BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

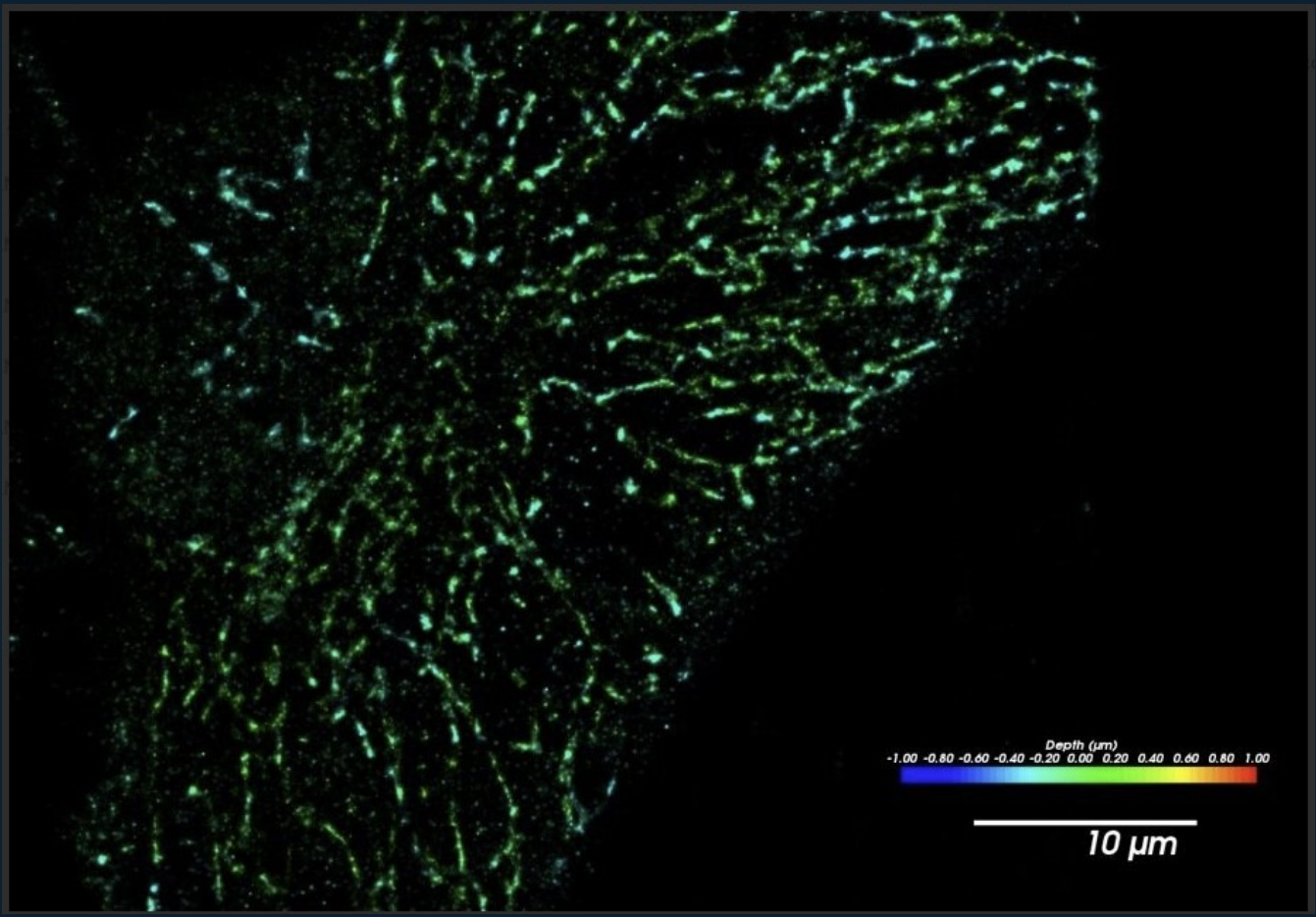
IDENTIFYN RESOLUTION STEPDOWN

# SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

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

| 8 | 1 | PENDING |
|---|---|---|
| ORDERED EVIDENCE TIERS | SUPPORTING CONTROL | SCIENTIST REVIEW |




REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

**SA-000013 | 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 Goat 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-000013 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-000013 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 | 2; 38 HE eGFP; 49 DAPI; 450-490; 335-383; 500-550; 420-470; 493 |
| Widefield SIM — Apotome | 405/DAPI; 488; 647 | 3; 38 HE eGFP; 350 Cy5; 49 DAPI; 450 - 490; 625 - 665; 335-383; 500 - 550 |
| Confocal | 405/DAPI; 488 | 2; None; 488nm @0.5%; 405nm @0.5%; 493; 353; 517; 465 |
| Airyscan | 405/DAPI; 488 | 2; None; 488nm @0.3%; 405nm @0.5%; 493; 353; 517; 465 |
| SIM | 405/DAPI; 488; 647 | 3; 488nm @10.00%,; 642nm @3.00%; 405nm @20.00%,; 488; 642; 405; 515 |
| SIM² | 405/DAPI; 488 | 1; 488nm @3.00%; 488; 570; 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: 2; Scaling (Per Pixel): 0.102µm X 0.102µm; Image size (Pixels): 455 X 446; Image Size (Pixels): 455 X 446; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms; 80ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @15%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 30.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

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 Xylis Lamp was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 797 X 708; Image Size (Pixels): 797 X 708; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807. Timing: Exposure Time: 340ms; 400ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @32.72%; X-Cite Xylis Lamp Intensity @81.66%. 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; 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 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: 43 Slices (2.94µm); Channels: 2; Scaling (Per Pixel): 70.60nm X 70.60nm X 70.00nm; Image size (Pixels): 1187 X 775; Image Size (Pixels): 1187 X 775; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsp-PMT; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.65µs; Frame Time: 8.72s. Illumination: Laser Wavelength: 488nm @0.5%; 405nm @0.5%. Optical/scan: Pinhole: 1.00AU / 45µm; 1.00 AU / 37µm; Scan Zoom: 1.2; LSM Scan Speed: 8; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: Projection: View Angle 48°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 45.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### 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: 48 Slices (3.29µm); Channels: 2; Scaling (Per Pixel): 35.30nm X 35.30nm X 70.00nm; Image size (Pixels): 2184 X 1979; Image Size (Pixels): 2184 X 1979; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.96µs; Frame Time: 21.88s. Illumination: Laser Wavelength: 488nm @0.3%; 405nm @0.5%. Optical/scan: Pinhole: 5.31 AU / 236µm; 5.00 AU / 182µm; Scan Zoom: 1.3; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 66°, 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). Structured source metadata values: 45.

### VISIBLE OBSERVATION

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

### SOURCE-REPORTED RESULT

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

### 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: Channels: 3; Scaling (Per Pixel): 31.30nm X 31.30nm X 60.00nm; Image size (Pixels): 2073 X 1401; Image Size (Pixels): 2073 X 1401; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 50ms; Frame Time: 34.54s. Illumination: Laser Wavelength: 488nm @10.00%,; 642nm @3.00%. 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: 64.

### 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; 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 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: Channels: 1; Scaling (Per Pixel): 31.30nm X 31.30nm; Image size (Pixels): 2000 X 2000; Image Size (Pixels): 2000 X 2000; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Edge 4.2 SIM; Detector Type: Camera. Timing: Exposure Time: 20ms; Frame Time: 14.36s. Illumination: Laser Wavelength: 488nm @3.00%. Optical/scan: Scan Zoom: 1.0; Scan Direction: Unidirectional; Averaging: 16; Effective NA: 1.46. Processing: Process Sampling: 2. Structured source metadata values: 46.

### 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.

### 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): 1255 X 1008; Image Size (Pixels): 1255 X 1008; 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-000013 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**

Confocal / Scaling (Per Pixel): 70.60nm X 70.60nm X 70.00nm -> 0.07060 µm X 0.07060 µm X 0.07000 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)=1187 X 775; Image Size (Scaled)=83.80µm X 54.72µm; 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 70.00nm -> 0.03530 µm X 0.03530 µm X 0.07000 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)=2184 X 1979; Image Size (Scaled)=77.09µm X 69.86µm; 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 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)=2073 X 1401; Image Size (Scaled)=64.89µm X 43.86µm; 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 -> 0.03130 µm X 0.03130 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)=2000 X 2000; Image Size (Scaled)=62.61µm X 62.61µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

# INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

# ORIGINAL RECORD BEGINS

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

## PRODUCT-LEVEL STEPDOWN MAP

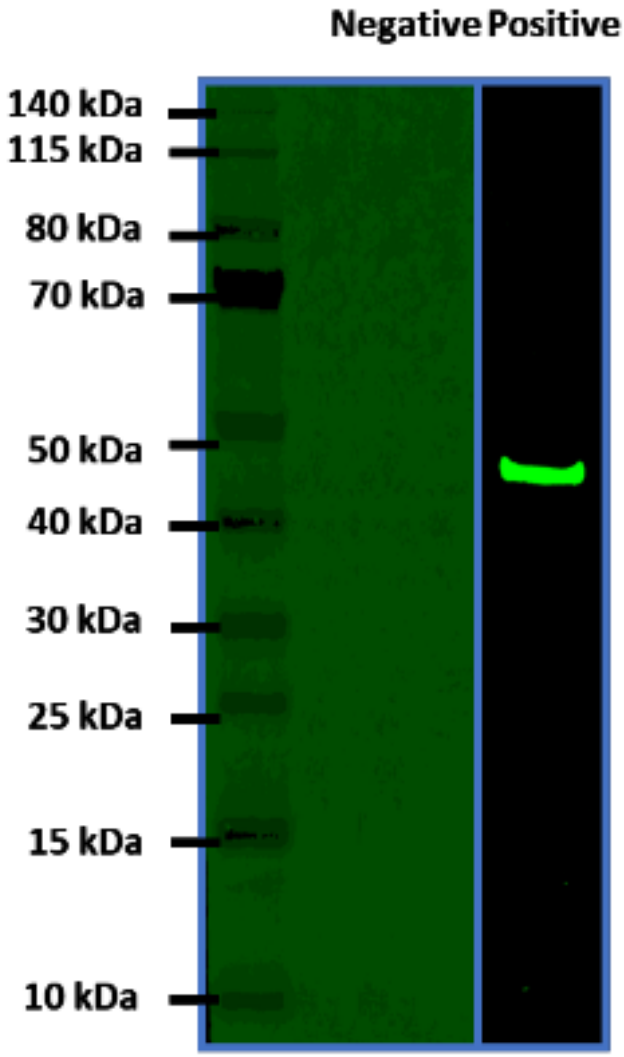
This dossier preserves the complete available evidence progression for SA-000013. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

| 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 |
| 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 | L2 |
| 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

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 Goat 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 = L2
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

None
---

Image: Identifyn® LLC

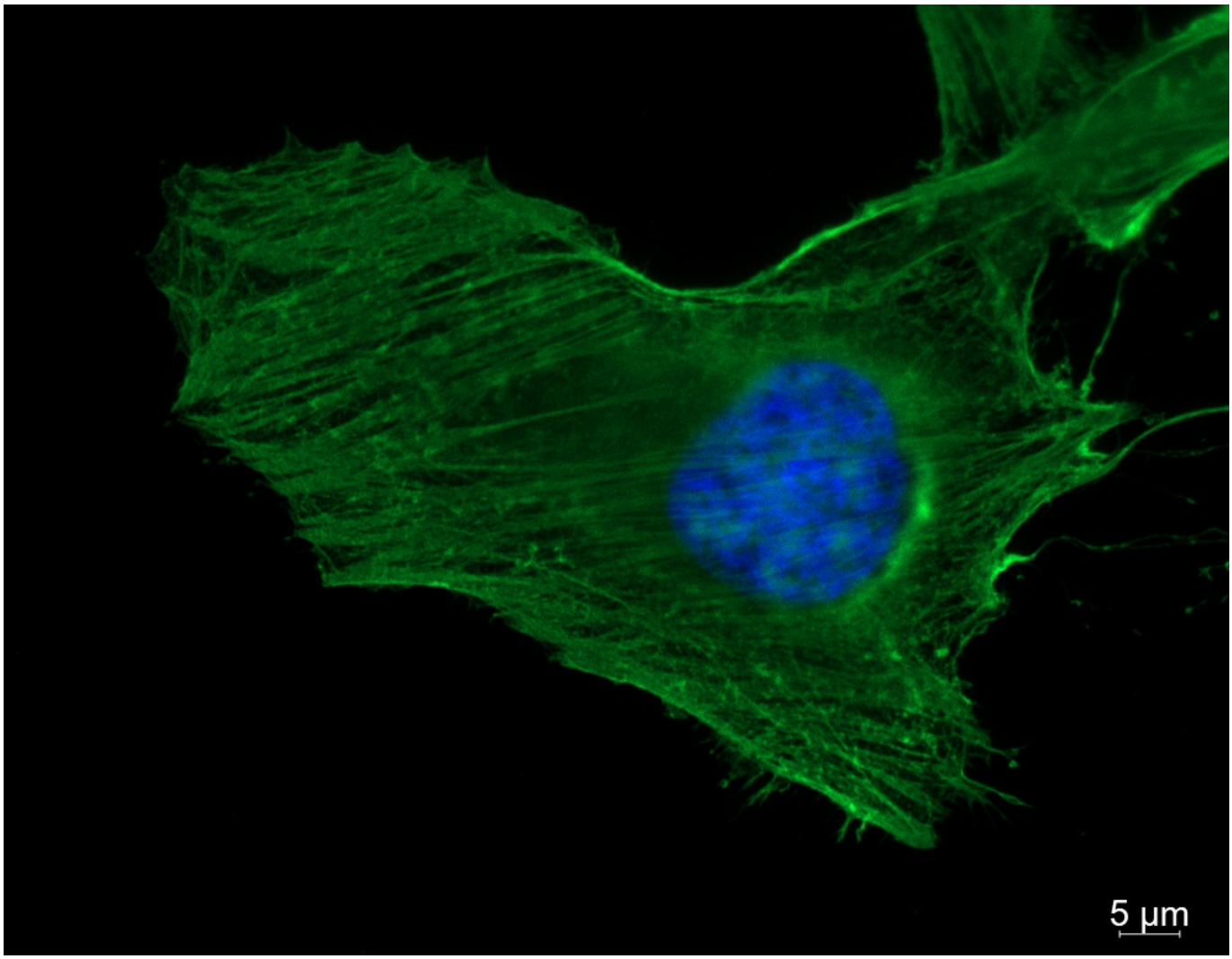

## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **Stage** | Western blot |
| **Instrument** | Azure Sapphire FL |

## SOURCE PROVENANCE

| | |
|---|---|
| **Microscope configuration** | Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s) |
| **Structured source metadata values** | 11 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | FEEB3F80D39617ECB1607C12474121285C5091EEB3CDF9642C8B3D138A43FB4D |
| **Method PDF SHA-256** | 4B1635559D4818A0B95D19ACD536E3C6D63961F7F35628D15994A61E7DB3B7DF |

## ORIGINAL IMAGE EVIDENCE / WIDEFIELD




| | |
|---|---|
| EVIDENCE TIER | Widefield |
| INSTRUMENT | ZEISS Axio Imager.M1 |
| CELL MODEL | HeLa |
| DETECTED DYES | 405/DAPI; 488 |
| METADATA VALUES | 30 |

# STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| Objective | Plan-Apochromat 63x/1.40 Oil M27 |
| Channels | 2 |
| Scaling (Per Pixel) | 0.102µm X 0.102µm |
| Image size (Pixels) | 455 X 446 |
| Image Size (Scaled) | 47.12µm X 45.19µm |
| 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 \| 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 @20% \| X-Cite Xylis Lamp Intensity @15% |
| Exposure Time | 150ms \| 80ms |
| Excitation Wavelength | 493 \| 353 |
| Emission Wavelength | 517 \| 465 |
| Effective NA | 1.4 |
| Imaging Device | Axiocam MR R3 |
| Camera Adapter | 1X |
| Depth of Focus | 0.80µm \| 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

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, 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® 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 = 2
Scaling (Per Pixel) = 0.102µm X 0.102µm
Image size (Pixels) = 455 X 446
Image Size (Scaled) = 47.12µm X 45.19µm
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 @20%
Exposure Time = 150ms
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

## 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

---

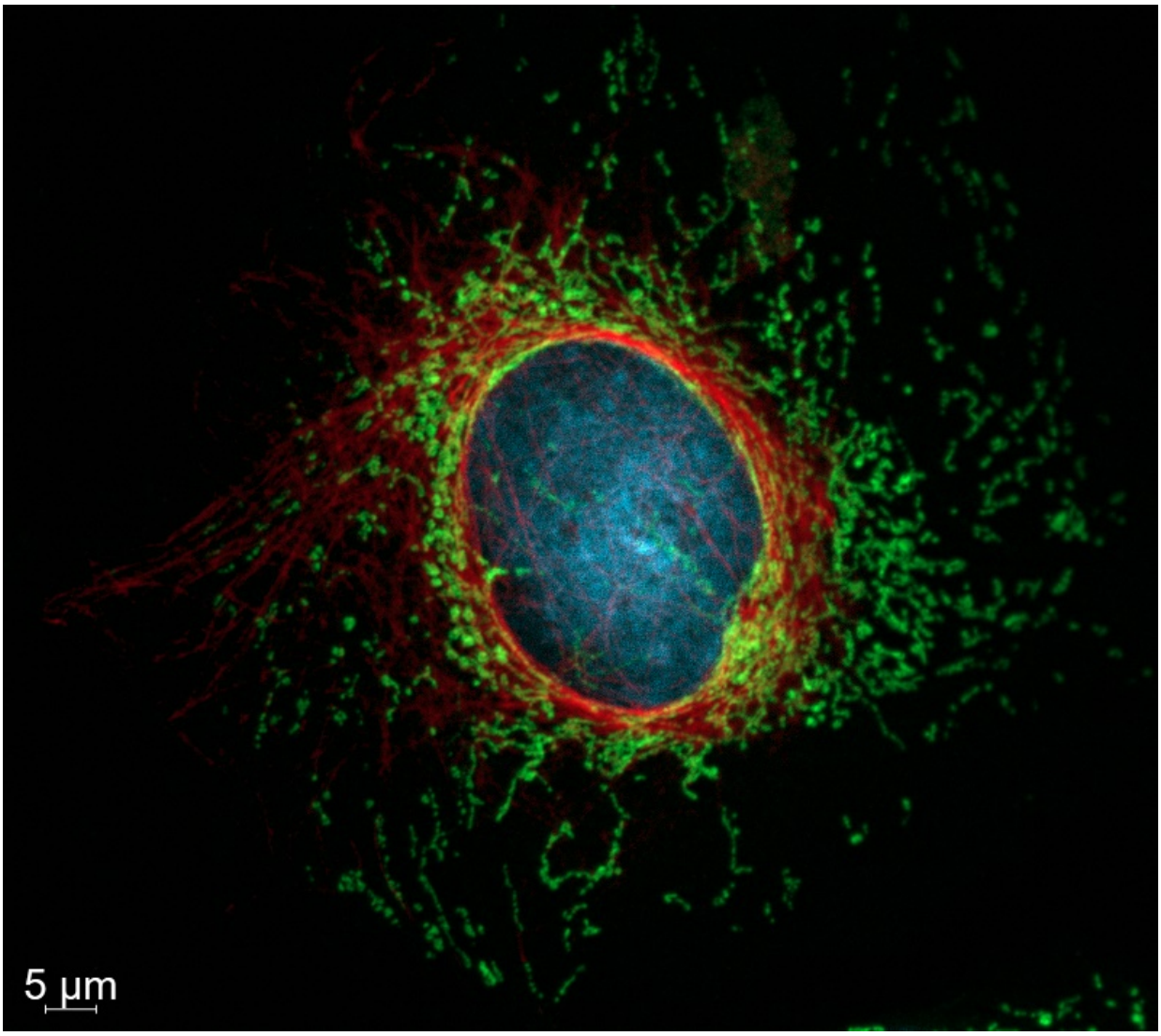
## Image by E.F. Simon



## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **Stage** | Widefield |
| **Instrument** | ZEISS Axio Imager.M1 |
| **Microscope configuration** | Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| **Structured source metadata values** | 30 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | EB9D2BBB0694BB02D5EED3D7B2481145D40596370E5EAF7443AD79308185BE88 |
| **Method PDF SHA-256** | C8152E08ED247AB6779BC8F03B8DE09D42834C2D20B629BA369A82B9CA18A980 |

# 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; 647 |
| 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) | 797 X 708 |
| Image Size (Scaled) | 113.86µm X 101.14µm |
| Bit Depth | 14 Bit |
| Image Center Position | X: 48.12mm, Y: 56.96mm |
| Stage Position | X: 48.12mm, Y: 56.96mm |
| ROI Center Offset | X: 1.14µm, Y: 0.00µm |
| Reflector | 38 HE eGFP \| 350 Cy5 \| 49 DAPI |
| Beam Splitter | 495 \| 660 \| 395 |
| Filter Excitation Wavelength | 450 - 490 \| 625 - 665 \| 335-383 |
| Filter Emission Wavelength | 500 - 550 \| 665 - 715 \| 420-470 |
| Contrast Method | Fluorescence |
| Light source | X-Cite Xylis Lamp Intensity @32.72% \| X-Cite Xylis Lamp Intensity @81.66% \| X-Cite Xylis Lamp Intensity = 5% |
| Exposure Time | 340ms \| 400ms \| 30ms |
| Excitation Wavelength | 493 \| 653 \| 353 |
| Emission Wavelength | 517 \| 668 \| 465 |
| Effective NA | 1.25 |
| Imaging Device | Axiocam 807 |
| Camera Adapter | 1X |
| Depth of Focus | 1.00µm \| 1.30µm \| 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse IgG (H + L)
Cat ID# - SA 000059

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), 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, beta-3 Tubulin Monoclonal Antibody (2G10), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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) = 797 X 708
Image Size (Scaled) = 113.86µm X 101.14µm
Bit Depth = 14 Bit
Image Center Position = X: 48.12mm, Y: 56.96mm
Stage Position = X: 48.12mm, Y: 56.96mm
ROI Center Offset = X: 1.14µm, 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 @32.72%
Exposure Time = 340ms
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

## 647 -

Reflector = 350 Cy5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 665
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @81.66%
Exposure Time = 400ms
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 = 5%
Exposure Time = 30ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.25
Imaging Device = Axiocam 807
Camera Adapter = 1X
Depth of Focus = 0.90µm
Binning Mode = 2,2

## Post Processing - SIM Processing

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

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

## Image Corrections

## None

---

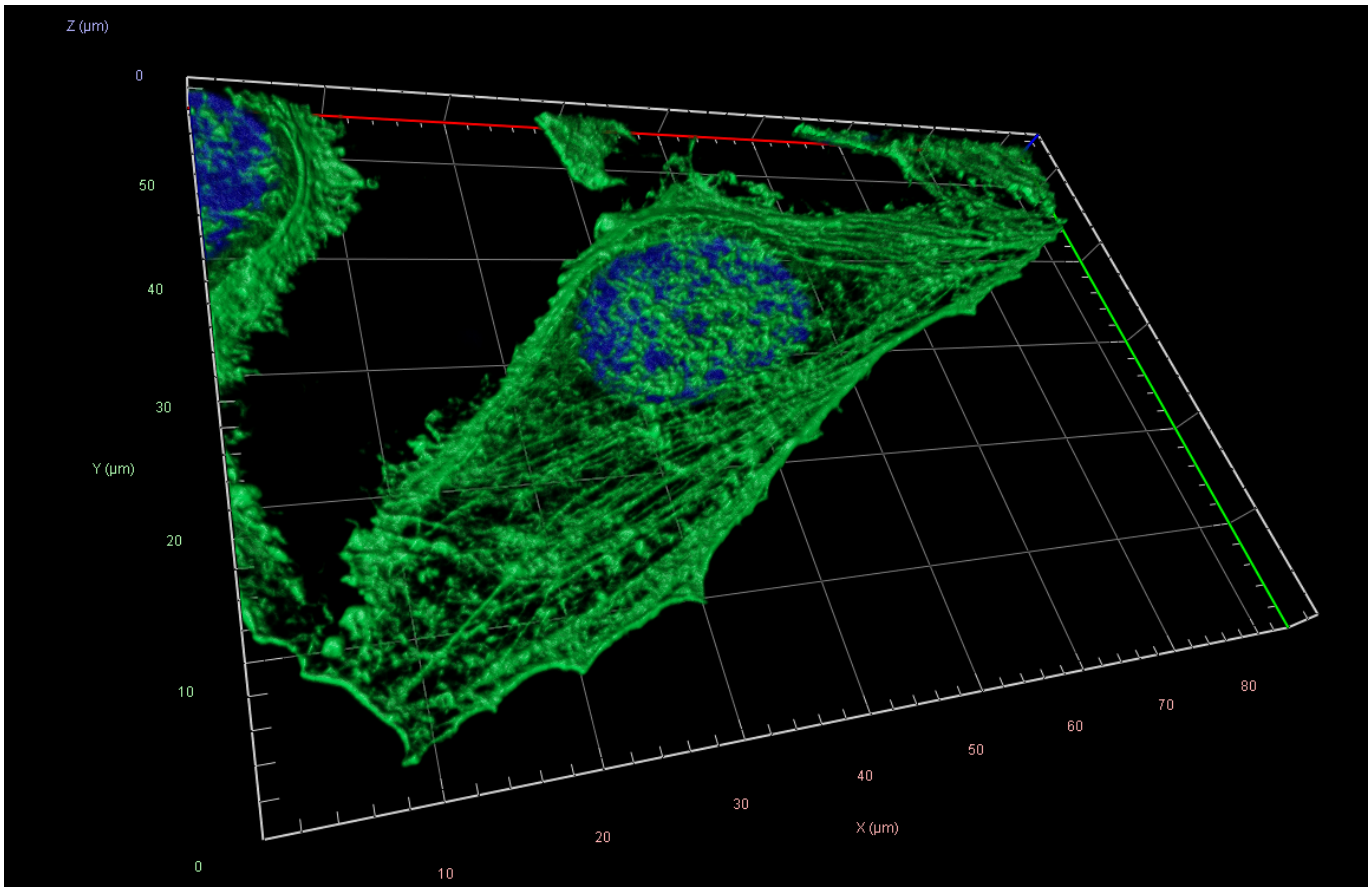
## Image by J.K. Bennett



## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | 379FFD0D5F28F54CCC8464D2E96425826E7FFEEBD87207932C47F6C9B490A2FB |
| **Method PDF SHA-256** | 67E998FB4E65C7414A0178ABF4A75DEA059F56196E31A9CFDD1E7C6A406F1419 |

# ORIGINAL IMAGE EVIDENCE / CONFOCAL




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

# STRUCTURED ACQUISITION METADATA / CONFOCAL

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye: |
| Objective | with a Plan-Apochromat 63X/1.40 oil DIC M27 |
| Z-Stack | 43 Slices (2.94µm) |
| Channels | 2 |
| Scaling (Per Pixel) | 0.07060 µm X 0.07060 µm X 0.07000 µm |
| Image Size (Pixels) | 1187 X 775 |
| Image Size (Scaled) | 83.80µm X 54.72µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 73.01mm, Y: 50.26mm |
| Stage Position | X: 73.01mm, Y: 50.26mm |
| ROI Center Offset | X: 0.00µm, Y: 0.00µm |
| Reflector | None |
| Contrast Method | Fluorescence |
| Pinhole | 1.00AU / 45µm \| 1.00 AU / 37µm |
| LMS MBS | MBS 405 + 488 + 561 + 640(T10/R90) |
| Laser Wavelength | 488nm @0.5% \| 405nm @0.5% |
| Laser Blanking | Enabled |
| Scan Mode | Frame |
| Scan Zoom | 1.2 |
| Rotation | 0° |
| Sampling | 1.00 |
| Pixel Time | 0.65µs |
| Frame Time | 8.72s |
| LSM Scan Speed | 8 |
| Scan Direction | Bidirectional |
| Line Step | 1 |
| Excitation Wavelength | 493 \| 353 |
| Emission Wavelength | 517 \| 465 |
| Effective NA | 1.4 |
| Detection Wavelength | 484 - 590 \| 410-470 |
| Imaging Device | Airyscan \| GaAsp-PMT |
| Detector Type | GaAsp-PMT |
| Detector Gain | 650 V |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Offset | 0 |
| Detector Digital Gain | 1.0 |
| Averaging | 8 |
| 3D Volume Projection | Precise |
| Appearance | Threshold 7% (488), Threshold 3% (DAPI), Ramp 93% (488), Ramp 97% (DAPI), Maximum 100% all channels |
| Background | Black |
| Light | Brightness133%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping |
| Projection | View Angle 48°, 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

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, 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® 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 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 = 43 Slices (2.94µm)

Channels = 2
Scaling (Per Pixel) = 70.60nm X 70.60nm X 70.00nm
Image Size (Pixels) = 1187 X 775
Image Size (Scaled) = 83.80µm X 54.72µm
Bit Depth = 16 Bit
Image Center Position = X: 73.01mm, Y: 50.26mm
Stage Position = X: 73.01mm, Y: 50.26mm
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 @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.65µs
Frame Time = 8.72s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 484 - 590
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 650 V
Detector Offset = 0
Detector Digital Gain = 1.0

## DAPI -

Reflector - None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 37µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.2
Rotation = 0°
Sampling = 1.00
Pixel Time = 0.65µs
Frame Time = 8.72s
LSM Scan Speed = 8
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
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

## 3D Image Processing

3D Volume Projection = Precise
Appearance = Threshold 7% (488), Threshold 3% (DAPI), Ramp 93% (488), Ramp 97% (DAPI), Maximum 100% all channels
Background = Black
Light = Brightness133%, Azimuth 80°,, elongation -130°, Enabled Directional Light and Tone Mapping
Projection = View Angle 48°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Image Corrections

## None

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## Image by J.K. Bennett



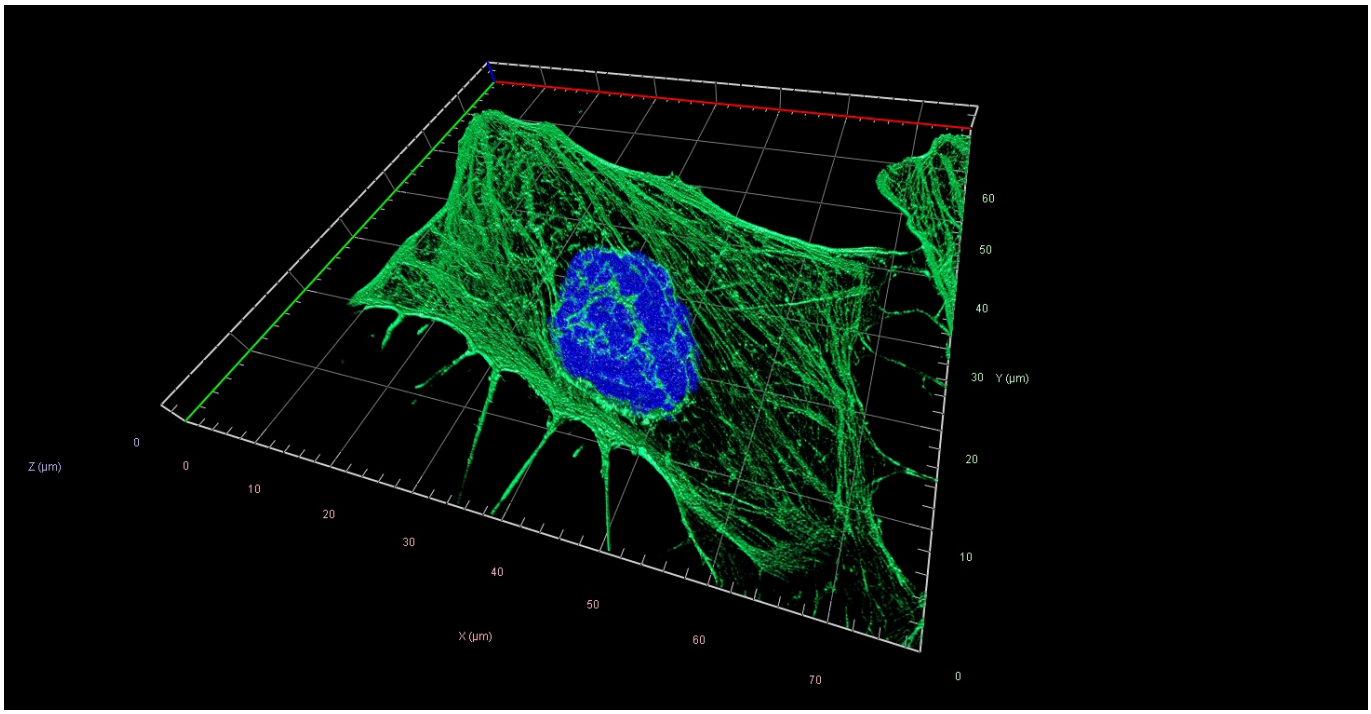
## SOURCE PROVENANCE

| | |
|---|---|
| Catalog | SA-000013 |
| Stage | Confocal |

## SOURCE PROVENANCE

| | |
|---|---|
| **Instrument** | ZEISS LSM 900 |
| **Microscope configuration** | Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye: |
| **Structured source metadata values** | 45 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 11DD1C341CB1226EB240CE151D0A18ECD113B8A0A87C8ED5284A32884668DF0B |
| **Method PDF SHA-256** | C29857500DB65F4F270B659B6ADA2833FA60834D0FC68E85B1CBE7CAFCDC53E1 |

# ORIGINAL IMAGE EVIDENCE / AIRYSCAN




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

# STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Platform / configuration | Zeiss LSM 900 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 | 48 Slices (3.29µm) |
| Channels | 2 |
| Scaling (Per Pixel) | 0.03530 µm X 0.03530 µm X 0.07000 µm |
| Image Size (Pixels) | 2184 X 1979 |
| Image Size (Scaled) | 77.09µm X 69.86µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 82.36mm, Y: 50.62mm |
| Stage Position | X: 82.36mm, Y: 50.62mm |
| ROI Center Offset | X: 0.00µm, Y: 0.00µm |
| Reflector | None |
| Contrast Method | Fluorescence |
| Pinhole | 5.31 AU / 236µm \| 5.00 AU / 182µm |
| LMS MBS | MBS 405 + 488 + 561 + 640(T10/R90) |
| Laser Wavelength | 488nm @0.3% \| 405nm @0.5% |
| Laser Blanking | Enabled |
| Scan Mode | Frame |
| Scan Zoom | 1.3 |
| Rotation | 0° |
| Sampling | 2.00 |
| Pixel Time | 0.96µs |
| Frame Time | 21.88s |
| LSM Scan Speed | 6 |
| Scan Direction | Bidirectional |
| Line Step | 1 |
| Averaging | 4 |
| Excitation Wavelength | 493 \| 353 |
| Emission Wavelength | 517 \| 465 |
| Effective NA | 1.4 |
| Detection Wavelength | 495-569 \| 400-464 |
| Imaging Device | Airyscan |
| Detector Type | GaAsp-PMT |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Gain | 850 V |
| Detector Offset | 0 |
| Detector Digital Gain | 1.0 |
| AiryScan Mode | Super Resolution 8.7 (3D, Auto) |
| 3D Maximum Projection | Precise |
| Appearance | Threshold 4% (488), Threshold 12% (405), Ramp 96% (488), Ramp 88% (405), Maximum 100% |
| Background | Black |
| Light | Brightness 213%, Enabled Directional Light and Tone Mapping |
| Projection | View Angle 66°, 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

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, 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® 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 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 = 48 Slices (3.29µm)

Channels = 2
Scaling (Per Pixel) = 35.30nm X 35.30nm X 70.00nm
Image Size (Pixels) = 2184 X 1979
Image Size (Scaled) = 77.09µm X 69.86µm
Bit Depth = 16 Bit
Image Center Position = X: 82.36mm, Y: 50.62mm
Stage Position = X: 82.36mm, Y: 50.62mm
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 @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.96µs
Frame Time = 21.88s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 495-569
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 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 / 182µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 405nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.3
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.96µs
Frame Time = 21.88s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 400-464
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.7 (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 4% (488), Threshold 12% (405), Ramp 96% (488), Ramp 88% (405), Maximum 100%
Background = Black
Light = Brightness 213%, Enabled Directional Light and Tone Mapping
Projection = View Angle 66°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Image Corrections -

## None

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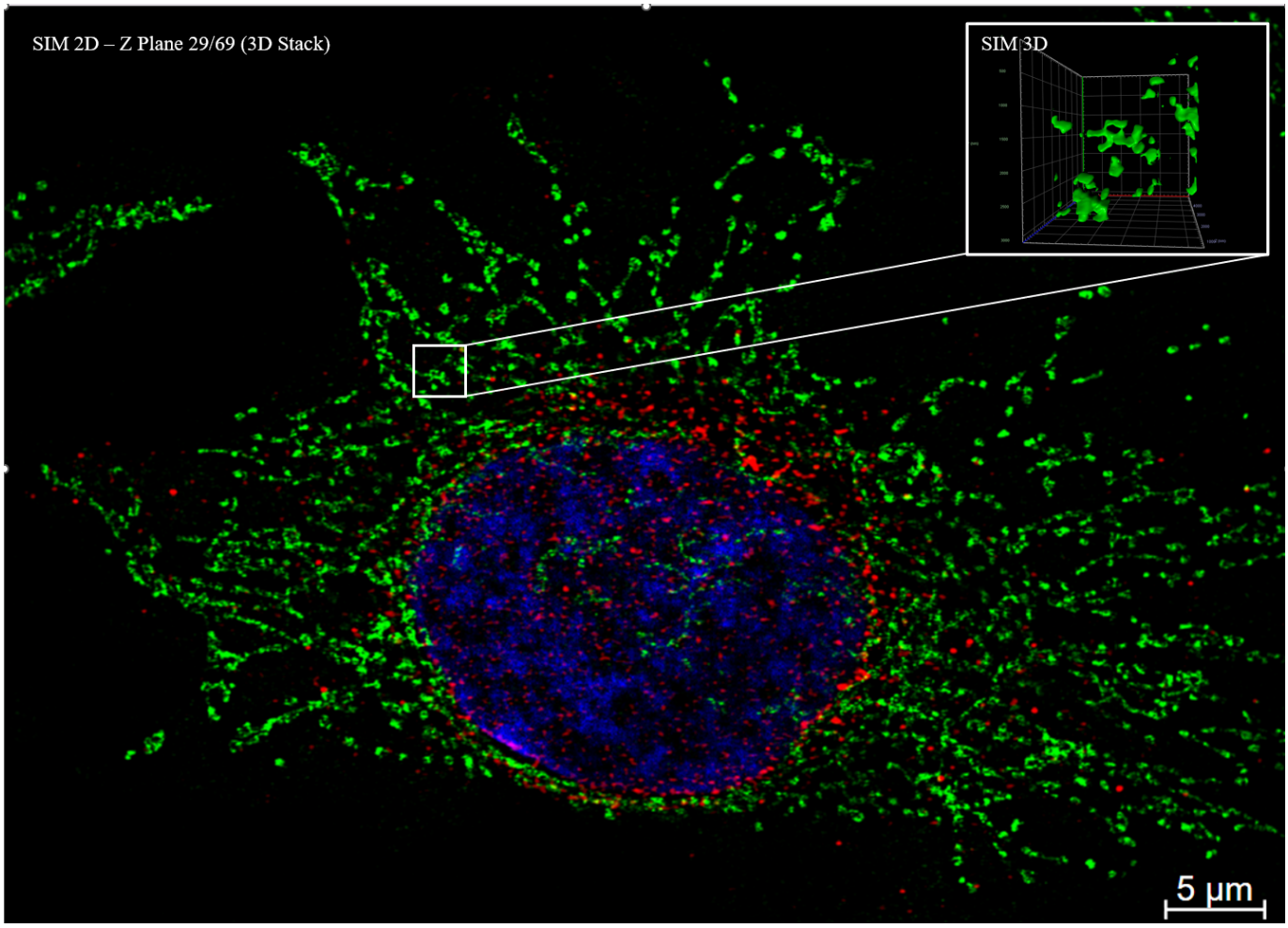
## Image by P. Raut


Copyright 2026 GMD12 LLC.

## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | 45 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | CC1C86B8046F0155FD5D4AEB83C538F2E42257F4F479F1610539CB71F0001160 |
| **Method PDF SHA-256** | 83BE83A2472880028D303B449DBE34BFFC29599764385FF0DC93342B3B9E82E3 |

## ORIGINAL IMAGE EVIDENCE / SIM




| | |
|---|---|
| EVIDENCE TIER | SIM |
| INSTRUMENT | ZEISS Elyra 7 |
| CELL MODEL | HeLa |
| DETECTED DYES | 405/DAPI; 488; 647 |
| METADATA VALUES | 64 |

## 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 488 | Main Beam Splitter Non-Descanned: LBF 405/488/561/642, |
| Dual Camera Beam Splitter | SBS LP 560 |
| Beam Splitter 647 | 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 |
| Single Plane (2D) | Plane 29/69 from Z- Stack |
| Z Stack - (3D) | 69 Slices (4.08µm) |
| Channels | 3 |
| Scaling (Per Pixel) | 0.03130 µm X 0.03130 µm X 0.06000 µm |
| Image Size (Pixels) | 2073 X 1401 |
| Image Size (Scaled) | 64.89µm X 43.86µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 4.01µm, Y: 924.80µm |
| Stage Position | X: 4.01µm, Y: 924.80µm |
| ROI Center Offset | X: 0.00µm, Y: 0.00µm |
| Contrast Method | Fluorescence |
| Laser Wavelength | 488nm @10.00%, \| 642nm @3.00% \| 405nm @20.00%, |
| Scan Mode | Other |
| Scan Zoom | 1.0 |
| Rotation | 0º |
| Frame Time | 34.54s |
| Scan Direction | Unidirectional |
| Line Step | N/A |
| Averaging | 16 |
| Excitation Wavelength | 488 \| 642 \| 405 |
| Emission Wavelength | 515 \| Longpass 570 |
| Effective NA | 1.46 |
| Detection Wavelength | 495-550 \| Beyond 570 Longpass |
| Imaging Device | Edge 4.2 SIM |
| Exposure Time | 50ms |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Depth of Focus | 0.73µm \| 0.81µm |
| 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 \| 32.000000 \| 23.000000 |
| Processing | 3D |
| Auto Sharpness | Yes |
| Sharpness | 9.329592 \| 8.518750 \| 9.3555612 |
| Detrend | No |
| Sectioning | 100.000000 |
| Baseline | Yes |
| Scale to Raw Image | No |
| Experimental PSF | No |
| 3D Surface Projection | Precise |
| Appearance | Threshold 36%, Ambient Light 1%, Specular Light 100%, Shininess 30% |
| Background | Black |
| Light | Azimuth 80°, Elongation -130°, 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Mouse IgG (H + L)
Cat ID# - SA 000059

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

The second primary antibody, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant 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 488 = Main Beam Splitter Non-Descanned: LBF 405/488/561/642,
Dual Camera Beam Splitter = SBS LP 560

Beam Splitter 647 = 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

### Single Plane (2D) = Plane 29/69 from Z- Stack

### Z Stack - (3D) = 69 Slices (4.08µm)

Channels = 3
Scaling (Per Pixel) = 31.30nm X 31.30nm X 60.00nm
Image Size (Pixels) = 2073 X 1401
Image Size (Scaled) = 64.89µm X 43.86µm
Bit Depth = 16 Bit
Image Center Position = X: 4.01µm, Y: 924.80µm
Stage Position = X: 4.01µm, Y: 924.80µm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

### 488 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @10.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 34.54s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 488
Emission Wavelength = 515
Effective NA = 1.46
Detection Wavelength = 495-550
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 = 488.000000
## Grating Period (µm): 27.500000

### SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 9.329592
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

## 647 -

Contrast Method = Fluorescence
Laser Wavelength = 642nm @3.00%
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 34.54s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 642
Emission Wavelength = Longpass 570
Effective NA = 1.46
Detection Wavelength = Beyond 570 Longpass
Imaging Device = Edge 4.2 SIM
Exposure Time = 50ms
Depth of Focus = 0.81µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

## Post Processing - SIM Processing

## Channel = 642.000000
## Grating Period (µm): 32.000000

## SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 8.518750
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

## DAPI -

Contrast Method = Fluorescence
Laser Wavelength = 405nm @20.00%,
Scan Mode = Other
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 34.54s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 405
Emission Wavelength = 515
Effective NA = 1.46
Detection Wavelength = 495-550
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): 23.000000

## SIM

Processing: 3D
Auto Sharpness: Yes
Sharpness: 9.3555612
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF : No

## 3D Image Processing

3D Surface Projection = Precise
Appearance = Threshold 36%, Ambient Light 1%, Specular Light 100%, Shininess 30%
Background = Black
Light = Azimuth 80°, Elongation -130°, 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

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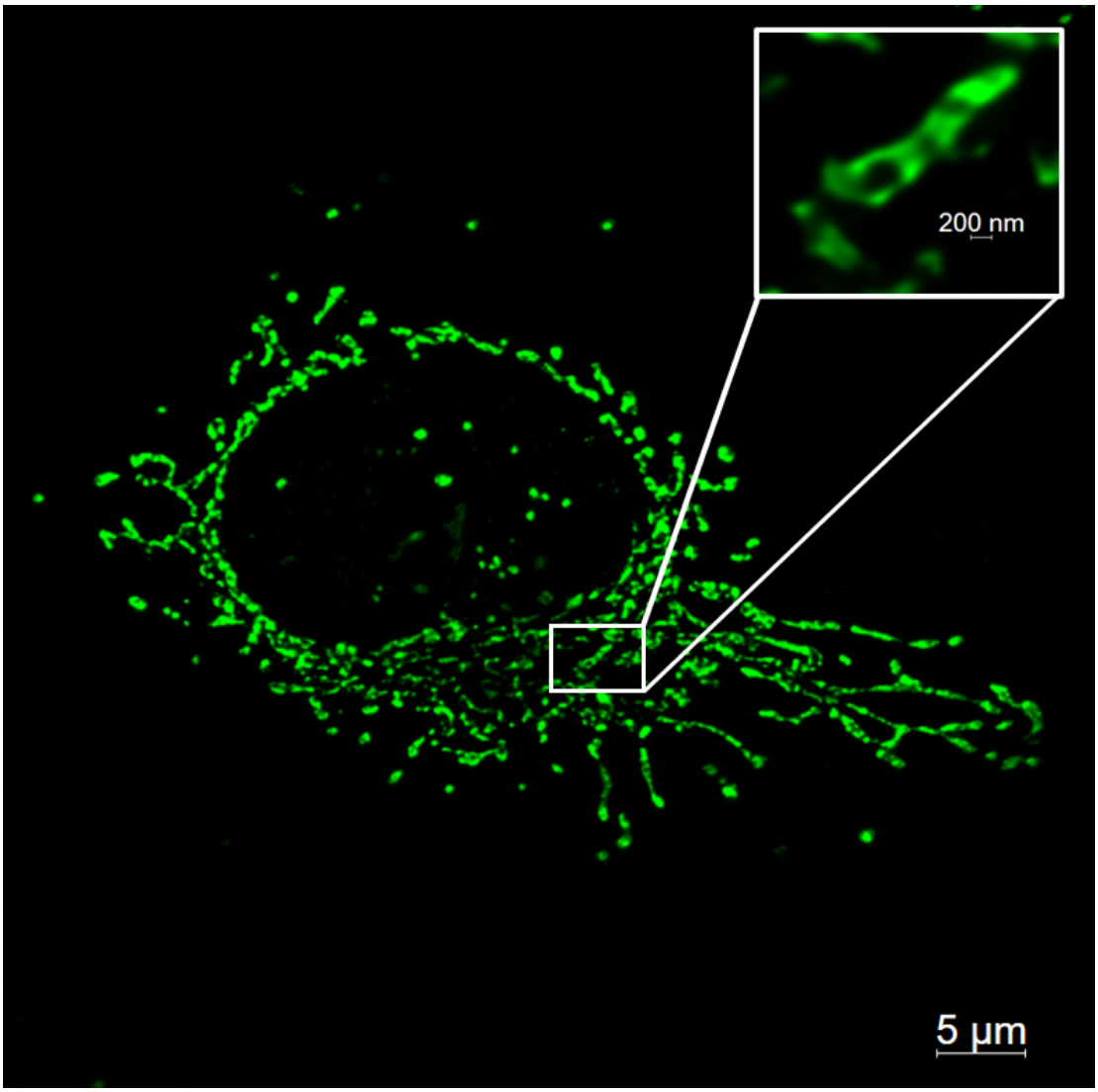
## Image by J.K. Bennett



## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | 64 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | 13A191D2D75C15FE0DCBC653183327327FF74719D186C5D3DCC1558FCEF5AF4A |
| **Method PDF SHA-256** | 61C8FB9F328F4299A3D4D8D1731E024D5DF0033A7E26316853380D07A73A73E2 |

## ORIGINAL IMAGE EVIDENCE / SIM²




| | |
|---|---|
| EVIDENCE TIER | SIM² |
| INSTRUMENT | ZEISS Elyra 7 |
| CELL MODEL | HeLa |
| DETECTED DYES | 405/DAPI; 488 |
| METADATA VALUES | 46 |

## 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 488 | 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 |
| Channels | 1 |
| Scaling (Per Pixel) | 0.03130 µm X 0.03130 µm |
| Image Size (Pixels) | 2000 X 2000 |
| Image Size (Scaled) | 62.61µm X 62.61µm |
| Bit Depth | 16 Bit |
| Image Center Position | X: 4.62µm, Y: 391.10µm |
| Stage Position | X: 4.62µm, Y: 391.10µm |
| ROI Center Offset | X: 0.00µm, Y: 0.00mm |
| Contrast Method | Fluorescence |
| Laser Wavelength | 488nm @3.00% |
| Scan Mode | Frame |
| Scan Zoom | 1.0 |
| Rotation | 0º |
| Frame Time | 14.36s |
| Scan Direction | Unidirectional |
| Line Step | N/A |
| Averaging | 16 |
| Excitation Wavelength | 488 |
| Emission Wavelength | 570 |
| Effective NA | 1.46 |
| Detection Wavelength | 570-1000 |
| Imaging Device | Edge 4.2 SIM |
| Exposure Time | 20ms |
| Depth of Focus | 0.81µm |
| Binning Mode | 1x1 |
| Detector Type | Camera |
| Depth Offset | 0 |

| FIELD | SOURCE-DOCUMENTED VALUE |
|---|---|
| Detector Digital Gain | 0.00 |
| Channel | 488.000000 |
| Grating Period (µm) | 23.000000 |
| Processing | 2D, Leap |
| Input SNR | Low |
| Iterations | 16 |
| Regularization Weight | 0.065000 |
| Process Sampling | 2 |
| Output Sampling | 2 |
| Filter | Median |
| Detrend | No |
| Sectioning | 100.000000 |
| Baseline | Yes |
| Scale to Raw Image | No |
| Experimental PSF | No |

## 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 Goat Anti-Rabbit IgG (H + L)
Cat ID# - SA 000013

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, Mitochondria Rabbit Recombinant Antibody (MTC02,2860R), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and 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 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 488 = 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

### Single Plane (2D)

Channels = 1
Scaling (Per Pixel) = 31.30nm X 31.30nm
Image Size (Pixels) = 2000 X 2000
Image Size (Scaled) = 62.61µm X 62.61µm
Bit Depth = 16 Bit
Image Center Position = X: 4.62µm, Y: 391.10µm
Stage Position = X: 4.62µm, Y: 391.10µm
ROI Center Offset = X: 0.00µm, Y: 0.00mm

### 488 -

Contrast Method = Fluorescence
Laser Wavelength = 488nm @3.00%
Scan Mode = Frame
Scan Zoom = 1.0
Rotation = 0º
Frame Time = 14.36s
Scan Direction = Unidirectional
Line Step = N/A
Averaging = 16
Excitation Wavelength = 488
Emission Wavelength = 570
Effective NA = 1.46
Detection Wavelength = 570-1000
Imaging Device = Edge 4.2 SIM
Exposure Time = 20ms
Depth of Focus = 0.81µm
Binning Mode = 1x1
Detector Type = Camera
Depth Offset = 0
Detector Digital Gain = 0.00

## Post Processing - SIM2 Processing

## Channel = 488.000000
## Grating Period (µm): 23.000000

### SIM2

Processing: 2D, Leap
Input SNR: Low
Iterations: 16
Regularization Weight: 0.065000
Process Sampling: 2
Output Sampling: 2
Filter: Median
Detrend: No
Sectioning: 100.000000
Baseline: Yes
Scale to Raw Image: No
Experimental PSF: No

## Image Corrections

## None

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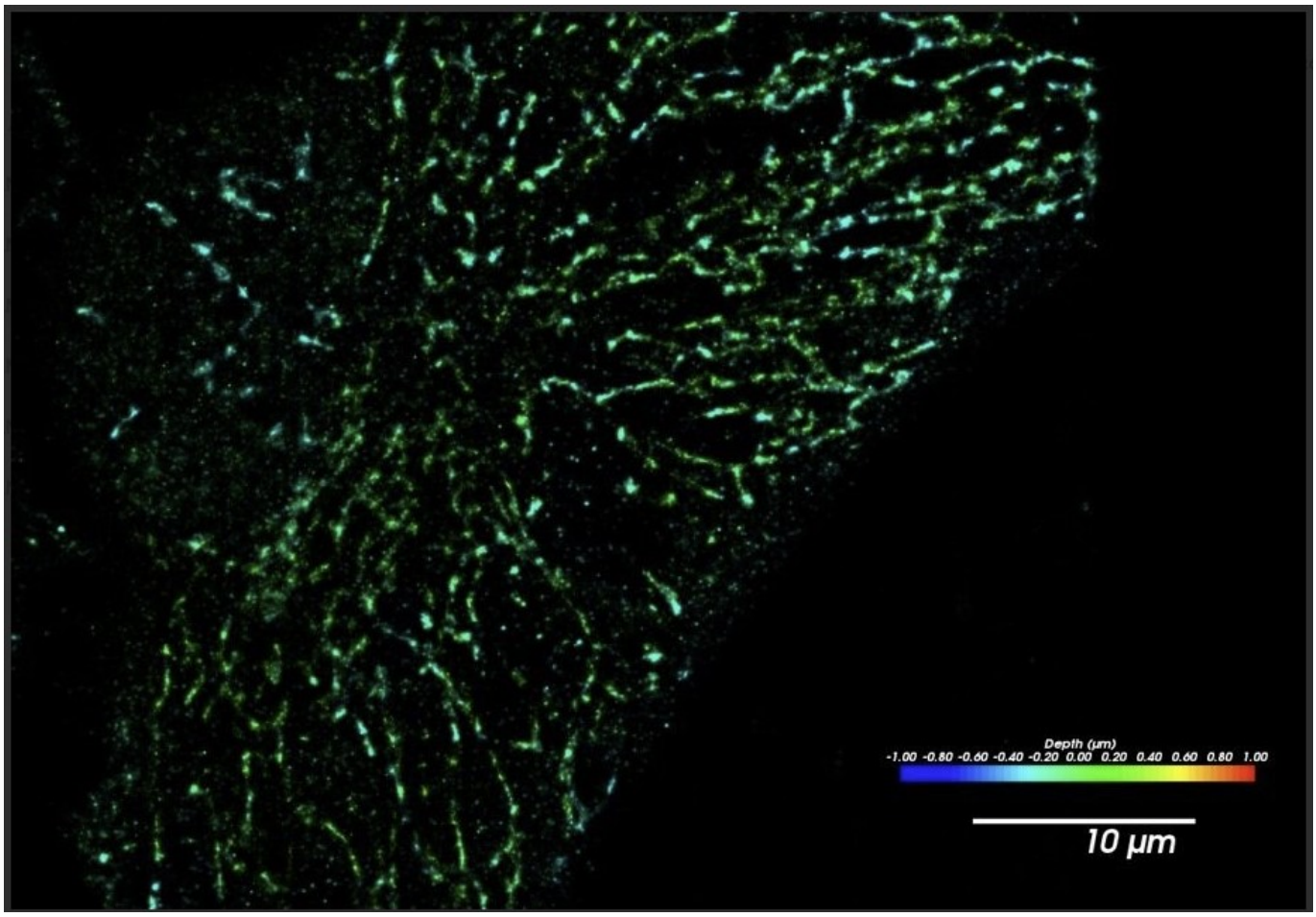
## Image by J.K. Bennett

## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | 46 |
| **Metadata source** | PRESERVED_SOURCE_METHOD_PDF_TEXT |
| **Cell model** | HeLa |
| **Image SHA-256** | CF1E0B2430360A026A71540EA309F3FC564F43B1114E2A5BB8A2425BAF415AD9 |
| **Method PDF SHA-256** | F7556B61787B2A20EAA9B95AF218E5AFE97D9C0DF1AA46ECBB933081730F9860 |

# 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 | 179.8 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: 179; End: 179 |
| 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 | 16px |
| 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: 02 |
| 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 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 Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

Hela cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber, followed by PFA fixation and blocked with Identifyn'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 Identifyn's™ proprietary Wash Buffer and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn'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 Identifyn's Calibration Standard Operating Procedure, which can be found here.

### https://identifyn.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: 179.8 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: 179; End: 179

## 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: 16px
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: 02
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

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## Image by S. Thakur


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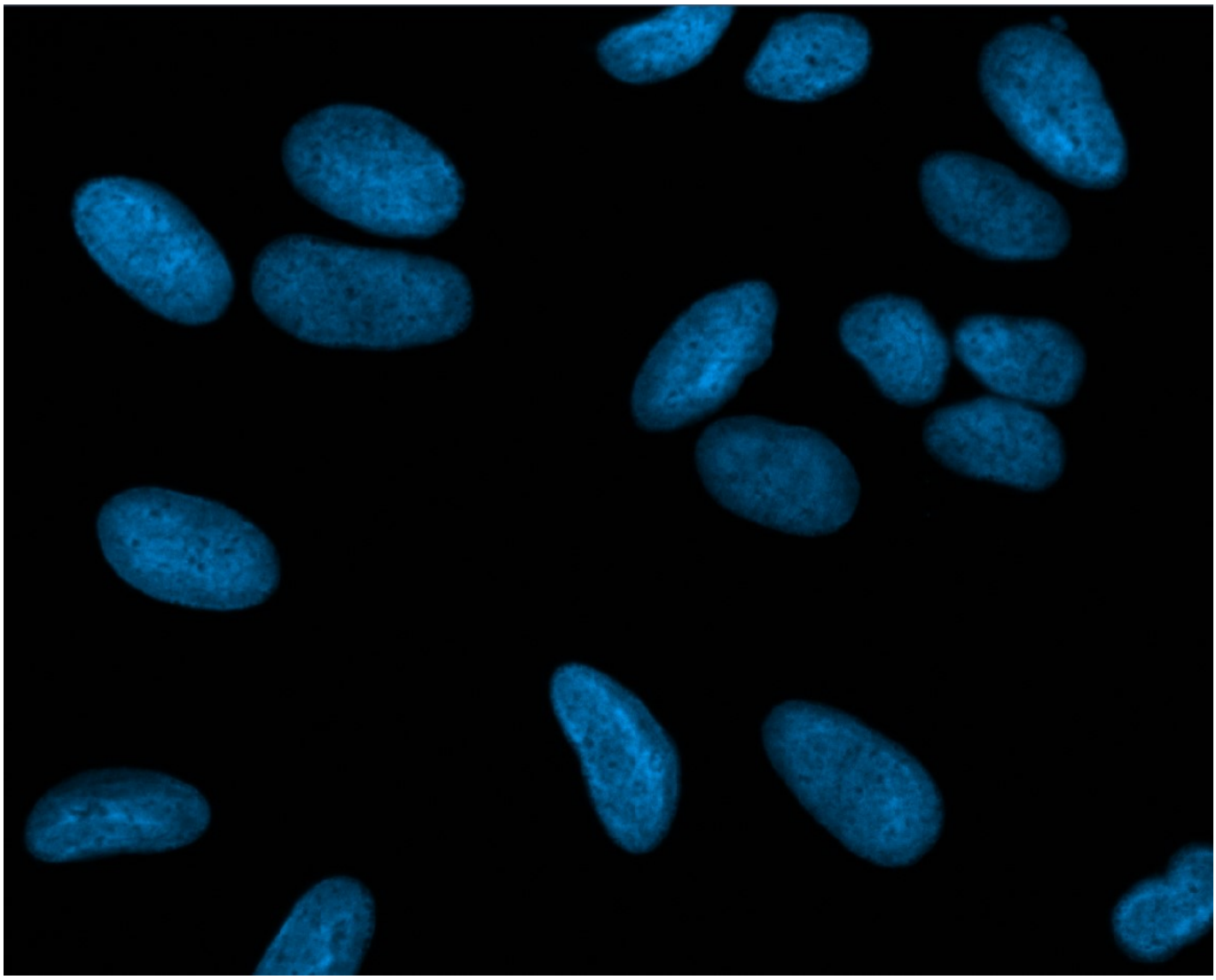
## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | BA32E3E6BBB91EB4332E2B3F59D0CC139A6274C04E40C7F52E8D4AE07D404F13 |
| **Method PDF SHA-256** | CFD4DDD11AB49F422DAE45ADF0B6BE0B229F1BE23A98DC4FD5E41CB9593E18B6 |

## 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) | 1255 X 1008 |
| Image Size (Scaled) | 179.29µm X 144.00µm |
| Bit Depth | 14 Bit |
| Image Center Position | X: 50.14mm, Y: 51.35mm |
| ROI Center Offset | X: 50.14µm, Y: 51.35µm |
| 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 Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000013

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) = 1255 X 1008
Image Size (Scaled) = 179.29µm X 144.00µm
Bit Depth = 14 Bit
Image Center Position = X: 50.14mm, Y: 51.35mm
ROI Center Offset = X: 50.14µm, Y: 51.35µm

### 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.

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## Image by J.K. Bennett



## SOURCE PROVENANCE

| | |
|---|---|
| **Catalog** | SA-000013 |
| **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** | B7F4D3B5D0F9E7C022E913AD7BBF2D4659991F63DBEEB359AEEB94BA899973FF |
| **Method PDF SHA-256** | 2BC8D4A255FA18929F3DF42BBEF104D27A43B4765A4613CB3A296103808DBE2C |