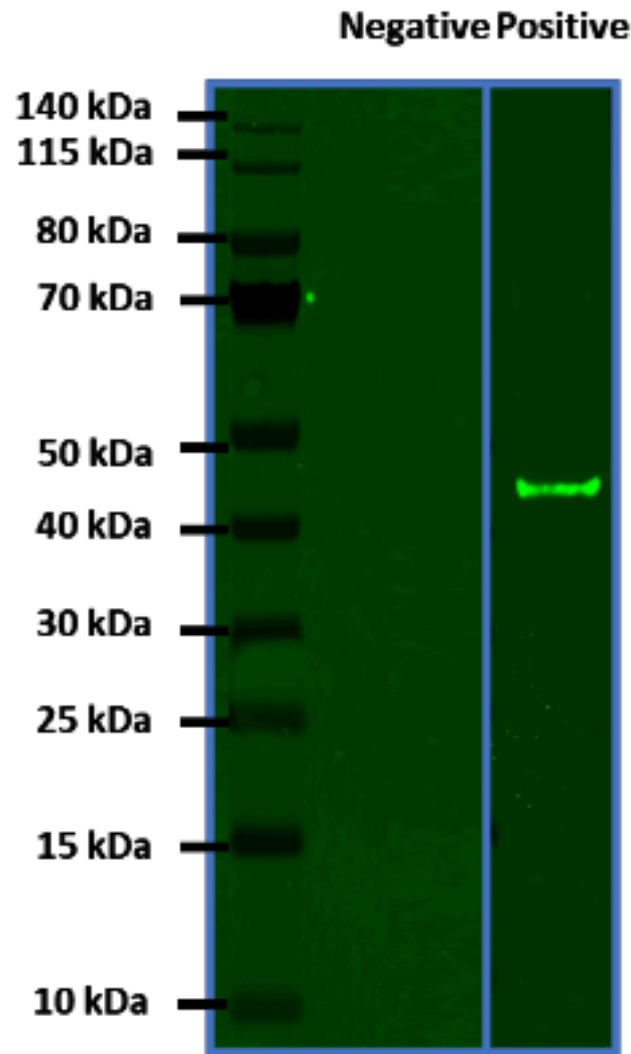
This dossier preserves the complete available evidence progression for SA-000022. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	488
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	488nm
Emission Filter	513±17nm
Detector	PMT
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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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™ 488 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 = 488nm

Emission Filter = 513±17nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

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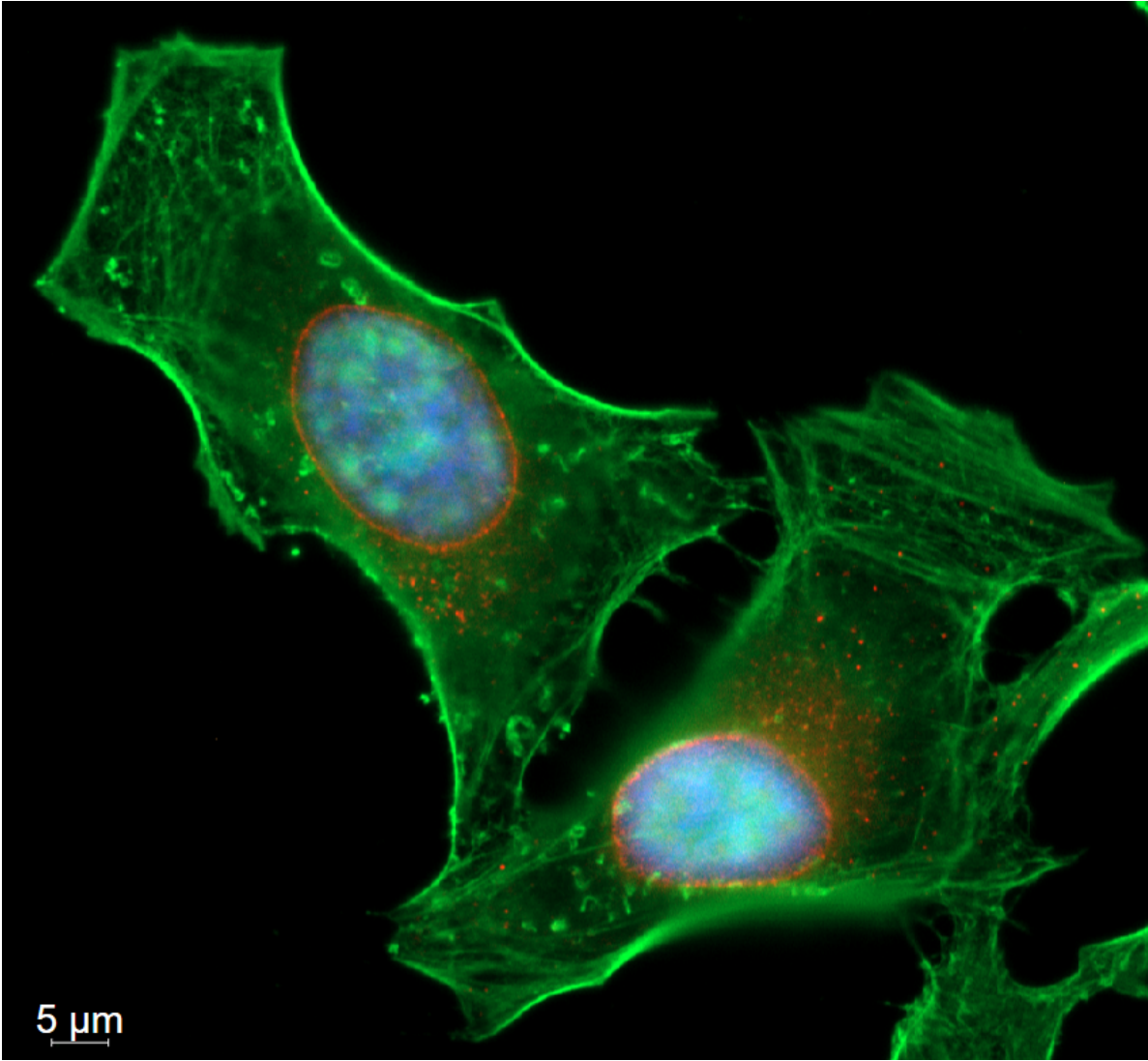
SOURCE PROVENANCE

Catalog	SA-000022
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	9FCC3305BF97D401CB73BEAFCC8CDDDB62CFFD156CD444FCDD7804DFE5D05AE1B
Method PDF SHA-256	74C63A7918E39CF88966DC196840DC179B39B424E74ECED99ED6AC81AFB28455

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	37

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)	1002 X 917
Image Size (Scaled)	102.59µm X 93.88µm
Bit Depth	12 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	38 HE eGFP 45 Texas Red 49 DAPI
Beam Splitter	495 585 395
Filter Excitation Wavelength	450 - 490 540 - 580 335 - 383
Filter Emission Wavelength	500 - 550 593 - 668 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @40% X-Cite Xylis Lamp Intensity @15%
Exposure Time	160ms 50ms
Excitation Wavelength	493 590 353
Emission Wavelength	517 618 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.80µm 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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse F(ab')₂ (H + L)

Cat ID# - SA 000051

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® 594 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), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 488 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) = 1002 X 917

Image Size (Scaled) = 102.59µm X 93.88µm

Bit Depth = 12 Bit

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

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

488 -

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 - 550

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @40%

Exposure Time = 160ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.4

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 0.80µm

Binning Mode = 1,1

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 = 50ms
 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 = 50ms
 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

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SOURCE PROVENANCE

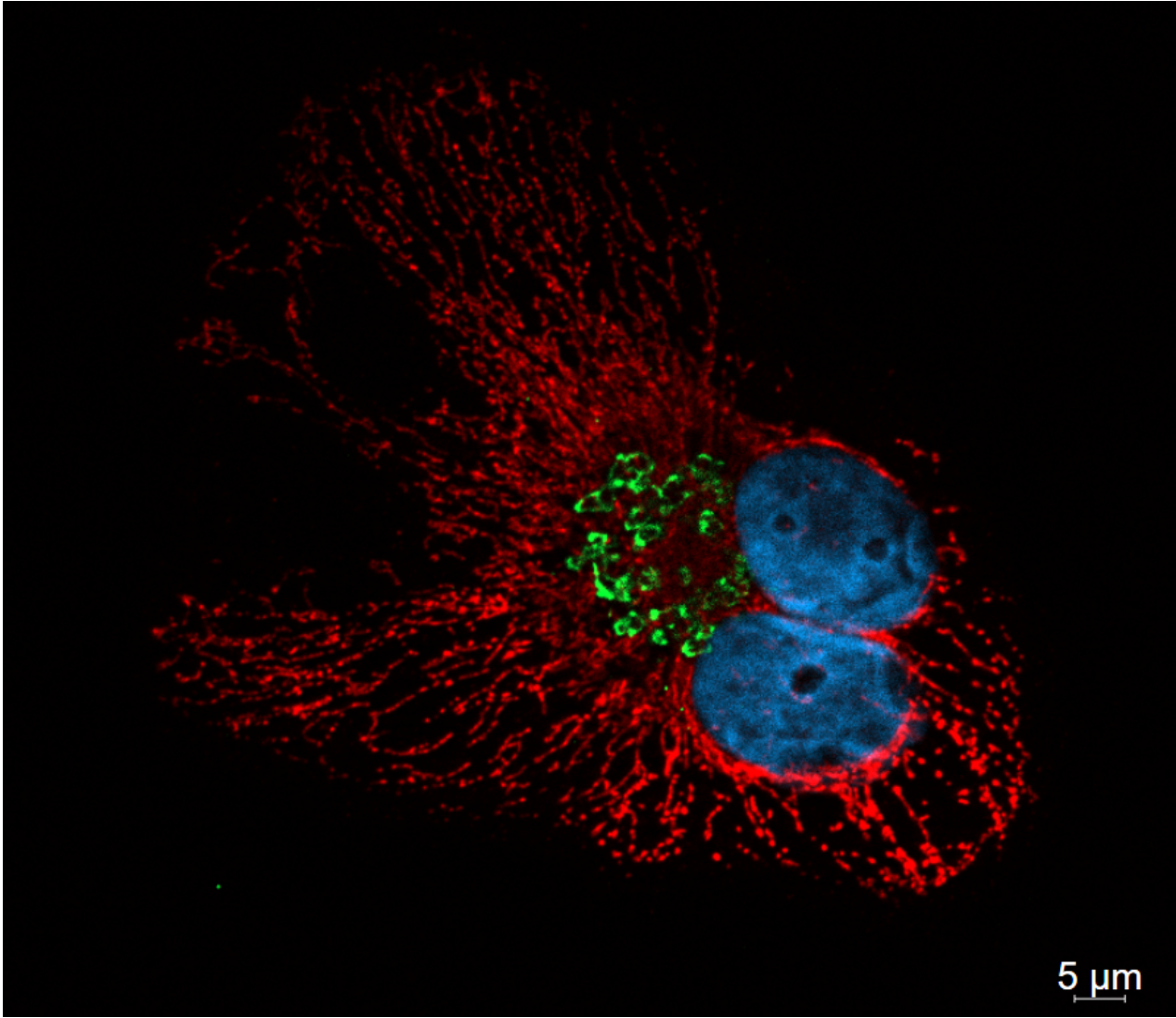
Catalog

SA-000022

SOURCE PROVENANCE

Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam MR R3, and X-Cite Xylys Lamp was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E96E881BF3DAD9F5B2D0522CFA622A164C4794282EFE53F203760E88BD7E1DD0
Method PDF SHA-256	9ED8D81A7E5EA51920D2A361D93246C83090594A059DDD2301C55E8C615C1FCA

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; 488; 594
METADATA VALUES	41

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	3
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	827 X 719
Image Size (Scaled)	118.14µm X 102.71µm
Bit Depth	14 Bit
Image Center Position	X: 80.61mm, Y: 45.69mm
Stage Position	X: 80.61mm, Y: 45.69mm
ROI Center Offset	X: 1.14, Y: 0.00
Reflector	38 HE eGFP 45 Texas Red 49 DAPI
Beam Splitter	495 585 395
Filter Excitation Wavelength	450 - 490 540 - 580 335-383
Filter Emission Wavelength	500 - 550 593 - 668 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @40% X-Cite Xylis Lamp Intensity @55% X-Cite Xylis Lamp Intensity @6%
Exposure Time	150ms 200ms 18ms
Excitation Wavelength	493 590 353
Emission Wavelength	517 618 465
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.00µm 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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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

Cat ID# - SA 000045

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 = 3

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) =827 X 719

Image Size (Scaled) = 118.14µm X 102.71µm

Bit Depth = 14 Bit

Image Center Position = X: 80.61mm, Y: 45.69mm

Stage Position = X: 80.61mm, Y: 45.69mm

ROI Center Offset = X: 1.14, Y: 0.00

488 -

Reflector = 38 HE eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @40%
 Exposure Time = 150ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.25
 Imaging Device = Axiocam 807
 Camera Adapter = 1X
 Depth of Focus = 1.00µm
 Binning Mode = 2,2

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 @55%
 Exposure Time = 200ms
 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 @6%
 Exposure Time = 18ms
 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.

Image Corrections

None

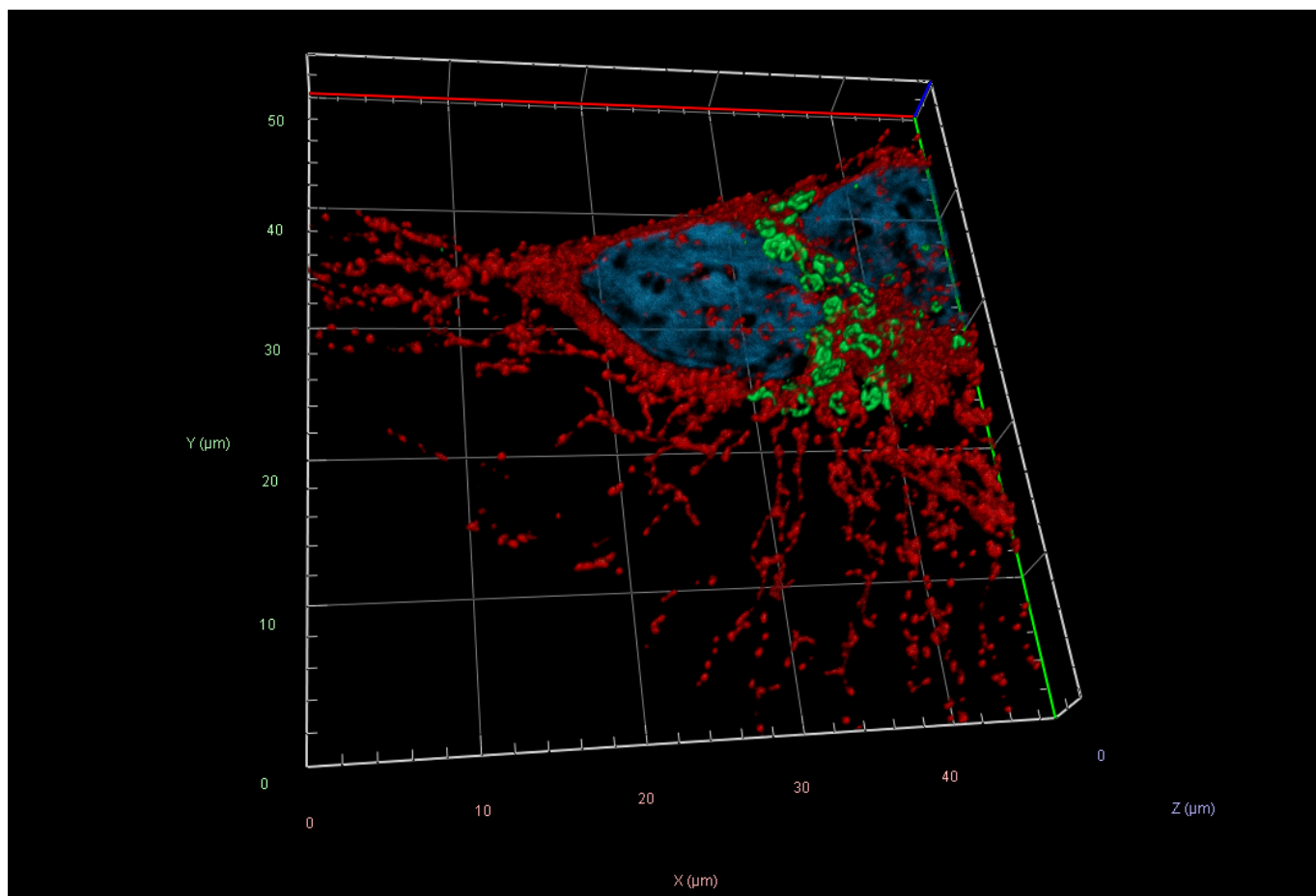
Image by E.F Simon

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SOURCE PROVENANCE

Catalog	SA-000022
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	41
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	11856B83AD1519C6AB952DC2574A630F8C2D5A9976461D703B826673C6E7A6B8
Method PDF SHA-256	73193547E3F65B41885773071623B3E7E79C11B0409F09CF79A35187027BF04E

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	50

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	59 Slices (4.06µm)
Channels	3
Scaling (Per Pixel)	70.58nm X 70.58nm X 70.00nm
Image Size (Pixels)	667 X 741
Image Size (Scaled)	47.08µm X 50.40µm
Bit Depth	16 Bit
Image Center Position	X: 73.49mm, Y: 49.37mm
Stage Position	X: 73.49mm, Y: 49.37mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 45µm 1.00AU / 54µm 1.00AU / 37µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @0.9% 561nm @0.6% 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	1.00
Pixel Time	0.59µs
Frame Time	4.44s
LSM Scan Speed	9
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	493 590 353
Emission Wavelength	517 618 465
Effective NA	1.4
Detection Wavelength	496 - 575 590 - 700 410 - 470
Imaging Device	Airyscan GaAsp-PMT 1
Detector Type	GaAsp-PMT
Detector Gain	650 V 700 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Volume Projection	Precise
Appearance	Threshold 12% (594), Threshold 13% (488), Threshold 12% (DAPI), Ramp 88% (594), Ramp 87% (488), Ramp 98% (DAPI), Maximum 100%
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)

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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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

Cat ID# - SA 000045

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution 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 followed this, Mitochondria Antibody (MA5-12017), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 = 59 Slices (4.06µm)

Channels = 3

Scaling (Per Pixel) = 70.58nm X 70.58nm X 70.00nm

Image Size (Pixels) = 667 X 741

Image Size (Scaled) = 47.08µm X 50.40µm

Bit Depth = 16 Bit

Image Center Position = X: 73.49mm, Y: 49.37mm

Stage Position = X: 73.49mm, Y: 49.37mm

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

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 45µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @0.9%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.59µs
 Frame Time = 4.44s
 LSM Scan Speed = 9
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 496 - 575
 Imaging Device = Airyscan
 Detector Type = GaAsp-PMT
 Detector Gain = 650 V
 Detector Offset = 0
 Detector Digital Gain = 1.0

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 54µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @0.6%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.59µs
 Frame Time = 4.44s
 LSM Scan Speed = 9
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 8
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 590 - 700
 Imaging Device = GaAsp-PMT 1
 Detector Type = GaAsp-PMT
 Detector Gain = 700 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 = 1.6
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.59µs
 Frame Time = 4.44s
 LSM Scan Speed = 9
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 9
 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 12% (594), Threshold 13% (488), Threshold 12% (DAPI), Ramp 88% (594), Ramp 87% (488), Ramp 98% (DAPI), Maximum 100%
 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)

Image Corrections

None

Image by J.K. Bennett

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SOURCE PROVENANCE

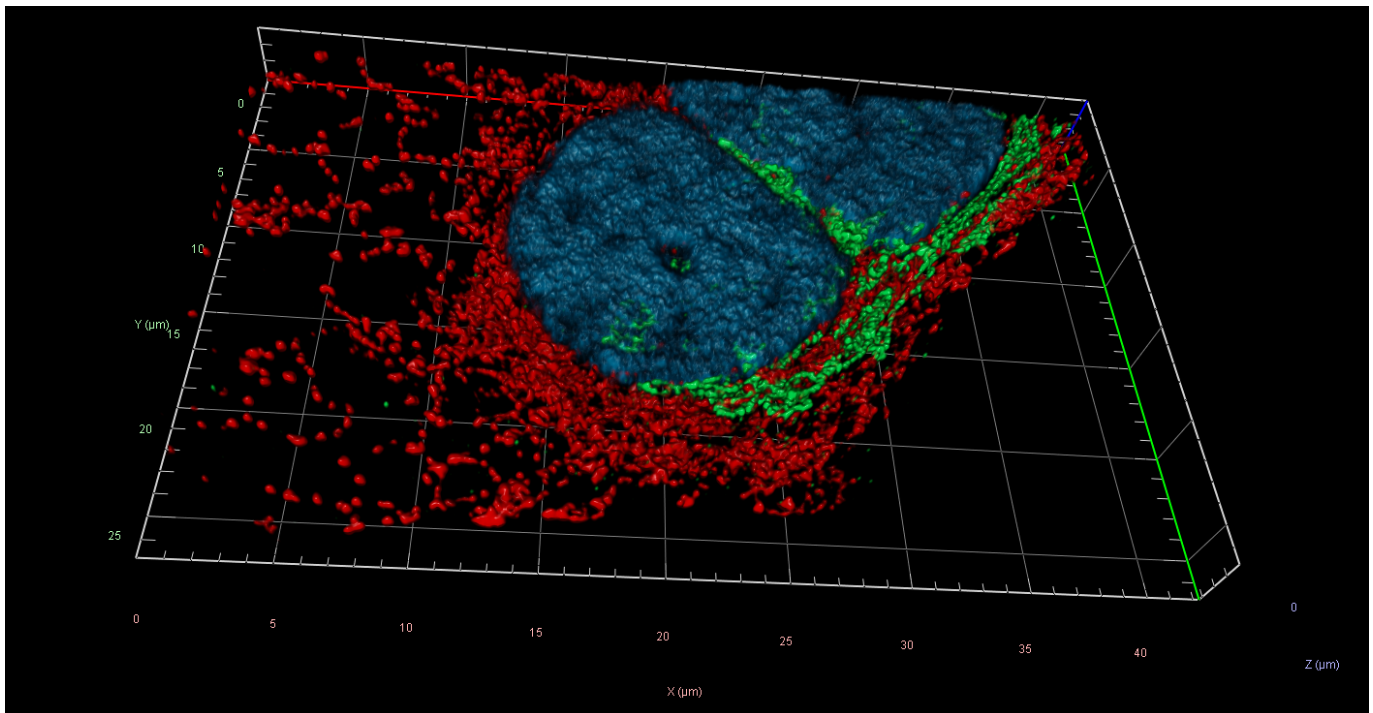
Catalog

SA-000022

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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	FFA6DA31FBF28FB044C92296A02AD5CFEE165C37F881BDA8CA6C0D1EEB339F60
Method PDF SHA-256	85EDDB0D1B040ABFA914E2F80148ECB3B7C264FDC518FA14F8ABC80488E24638

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	53

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	82Slices (3.24µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.04000 µm
Image Size (Pixels)	1197 X 758
Image Size (Scaled)	42.26µm X 26.76µm
Bit Depth	16 Bit
Image Center Position	X: 68.26mm, Y: 53.00mm
Stage Position	X: 68.26mm, Y: 53.00mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.31 AU / 236µm 5.00 AU / 272µm 5.00 AU / 182µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @.02% 561nm @1.5% 405nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.4
Rotation	0°
Sampling	2.00
Pixel Time	0.51µs
Frame Time	5.07s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	493 590 353
Emission Wavelength	517 618 465
Effective NA	1.4
Detection Wavelength	495-569 598-700 400-464
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 9.6 (3D, Auto) Super Resolution 9.0 (3D, Auto) Super Resolution 8.5 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 8% (488), Threshold 12% (594), Threshold 0% (DAPI), Ramp 92% (488), Ramp 88% (594), Ramp 100% (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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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

Cat ID# - SA 000045

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 = 82Slices (3.24µm)

Channels = 3

Scaling (Per Pixel) = 35.30nm X 35.30nm X 40nm

Image Size (Pixels) = 1197 X 758

Image Size (Scaled) = 42.26µm X 26.76µm

Bit Depth = 16 Bit

Image Center Position = X: 68.26mm, Y: 53.00mm

Stage Position = X: 68.26mm, Y: 53.00mm

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

488 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.31 AU / 236µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 488nm @.02%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.51µs
 Frame Time = 5.07s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Detection Wavelength = 495-569
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.6 (3D, Auto)

594 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 272µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 561nm @1.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.51µs
 Frame Time = 5.07s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 590
 Emission Wavelength = 618
 Effective NA = 1.4
 Detection Wavelength = 598-700
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 9.0 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 182µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.4
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.51µs
 Frame Time = 5.07s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 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 8.5 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 8% (488), Threshold 12% (594), Threshold 0% (DAPI), Ramp 92% (488), Ramp 88% (594), Ramp 100% (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 -

DAPI brightness was decreased by 10% from "Best Fit" to create contrast with the 488 signal where the Golgi sits close to the DAPI staining (nuclear compartment)

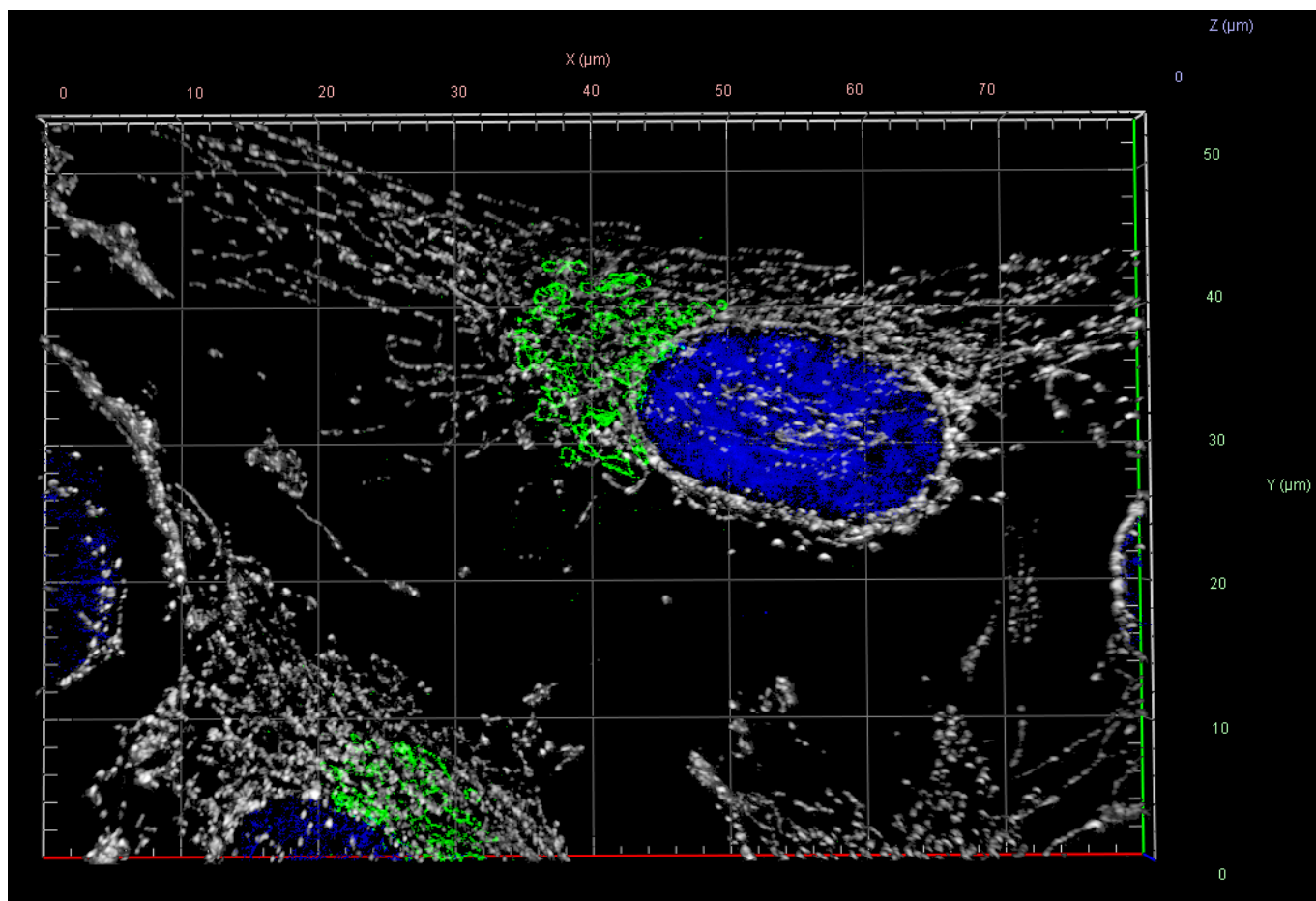
Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

SOURCE PROVENANCE

Catalog	SA-000022
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	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F49EE5568D44C205575796630B86EFAA3A6D066826C0E8648296E21C51AC1F26
Method PDF SHA-256	C7E534F783708FF8F25EF026D35201BE456531E555008953DAEC5314CABEB684

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	62

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
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	36Slices (2.1µm)
Channels	3
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.06000 µm
Image Size (Pixels)	2555 X 1716
Image Size (Scaled)	79.98µm X 53.72µm
Bit Depth	16 Bit
Image Center Position	X: 5.63mm, Y: -2.94mm
Stage Position	X: 5.63mm, Y: -2.94mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	488nm @2.0% 561nm @9.0% 405nm @10.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.00s 1.0s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	16
Excitation Wavelength	488 561 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	50ms 100ms
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 561.000000 405.000000
Grating Period (µm)	27.500000 36.500000
Processing	3D
Auto Sharpness	Yes
Sharpness	11.919812 10.265160 11.840482
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 4% (594), Threshold 16% (488), Threshold 8% (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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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

Cat ID# - SA 000045

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Golgi GOLPH2 Polyclonal Antibody, was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Mitochondria Antibody (MA5-12017), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 594 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 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 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 = 36Slices (2.1µm)

Channels = 3

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

Image Size (Pixels) = 2555 X 1716

Image Size (Scaled) = 79.98µm X 53.72µm

Bit Depth = 16 Bit

Image Center Position = X: 5.63mm, Y: -2.94mm

Stage Position = X: 5.63mm, Y: -2.94mm

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

488 -

Contrast Method = Fluorescence
 Laser Wavelength = 488nm @2.0%
 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
 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 - SIM Processing

Channel = 488.000000

Grating Period (µm): 27.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 11.919812
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

594 -

Contrast Method = Fluorescence
 Laser Wavelength = 561nm @9.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.0s
 Scan Direction = Unidirectional
 Line Step = N/A
 Averaging = 16
 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 - SIM Processing

Channel = 561.000000

Grating Period (µm): 27.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 10.265160
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @10.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.00s
 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 - SIM Processing

Channel = 405.000000
Grating Period (µm): 36.500000

SIM

Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 11.840482
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 4% (594), Threshold 16% (488), Threshold 8% (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

Identifyn® SRM Alexa Fluor™ 594 was represented as white instead of its default red coloring to demonstrate contrast and show the specificity and brightness of the Identifyn® SRM Alexa Fluor™ 488 dye product.

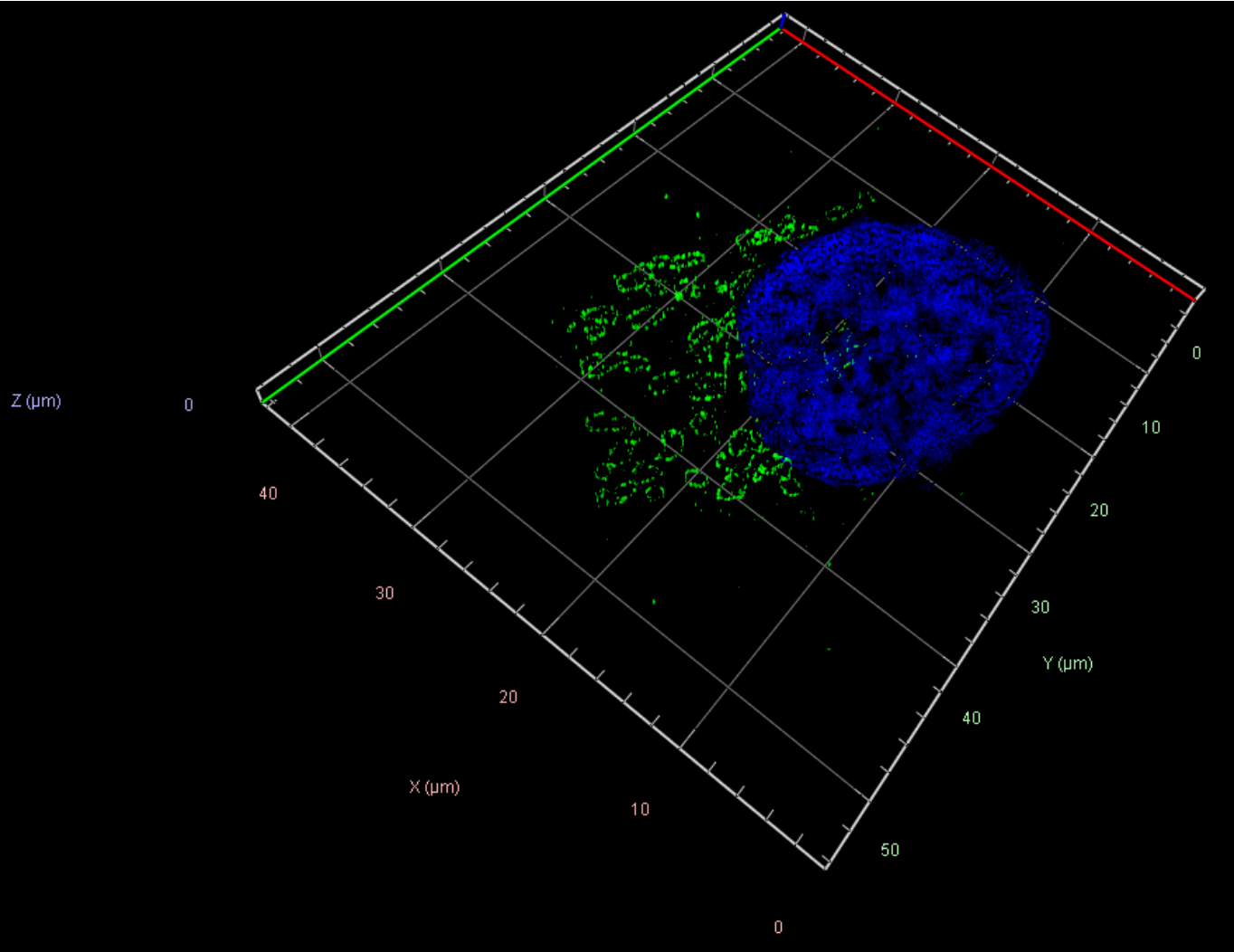
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000022
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	62
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F5FEC960BBDFD9877774912D62C7D4B7D3F60A06B15483DB9466ED91A5FD0E0F
Method PDF SHA-256	A4A86802DDD69D8F382FDBD74C06DE4E17CAF44F37292EC1470E069E4504ECC7

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra 7
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594; 647
METADATA VALUES	56

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 560
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	36Slices (2.1µm)
Channels	2
Scaling (Per Pixel)	0.03130 µm X 0.03130 µm X 0.06000 µm
Image Size (Pixels)	2555 X 1716
Image Size (Scaled)	79.98µm X 53.72µm
Bit Depth	16 Bit
Image Center Position	X: 5.63mm, Y: -2.94mm
Stage Position	X: 5.63mm, Y: -2.94mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Contrast Method	Fluorescence
Laser Wavelength	488nm @2.0% 405nm @10.0%
Scan Mode	Other
Scan Zoom	1.0
Rotation	0°
Frame Time	1.00s
Scan Direction	Unidirectional
Line Step	N/A
Averaging	16
Excitation Wavelength	488 405
Emission Wavelength	655 515
Effective NA	1.46
Imaging Device	Edge 4.2 SIM
Exposure Time	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 405.000000
Grating Period (µm)	27.500000 36.500000
Processing	3D
Auto Sharpness	Yes
Sharpness	11.919812 11.840482
Detrend	No
Sectioning	100.000000
Baseline	Yes
Scale to Raw Image	No
Experimental PSF	No
3D Maximum Projection	Precise
Appearance	Threshold 4% (594), Threshold 16% (488), Threshold 8% (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™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 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 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 = 36Slices (2.1µm)

Channels = 2

Scaling (Per Pixel) = 31.30nm X 31.30nm X 60.00nm

Image Size (Pixels) = 2555 X 1716

Image Size (Scaled) = 79.98µm X 53.72µm

Bit Depth = 16 Bit

Image Center Position = X: 5.63mm, Y: -2.94mm

Stage Position = X: 5.63mm, Y: -2.94mm

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

488 -

Contrast Method = Fluorescence
 Laser Wavelength = 488nm @2.0%
 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
 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
 Auto Sharpness: Yes
 Sharpness: 11.919812
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

DAPI -

Contrast Method = Fluorescence
 Laser Wavelength = 405nm @10.0%
 Scan Mode = Other
 Scan Zoom = 1.0
 Rotation = 0°
 Frame Time = 1.00s
 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

SIM 2
 Processing: 3D
 Auto Sharpness: Yes
 Sharpness: 11.840482
 Detrend: No
 Sectioning: 100.000000
 Baseline: Yes
 Scale to Raw Image: No
 Experimental PSF: No

3D Image Processing

3D Maximum Projection = Precise
 Appearance = Threshold 4% (594), Threshold 16% (488), Threshold 8% (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

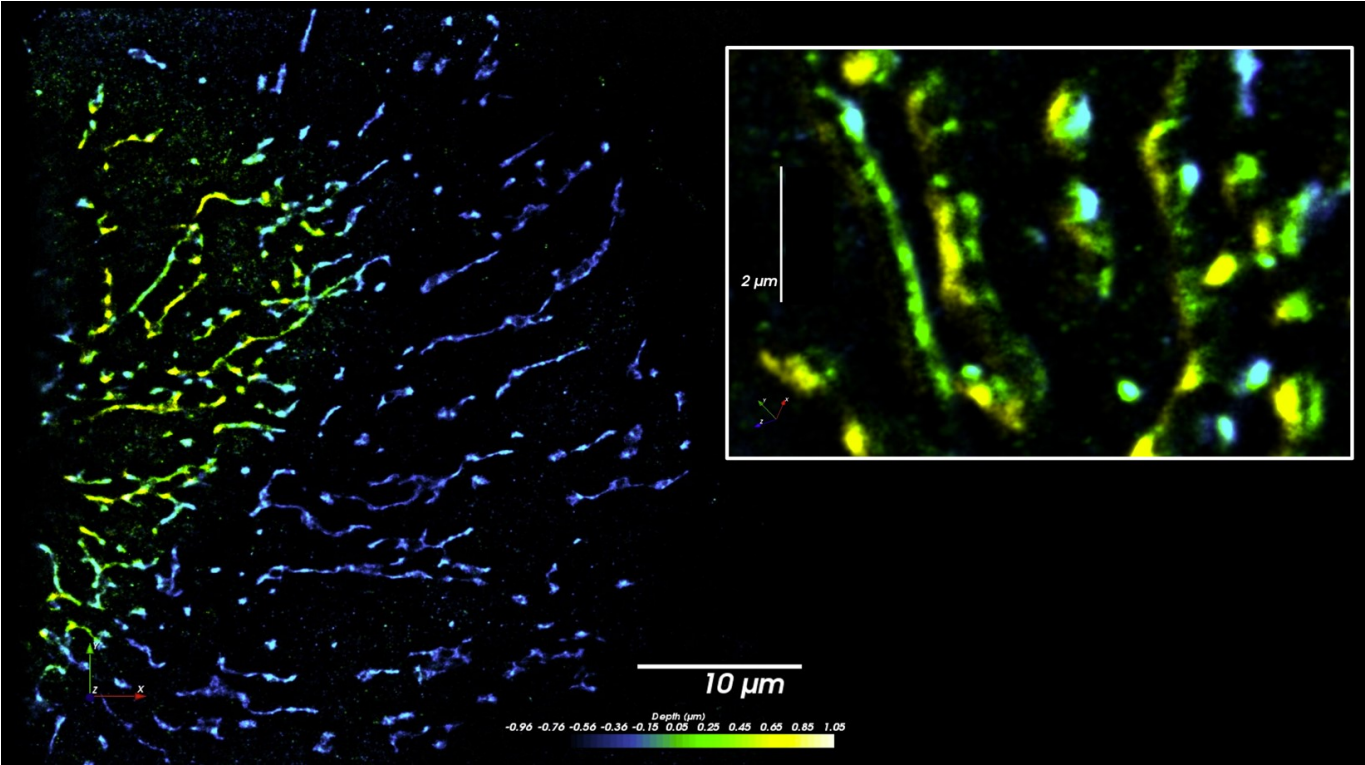
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000022
Stage	SIM ²
Instrument	ZEISS Elyra 7
Microscope configuration	Zeiss Elyra 7, Andor1, AxioObserver, using Plan-Apochromat 63X/1.46 oil Korr M27 was used with the following parameters for each dye:
Structured source metadata values	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B386E96599E3DD24E83AB3DC2A7848529E86170C4689FD2182E878AA1A14DD49
Method PDF SHA-256	F6615B0139F45536EA9453B67A182DCB2A04207645488923BF05AA2E2DF7D36B

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	488
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange, Green)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 488 nm (635 nm Dichroic); First reference probe: 488 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	180 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 488nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Reference Probe 1 Laser control (488 nm (635 nm Dichroic))	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 488nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5084 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 178.5; End: 178.5
SuperRes 488nm	0.1X Neutral Density Filter: Selected, @0.01% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 20000
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	9%
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 7
Sizing Mode	Radial Precision
Particle size	4nm
Color by	Constant
Color Table	Single
Opacity Mode	Constant; Opacity 1: 04
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	5 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	20nm
Smoothing	8.00
PSF Z offset	-450 to +450
Radial precision	35
Axial precision	65

SOURCE METHOD

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

Identifyfyn Standards for Optical Microscopy Methods™

Identifyfyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)

Cat ID# - SA 000022

Hela cells were grown on μ -Slide 8 Well high Glass Bottom Ibidi chamber, followed by PFA fixation and blocked with Identifyfyn's™ proprietary Block Buffer. The primary antibody, TOMM20 Recombinant Monoclonal Antibody (3G5) (MA5-24859), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyfyn's™ proprietary Wash Buffer and Identifyfyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyfyn's™ proprietary Wash Buffer followed by the addition of Identifyfyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

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

Calibration -

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

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

Image Acquisition -

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

Configuration Tab - Capture Configuration

Number of Probes: 1

Number of Simultaneous Probes: 1

Number of Reference Probes: 1

Camera: Both Cameras

Color Channel: Both Channels

Probe Capture Mode: Interleaved

Z -Stack Capture Mode: Interleaved

Multiple Location Capture: Not selected

Post Record Reference: Not Selected

Fluidics: Not Selected

Autofocus Drift Correction: On

Microscope configuration

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

Camera Configuration

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

Probe Configuration

Predefined Probe: First probe: 488 nm (635 nm Dichroic); First reference probe: 488 nm (635 nm Dichroic)
 Photoactivation/Imaging Laser: 0% Selected
 Exposure Time: 20ms Selected

Experiment Configuration

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

Stage and Piezo Selection

Examination of Sample

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

Piezo Current: 180 µm

Probe 1 Laser control (488 nm (635 nm Dichroic))

Widefield 488nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input
 Reference Probe 1 Laser control (488 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100ms
 Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 488nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position" -0.979

Current Intensity: 4.5

Calibration Value: -5084 nm/unit

Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50µm is Selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - Positioning of the target Cell or Subsection of the Target Cell is optimized and optimally focused.

Piezo Record: Begin: 178.5; End: 178.5

Probe 1 Laser control (488 nm (635 nm Dichroic)

SuperRes 488nm: 0.1X Neutral Density Filter: Selected, @0.01% Laser Input

Reference Probe 1 Laser control (488 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms

Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 20000

Z Positions: 1 using Steps: 0.2µm (200nm)

Cycles: 1

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control (488 nm (635 nm Dichroic)

Laser Power: 9%

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16 px
 Max Frame Accumulation: 1 frame
 Max Accumulation Offset: 1px
 Fiducial at Max Accumulation: Not selected
 Max Particles Per Frame: No Max
 Background SLPE Removal: Not Selected
 Cutout/Background Removal: Not Selected
 Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 7

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
 Sizing Mode: Radial Precision
 Particle size: 4nm
 Color by: Constant
 Color Table: Single
 Opacity Mode: Constant; Opacity 1: 04
 Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
 Time Intervals: 5 Intervals
 Pixel Size: 20nm
 Smoothing: 8.00

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

PSF Z offset: -450 to +450

Radial precision: 35

Axial precision: 65

The other parameters were not applied.

Statistical analysis - N/A

Image by S. Thakur

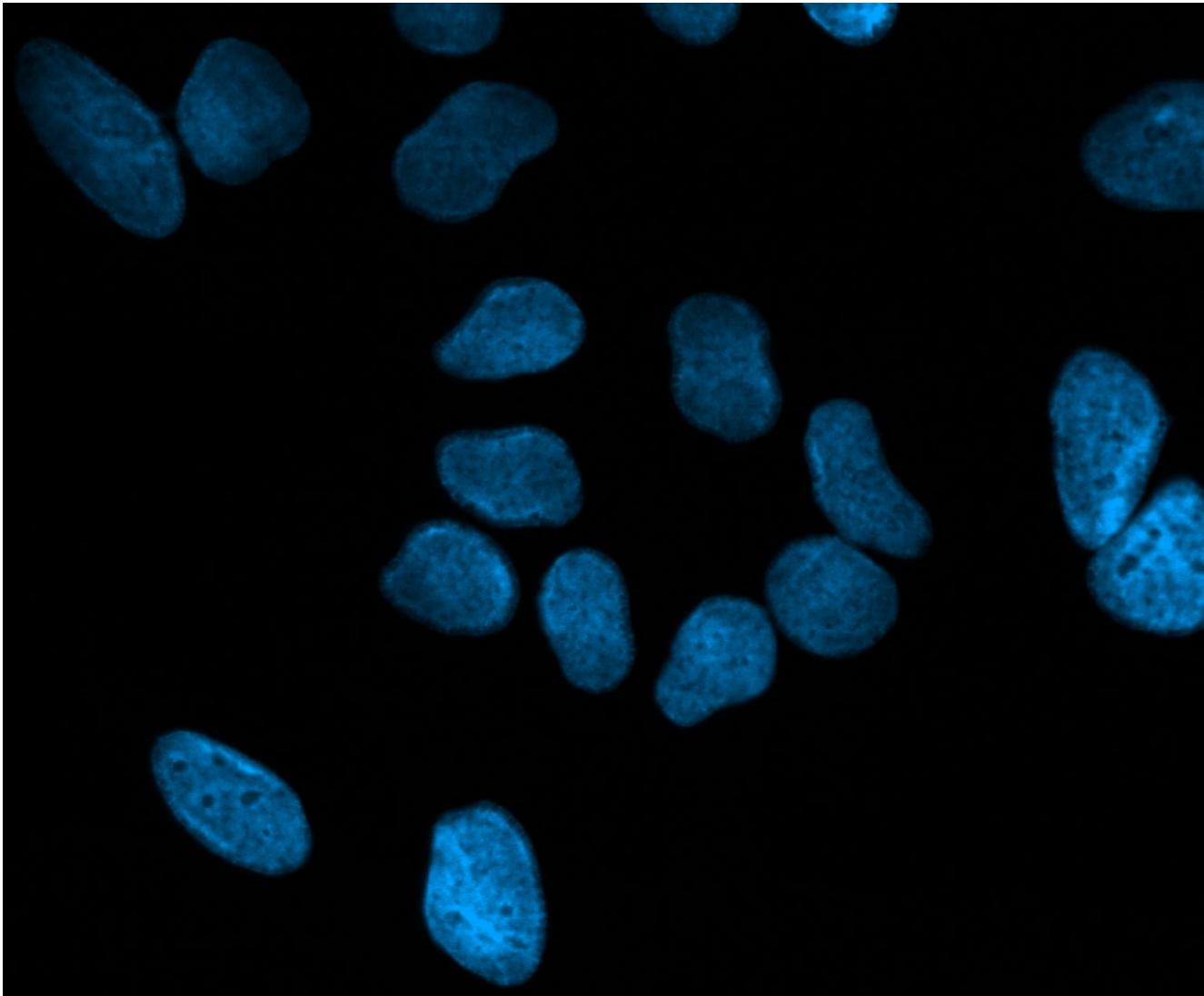
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SOURCE PROVENANCE

Catalog	SA-000022
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A383D98F52BF44002EA97FCD2ECD610AB5E69C19B388FE25E5415D1B16CF53EB
Method PDF SHA-256	2F021BC46A663DDE7F09089050D427CAD51698B56A125618F4A131CAF8A36223

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	30

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)	1171 X 968
Image Size (Scaled)	167.29µm X 138.29µm
Bit Depth	14 Bit
Image Center Position	X: 52.35mm, Y: 51.57mm
ROI Center Offset	X: 52.35µm, Y: 51.57µm
Reflector	38 HE eGFP 49 DAPI
Beam Splitter	495 395
Filter Excitation Wavelength	450 - 490 335-383
Filter Emission Wavelength	500 -550 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	493 353
Emission Wavelength	517 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.0µm 0.90µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit IgG (H + L)

Cat ID# - SA 000022

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® 488 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) = 1171 X 968

Image Size (Scaled) = 167.29µm X 138.29µm

Bit Depth = 14 Bit

Image Center Position = X: 52.35mm, Y: 51.57mm

ROI Center Offset = X: 52.35µm, Y: 51.57µm

488 -

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 -550

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @25%

Exposure Time = 200ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.0µm

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 -

None

Image Corrections

None

Control Summary -

In the absence of a primary antibody, Identifyn Standards for Optical Microscopy Methods™ were applied using standard staining methodology and image acquisition settings using the Zeiss Axio Imager.M1 Widefield. With no image correction, the image demonstrates that the secondary antibody, minus a primary target, had no off-target binding and no significant presence of free dye within the sample.

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

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000022
Stage	Control
Instrument	ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	CE474EECA67153ECE03B97163A5C106F4A2ECCFC6C4602D587EB972F9093D6EC
Method PDF SHA-256	F97BC3EFA853927ACC86E885954B56588E8F501F423E167F280A092229C67670