

IDENTIFYN RESOLUTION STEPDOWN

## SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (H + L)

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN  
SIM -> SIM<sup>2</sup> -> SINGLE-MOLECULE STORM

8

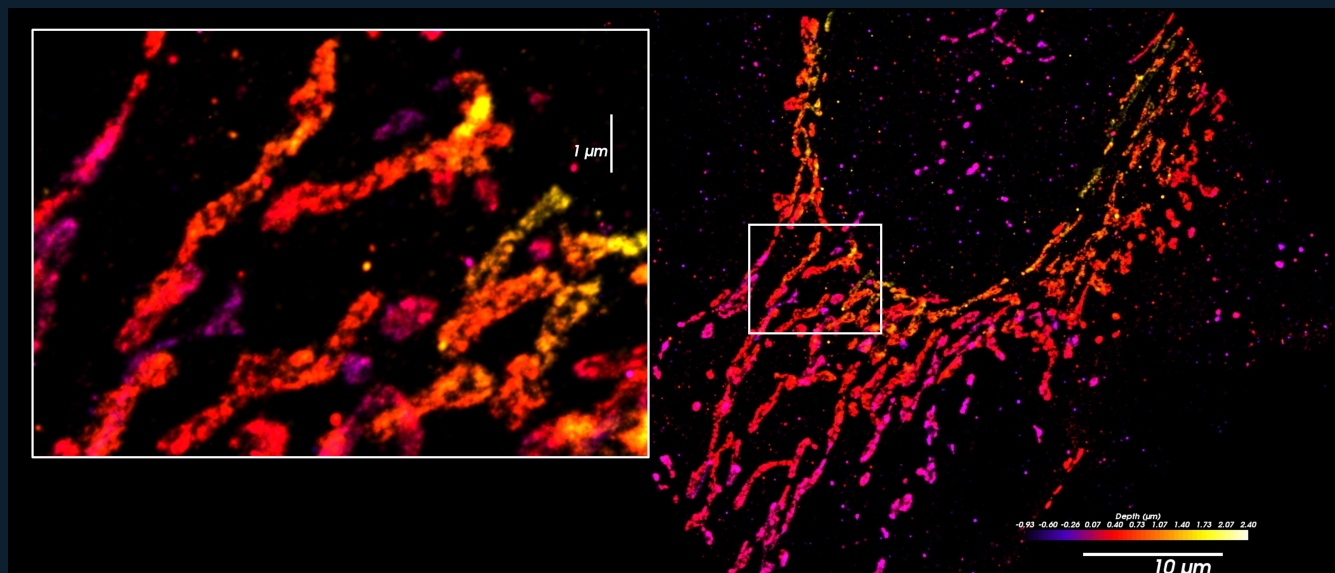
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000066 | 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
- SIM<sup>2</sup> reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

## SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (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 -> SIM<sup>2</sup> -> Single-molecule STORM -> Control

## BENNETT ASSESSMENT

The preserved SA-000066 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-000066 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            | 647                             | Not deterministically stated in the acquisition block                                                                                                               |
| Widefield               | 405/DAPI; 488; 647              | 2; 50 Cy5; 38 HE eGFP; 625 - 655; 450 - 490; 655 - 715; 500 - 550; 653                                                                                              |
| Widefield SIM — Apotome | 405/DAPI; 488; 647              | 2; 50 Cy5; 38 HE eGFP; 625 - 655; 450 - 490; 665 - 715; 500 - 550; 653                                                                                              |
| Confocal                | 405/DAPI; 488; 647              | 2; None; 640nm @0.5%; 488nm @0.5%; 653; 493; 668; 517                                                                                                               |
| Airyscan                | 405/DAPI; 488; 647; 680         | 1; None; 640nm @0.6%; 653; 668                                                                                                                                      |
| SIM                     | 405/DAPI; 488; 647              | 2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T1-Axiocam 820-SR; 640 |
| SIM <sup>2</sup>        | 405/DAPI; 488; 647              | 2; 1; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820 -SR; T1-Axiocam 820-SR; 640 |
| Single-molecule STORM   | 647                             | Both Channels                                                                                                                                                       |
| Control                 | 405/DAPI; 647                   | 2; 50 Cy5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653                                                                                                 |

## 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 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

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): 1359 X 809; Image Size (Pixels): 1359 X 809; Bit Depth: 12 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @50%; X-Cite Xylis Lamp Intensity @40%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 29.

#### 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; 647.

#### LITERATURE CORRELATION

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

#### INTERPRETIVE LIMIT

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

#### CLAIM CEILING

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

## MODALITY ASSESSMENT / PART 3 OF 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: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1250 X 723; Image Size (Pixels): 1250 X 723; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 60ms; 200ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @5%; X-Cite Xylis Lamp Intensity @40%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

#### VISIBLE OBSERVATION

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

#### SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 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: Channels: 2; Scaling (Per Pixel): 85.07nm X 85.07nm; Image size (Pixels): 917 X 917; Image Size (Pixels): 917 X 917; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsp-PMT 2; Airyscan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.58µs; Frame Time: 9.09s. Illumination: Laser Wavelength: 640nm @0.5%; 488nm @0.5%. Optical/scan: Pinhole: 1.00AU / 57µm; 1.00AU / 43µm; Scan Zoom: 1.3; LSM Scan Speed: 9; Scan Direction: Bidirectional; Effective NA: 1.4. Structured source metadata values: 38.

#### 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; 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

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 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: 65 Slices (3.2µm); Channels: 1; Scaling (Per Pixel): 42.52nm X 42.52nmµm X 50.00nm; Image size (Pixels): 3383 X 3383; Image Size (Pixels): 3383 X 3383; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.62µs; Frame Time: 16.86s. Illumination: Laser Wavelength: 640nm @0.6%. Optical/scan: Pinhole: 5.00AU / 280µm; Scan Zoom: 0.70; LSM Scan Speed: 6; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 54°, Scale Z 110%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.6 (3D, Auto). Structured source metadata values: 39.

#### 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; 647; 680.

#### 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. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 47 Slices (2.3µm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 5056 X 5056; Image Size (Pixels): 5056 X 5056; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820. Timing: Exposure Time: 120ms; Frame Time: 1.59s. Illumination: Laser Wavelength: 640nm @2.5%; 561nm @3.0%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: 2; Result Sampling: 4; Projection: View Angle 42°, Scale Z 60%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

#### VISIBLE OBSERVATION

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

#### SOURCE-REPORTED RESULT

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

### SIM<sup>2</sup> / SOURCE EVIDENCE PRESERVED

#### DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 67 Slices (3.3µm); Channels: 2; 1; Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm; Image size (Pixels): 1569 X 1455; Image Size (Pixels): 1569 X 1455; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820. Timing: Exposure Time: 120ms; Frame Time: 1.59s. Illumination: Laser Wavelength: 640nm @2.5%; 561nm @3.0%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

#### VISIBLE OBSERVATION

A preserved presentation image is available for SIM<sup>2</sup>. BENNETT records the visible source presentation without creating a new native measurement.

#### SOURCE-REPORTED RESULT

The source identifies this object as SIM<sup>2</sup>, documents platform context as ZEISS Elyra, 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

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 SIM<sup>2</sup> 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 647.

#### 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 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): 1261 X 980; Image Size (Pixels): 1261 X 980; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 32.

#### VISIBLE OBSERVATION

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

#### SOURCE-REPORTED RESULT

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

#### LITERATURE CORRELATION

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

#### INTERPRETIVE LIMIT

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

#### CLAIM CEILING

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

## CROSS-MODALITY SYNTHESIS

The preserved SA-000066 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): 85.07nm X 85.07nm -> 0.08507  $\mu$ m X 0.08507  $\mu$ m. 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)=917 X 917; Image Size (Scaled)=78.01 $\mu$ m X 78.01 $\mu$ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

### HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 42.52nm X 42.52nm $\mu$ m X 50.00nm -> 0.04252  $\mu$ m X 0.04252  $\mu$ m X 0.05000  $\mu$ m. 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)=3383 X 3383; Image Size (Scaled)=143.85 $\mu$ m X 143.85 $\mu$ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

### HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689  $\mu$ m X 0.01689  $\mu$ m X 0.05000  $\mu$ m. 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)=5056 X 5056; Image Size (Scaled)=85.40 $\mu$ m X 85.40 $\mu$ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

### HIGH CONFIDENCE - AUTO-RECONCILED

SIM<sup>2</sup> / Scaling (Per Pixel): 16.89nm X 16.89nm X 50.00nm -> 0.01689  $\mu$ m X 0.01689  $\mu$ m X 0.05000  $\mu$ m. 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)=1569 X 1455; Image Size (Scaled)=26.50 $\mu$ m X 24.58 $\mu$ m; 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

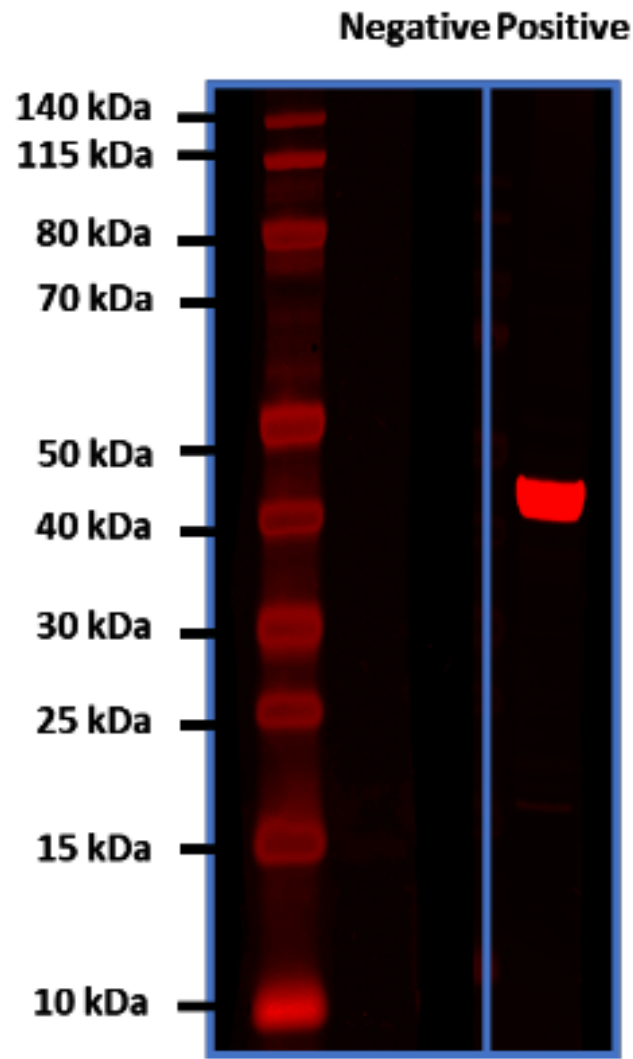
This dossier preserves the complete available evidence progression for SA-000066. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

| 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                      | PRESERVED |
| 7                  | SIM <sup>2</sup>        | ZEISS Elyra                      | 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   | 647               |
| 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                    | 638nm                                                                                         |
| Emission Filter          | 676±37nm                                                                                      |
| Detector                 | Avalanche Photodiode                                                                          |
| Intensity                | L1                                                                                            |
| 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™ 647 Affinity Purified Rabbit Anti-Mouse F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

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 Monoclonal Antibody (mAbGEa) (MA1-744) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit anti-Mouse F(ab)<sub>2</sub> was incubated for 1 hour and washed 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

#### Scan Settings

Detection mode = Fluorescence

Laser = 638nm

Emission Filter = 676±37nm

Detector = Avalanche Photodiode

Intensity = L1

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

#### Post Processing

None

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Image: Identifyn® LLC

Copyright 2026 GMD12 LLC.

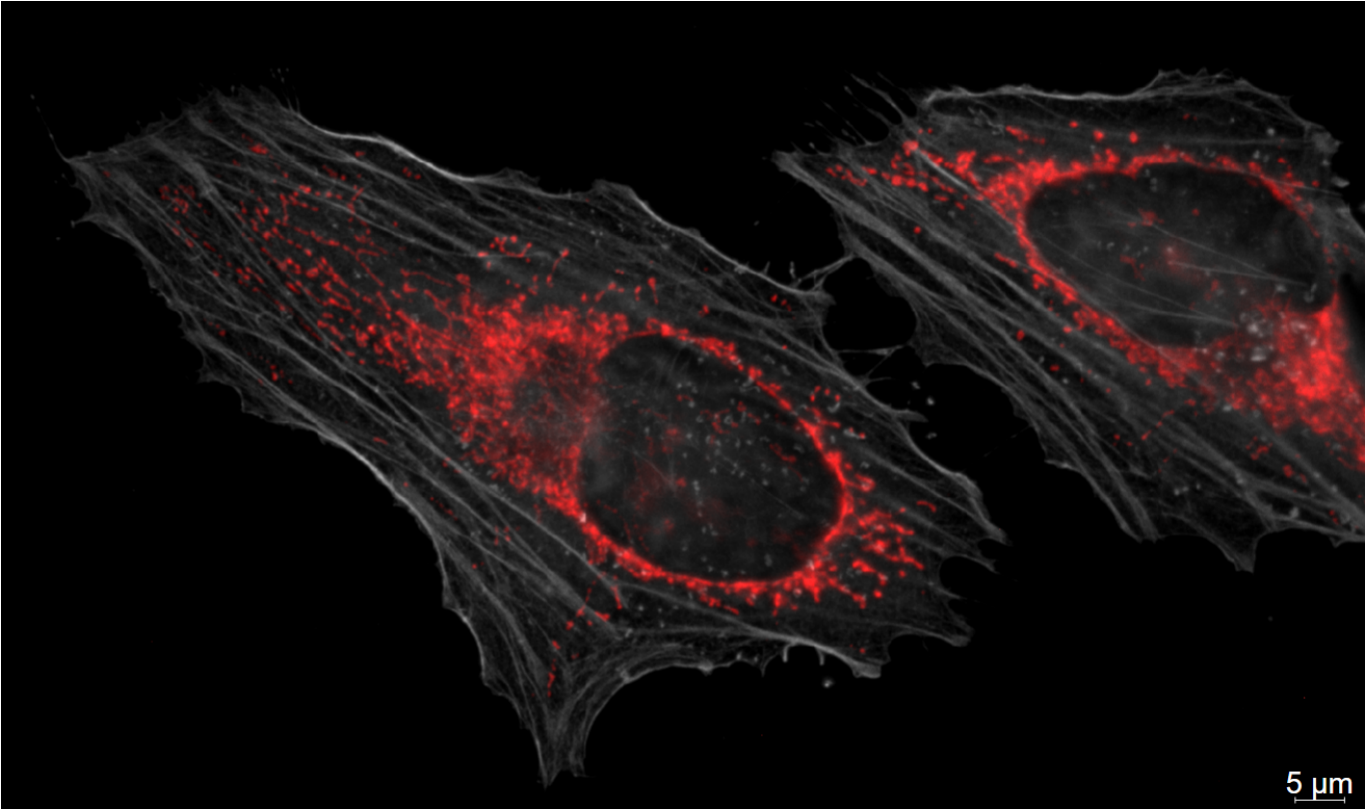
### SOURCE PROVENANCE

|            |                   |
|------------|-------------------|
| Catalog    | SA-000066         |
| 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                     | B22B0B682ABFAB01A170638A8BE7DF85354EB643E1EC87DD080BF1808969FECF                              |
| Method PDF SHA-256                | D15D3D9A3D464CC594AEE063592191C2E741E1E20CEBAF92D0DCA7A0E254CC63                              |

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



|                 |                      |
|-----------------|----------------------|
| EVIDENCE TIER   | Widefield            |
| INSTRUMENT      | ZEISS Axio Imager.M1 |
| CELL MODEL      | HeLa                 |
| DETECTED DYES   | 405/DAPI; 488; 647   |
| METADATA VALUES | 29                   |

## 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)          | 1359 X 809                                                                                                                                                          |
| Image Size (Scaled)          | 139.14µm X 82.83µm                                                                                                                                                  |
| Bit Depth                    | 12 Bit                                                                                                                                                              |
| Image Center Position        | X: 0.00µm, Y: 0.00µm                                                                                                                                                |
| ROI Center Offset            | X: 0.00µm, Y: 0.00µm                                                                                                                                                |
| Reflector                    | 50 Cy5   38 HE eGFP                                                                                                                                                 |
| Beam Splitter                | 660   495                                                                                                                                                           |
| Filter Excitation Wavelength | 625 - 655   450 - 490                                                                                                                                               |
| Filter Emission Wavelength   | 655 - 715   500 - 550                                                                                                                                               |
| Contrast Method              | Fluorescence                                                                                                                                                        |
| Light source                 | X-Cite Xylis Lamp Intensity @50%   X-Cite Xylis Lamp Intensity @40%                                                                                                 |
| Exposure Time                | 150ms                                                                                                                                                               |
| Excitation Wavelength        | 653   493                                                                                                                                                           |
| Emission Wavelength          | 668   517                                                                                                                                                           |
| Effective NA                 | 1.4                                                                                                                                                                 |
| Imaging Device               | Axiocam MR R3                                                                                                                                                       |
| Camera Adapter               | 1X                                                                                                                                                                  |
| Depth of Focus               | 1.03µm   0.80µm                                                                                                                                                     |
| 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™ 647 Affinity Purified Rabbit Anti-Mouse F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000020

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 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) = 1359 X 809

Image Size (Scaled) = 139.14µm X 82.83µm

Bit Depth = 12 Bit

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

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

### 647 -

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 655 - 715

Contrast Method = Fluorescence

Light source = X-Cite Xylis Lamp Intensity @50%

Exposure Time = 150ms

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Imaging Device = Axiocam MR R3

Camera Adapter = 1X

Depth of Focus = 1.03µm

Binning Mode = 1,1

## 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 = 1.4  
 Imaging Device = AxioCam MR R3  
 Camera Adapter = 1X  
 Depth of Focus = 0.80µm  
 Binning Mode = 1,1

## Post Processing

Identifyn® SRM Alexa Fluor™ 488 was pseudo-colored in Zen Software from green, the default color, to light grey to provide visual contrast between the 647/488 channels.

## Image Correction

None

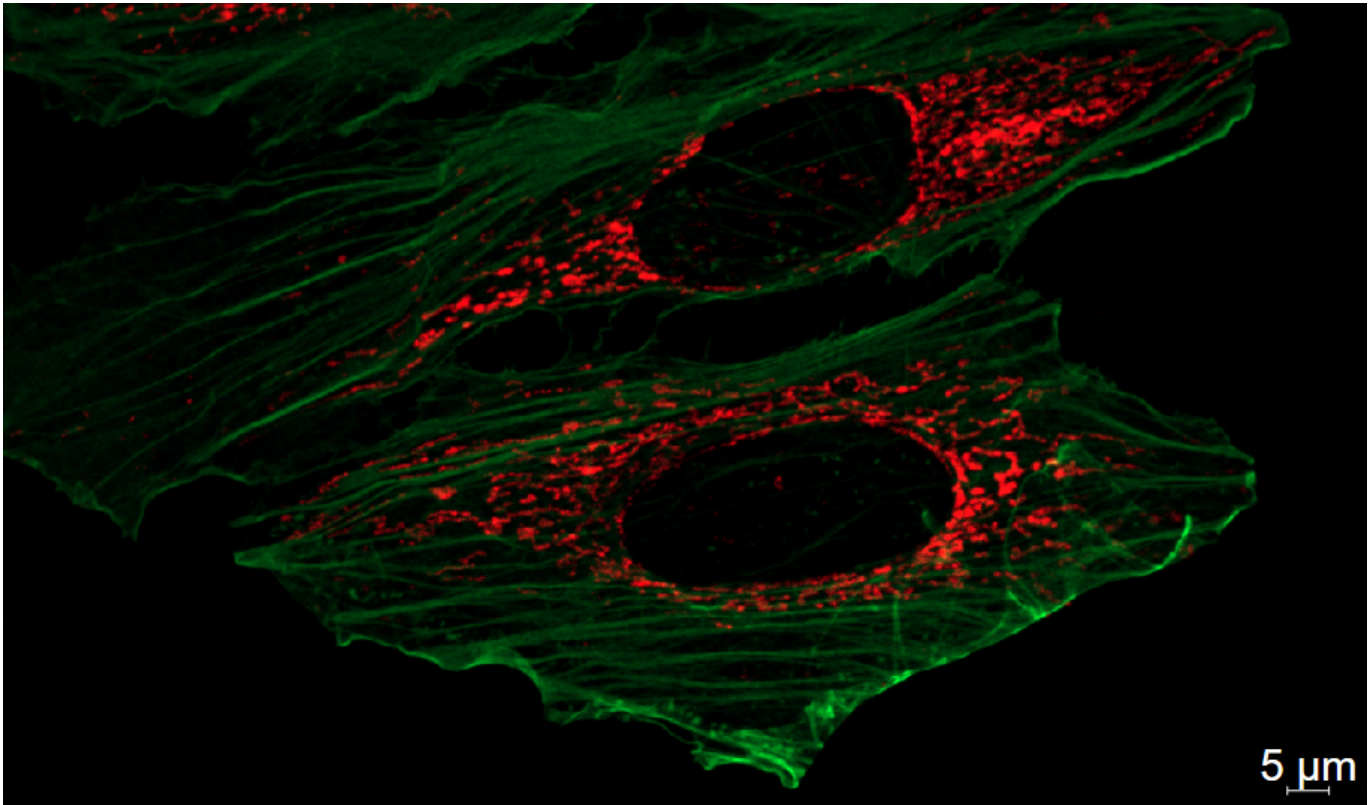
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## Image by E.F. Simon

Copyright 2026 GMD12 LLC.

| SOURCE PROVENANCE                 |                                                                                                                                                                     |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                                           |
| 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 | 29                                                                                                                                                                  |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                    |
| Cell model                        | HeLa                                                                                                                                                                |
| Image SHA-256                     | 2F72A19F6C393817DB6A710165F6E5D1490844B5F3918C480060F4F48C7D8E0C                                                                                                    |
| Method PDF SHA-256                | A3EF1F34ED0E88BAF4B9EF1001D1EB7093F5BE3086F8EE4CE950D06590BD6055                                                                                                    |

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 | 32                               |

## STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

| FIELD                        | SOURCE-DOCUMENTED VALUE                                                                                                                                                   |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Platform / configuration     | Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| Objective                    | EC Plan-Neofluar 63X/1.25 oil M27                                                                                                                                         |
| Channels                     | 2                                                                                                                                                                         |
| Scaling (Per Pixel)          | 0.143µm X 0.143µm                                                                                                                                                         |
| Image Size (Pixels)          | 1250 X 723                                                                                                                                                                |
| Image Size (Scaled)          | 178.57µm X 103.29µm                                                                                                                                                       |
| Bit Depth                    | 14 Bit                                                                                                                                                                    |
| Image Center Position        | X: 66.24mm, Y: 42.78mm                                                                                                                                                    |
| Stage Position               | X: 66.24mm, Y: 42.78mm                                                                                                                                                    |
| ROI Center Offset            | X: 1.14µm, Y: 0.00µm                                                                                                                                                      |
| Reflector                    | 50 Cy5   38 HE eGFP                                                                                                                                                       |
| Beam Splitter                | 660   495                                                                                                                                                                 |
| Filter Excitation Wavelength | 625 - 655   450 - 490                                                                                                                                                     |
| Filter Emission Wavelength   | 665 - 715   500 - 550                                                                                                                                                     |
| Contrast Method              | Fluorescence                                                                                                                                                              |
| Light source                 | X-Cite Xylis Lamp Intensity @5%   X-Cite Xylis Lamp Intensity @40%                                                                                                        |
| Exposure Time                | 60ms   200ms                                                                                                                                                              |
| Excitation Wavelength        | 653   493                                                                                                                                                                 |
| Emission Wavelength          | 668   517                                                                                                                                                                 |
| Effective NA                 | 1.25                                                                                                                                                                      |
| Imaging Device               | Axiocam 807                                                                                                                                                               |
| Camera Adapter               | 1X                                                                                                                                                                        |
| Depth of Focus               | 1.30µm   1.00µm                                                                                                                                                           |
| 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 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™ 647 Affinity Purified Rabbit Anti-Mouse F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

Identifyfyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000020

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyfyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

The second primary antibody followed this, Actin Recombinant Rabbit Monoclonal Antibody (JF47-01), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyfyn® 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) = 1250 X 723

Image Size (Scaled) = 178.57µm X 103.29µm

Bit Depth = 14 Bit

Image Center Position = X: 66.24mm, Y: 42.78mm

Stage Position = X: 66.24mm, Y: 42.78mm

ROI Center Offset = X: 1.14µm, Y: 0.00µm

## 647 -

Reflector = 50 Cy5  
 Beam Splitter = 660  
 Filter Excitation Wavelength = 625 - 655  
 Filter Emission Wavelength = 665 - 715  
 Contrast Method = Fluorescence  
 Light source = X-Cite Xylis Lamp Intensity @5%  
 Exposure Time = 60ms  
 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

## 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 = 200ms  
 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

## 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.

## Post Processing

None

## Image Corrections

None

----

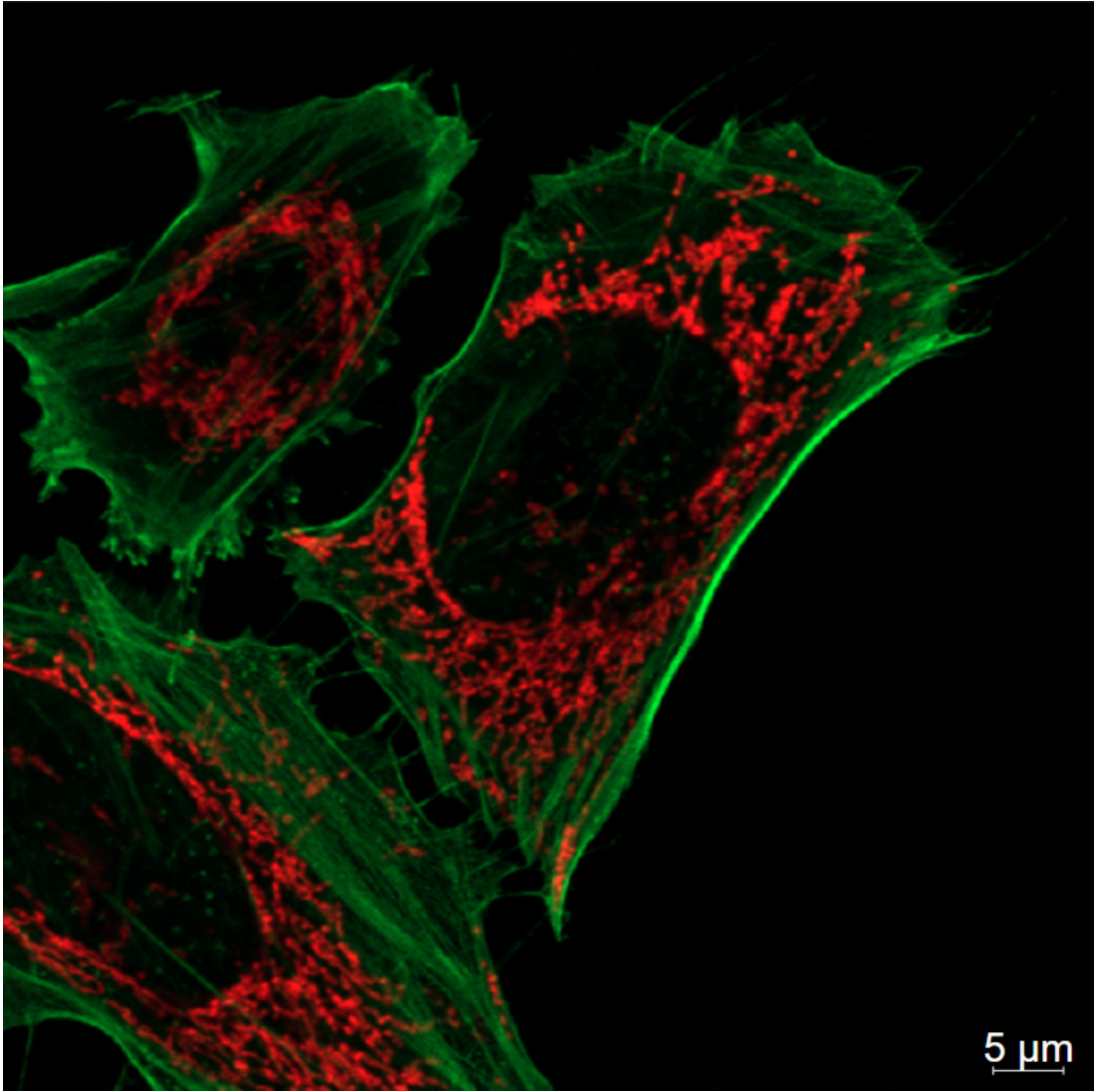
## Image by E.F. Simon

Copyright 2026 GMD12 LLC.

## SOURCE PROVENANCE

|                                   |                                                                                                                                                                          |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                                                |
| 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, Xiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| Structured source metadata values | 32                                                                                                                                                                       |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                                         |
| Cell model                        | HeLa                                                                                                                                                                     |
| Image SHA-256                     | 661A31E5896C70DCCFCD002E70EA548D0B108118C631649A214FBB1D6FE4F5BE                                                                                                         |
| Method PDF SHA-256                | 2BA85FE3A8FC34AFFB4CFA9C271FFF4CB691916D09479CB00B7B897694342032                                                                                                         |

ORIGINAL IMAGE EVIDENCE / CONFOCAL



|                 |                    |
|-----------------|--------------------|
| EVIDENCE TIER   | Confocal           |
| INSTRUMENT      | ZEISS LSM 900      |
| CELL MODEL      | HeLa               |
| DETECTED DYES   | 405/DAPI; 488; 647 |
| METADATA VALUES | 38                 |

## STRUCTURED ACQUISITION METADATA / CONFOCAL

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

| FIELD                    | SOURCE-DOCUMENTED VALUE                                                                                                                            |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Platform / configuration | Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye: |
| Objective                | with a Plan-Apochromat 63X/1.40 oil DIC M27                                                                                                        |
| Channels                 | 2                                                                                                                                                  |
| Scaling (Per Pixel)      | 0.08507 $\mu\text{m}$ X 0.08507 $\mu\text{m}$                                                                                                      |
| Image Size (Pixels)      | 917 X 917                                                                                                                                          |
| Image Size (Scaled)      | 78.01 $\mu\text{m}$ X 78.01 $\mu\text{m}$                                                                                                          |
| Bit Depth                | 16 Bit                                                                                                                                             |
| Image Center Position    | X: 76.02mm, Y: 48.93mm                                                                                                                             |
| Stage Position           | X: 76.02mm, Y: 48.93mm                                                                                                                             |
| ROI Center Offset        | X: 0.00 $\mu\text{m}$ , Y: 0.00 $\mu\text{m}$                                                                                                      |
| Reflector                | None                                                                                                                                               |
| Contrast Method          | Fluorescence                                                                                                                                       |
| Pinhole                  | 1.00AU / 57 $\mu\text{m}$   1.00AU / 43 $\mu\text{m}$                                                                                              |
| LMS MBS                  | MBS 405 + 488 + 561 + 640(T10/R90)                                                                                                                 |
| Laser Wavelength         | 640nm @0.5%   488nm @0.5%                                                                                                                          |
| Laser Blanking           | Enabled                                                                                                                                            |
| Scan Mode                | Frame                                                                                                                                              |
| Scan Zoom                | 1.3                                                                                                                                                |
| Rotation                 | 0°                                                                                                                                                 |
| Sampling                 | 1.00                                                                                                                                               |
| Pixel Time               | 0.58 $\mu\text{s}$                                                                                                                                 |
| Frame Time               | 9.09s                                                                                                                                              |
| LSM Scan Speed           | 9                                                                                                                                                  |
| Scan Direction           | Bidirectional                                                                                                                                      |
| Line Step                | 1                                                                                                                                                  |
| Excitation Wavelength    | 653   493                                                                                                                                          |
| Emission Wavelength      | 668   517                                                                                                                                          |
| Effective NA             | 1.4                                                                                                                                                |
| Detection Wavelength     | 656 - 700   478 - 539                                                                                                                              |
| Imaging Device           | GaAsp-PMT 2   Airyscan                                                                                                                             |
| Detector Type            | GaAsp-PMT                                                                                                                                          |
| Detector Gain            | 650 V                                                                                                                                              |
| Detector Offset          | 0                                                                                                                                                  |

| FIELD                 | SOURCE-DOCUMENTED VALUE |
|-----------------------|-------------------------|
| Detector Digital Gain | 1.0                     |

## SOURCE METHOD

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

### Identifyn Standards for Optical Microscopy Methods™

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit Anti-Mouse F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Rabbit F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000020

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher, Fish Serum Blocker cat#37527X3. The first primary antibody, Mitochondria Antibody (MA5-12017), was incubated overnight at 4°C at a dilution of 1:100. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000.

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

#### Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 85.07nm X 85.07nm

Image Size (Pixels) = 917 X 917

Image Size (Scaled) = 78.01µm X 78.01µm

Bit Depth = 16 Bit

Image Center Position = X: 76.02mm, Y: 48.93mm

Stage Position = X: 76.02mm, Y: 48.93mm

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

## 647 -

Reflector = None  
 Contrast Method = Fluorescence  
 Pinhole = 1.00AU / 57µm  
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)  
 Laser Wavelength = 640nm @0.5%  
 Laser Blanking = Enabled  
 Scan Mode = Frame  
 Scan Zoom = 1.3  
 Rotation = 0°  
 Sampling = 1.00  
 Pixel Time = 0.58µs  
 Frame Time = 9.09s  
 LSM Scan Speed = 9  
 Scan Direction = Bidirectional  
 Line Step = 1  
 Averaging - 16  
 Excitation Wavelength = 653  
 Emission Wavelength = 668  
 Effective NA = 1.4  
 Detection Wavelength = 656 - 700  
 Imaging Device = GaAsp-PMT 2  
 Detector Type = GaAsp-PMT  
 Detector Gain = 650 V  
 Detector Offset = 0  
 Detector Digital Gain = 1.0

## 488 -

Reflector = None  
 Contrast Method = Fluorescence  
 Pinhole = 1.00AU / 43µm  
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)  
 Laser Wavelength = 488nm @0.5%  
 Laser Blanking = Enabled  
 Scan Mode = Frame  
 Scan Zoom = 1.3  
 Rotation = 0°  
 Sampling = 1.00  
 Pixel Time = 0.58µs  
 Frame Time = 9.09s  
 LSM Scan Speed = 9  
 Scan Direction = Bidirectional  
 Line Step = 1  
 Averaging - 16  
 Excitation Wavelength = 493  
 Emission Wavelength = 517  
 Effective NA = 1.4  
 Detection Wavelength = 478 - 539  
 Imaging Device = Airyscan  
 Detector Type = GaAsP-PMT  
 Detector Gain = 650 V  
 Detector Offset = 0  
 Detector Digital Gain = 1.0

## Post Processing

None

## Image Corrections

None

----

## Image by J.K. Bennett

Copyright 2026 GMD12 LLC.

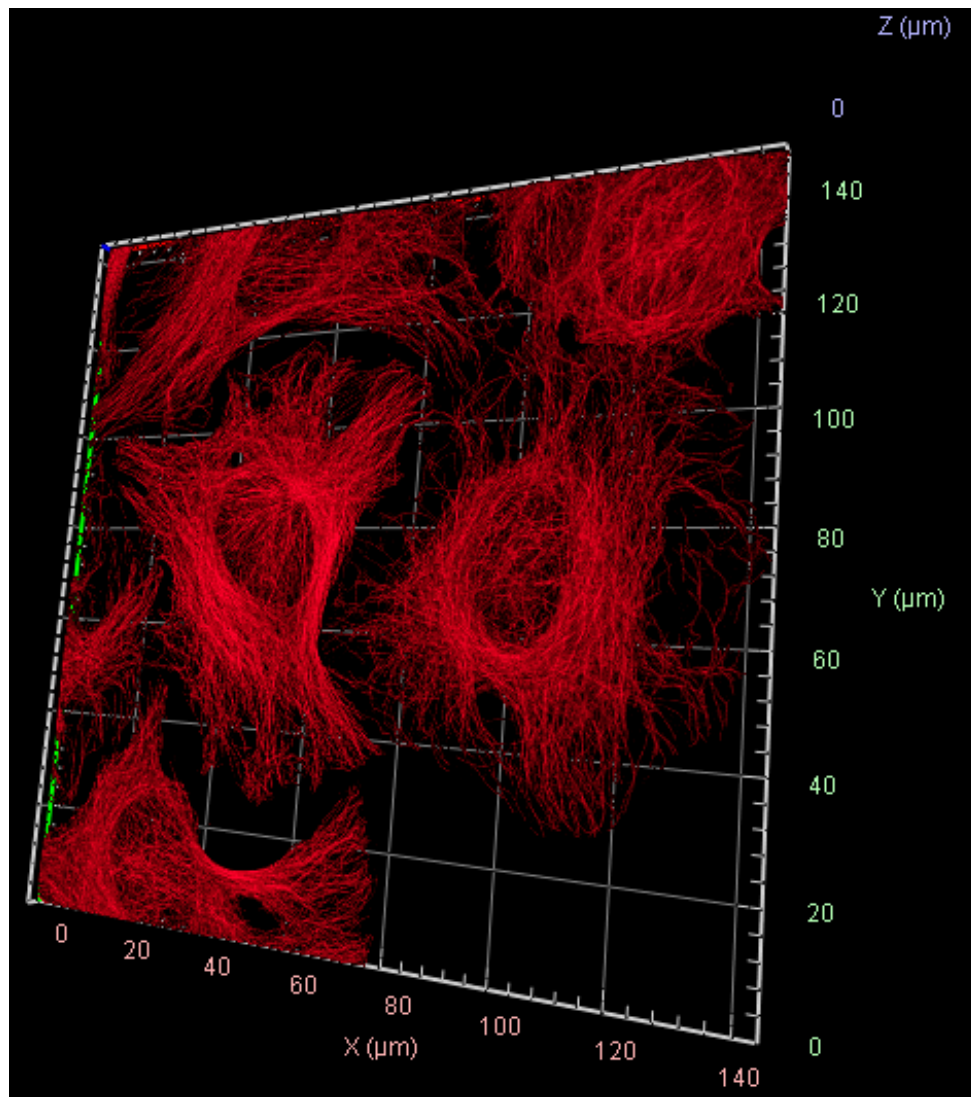
### SOURCE PROVENANCE

|                          |                                                                                                                                                    |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                  | SA-000066                                                                                                                                          |
| Stage                    | Confocal                                                                                                                                           |
| Instrument               | ZEISS LSM 900                                                                                                                                      |
| Microscope configuration | Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye: |

## SOURCE PROVENANCE

|                                   |                                                                   |
|-----------------------------------|-------------------------------------------------------------------|
| Structured source metadata values | 38                                                                |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                  |
| Cell model                        | HeLa                                                              |
| Image SHA-256                     | C4B134E9DEA4ACC5E3BB6AEF71EBD36C9296D9FAA1CA8FB82E07427F131D7E01  |
| Method PDF SHA-256                | E427B00334D6A3D1F954F07FB61BFDEFBFDCC2CF6DD3E5E93A89CC5409178477C |

## ORIGINAL IMAGE EVIDENCE / AIRYSCAN



|                 |                         |
|-----------------|-------------------------|
| EVIDENCE TIER   | Airyscan                |
| INSTRUMENT      | ZEISS LSM 900           |
| CELL MODEL      | HeLa                    |
| DETECTED DYES   | 405/DAPI; 488; 647; 680 |
| METADATA VALUES | 39                      |

## 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 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                  | 65 Slices (3.2µm)                                                                                                                                  |
| Channels                 | 1                                                                                                                                                  |
| Scaling (Per Pixel)      | 0.04252 µm X 0.04252 µm X 0.05000 µm                                                                                                               |
| Image Size (Pixels)      | 3383 X 3383                                                                                                                                        |
| Image Size (Scaled)      | 143.85µm X 143.85µm                                                                                                                                |
| Bit Depth                | 16 Bit                                                                                                                                             |
| Image Center Position    | X: 75.84mm, Y: 48.21mm                                                                                                                             |
| Stage Position           | X: 75.84mm, Y: 48.21mm                                                                                                                             |
| ROI Center Offset        | X: 0.00µm, Y: 0.00µm                                                                                                                               |
| Reflector                | None                                                                                                                                               |
| Contrast Method          | Fluorescence                                                                                                                                       |
| Pinhole                  | 5.00AU / 280µm                                                                                                                                     |
| LMS MBS                  | MBS 405 + 488 + 561 + 640(T10/R90)                                                                                                                 |
| Laser Wavelength         | 640nm @0.6%                                                                                                                                        |
| Laser Blanking           | Enabled                                                                                                                                            |
| Scan Mode                | Frame                                                                                                                                              |
| Scan Zoom                | 0.70                                                                                                                                               |
| Rotation                 | 0°                                                                                                                                                 |
| Sampling                 | 2.00                                                                                                                                               |
| Pixel Time               | 0.62µs                                                                                                                                             |
| Frame Time               | 16.86s                                                                                                                                             |
| LSM Scan Speed           | 6                                                                                                                                                  |
| Scan Direction           | Bidirectional                                                                                                                                      |
| Line Step                | 1                                                                                                                                                  |
| Excitation Wavelength    | 653                                                                                                                                                |
| Emission Wavelength      | 668                                                                                                                                                |
| Effective NA             | 1.4                                                                                                                                                |
| Detection Wavelength     | 630 - 700                                                                                                                                          |
| Imaging Device           | Airyscan                                                                                                                                           |
| Detector Type            | GaAsp-PMT                                                                                                                                          |
| Detector Gain            | 800 V                                                                                                                                              |

| FIELD                 | SOURCE-DOCUMENTED VALUE                                                                                                                                    |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Detector Offset       | 0                                                                                                                                                          |
| Detector Digital Gain | 1.0                                                                                                                                                        |
| AiryScan Mode         | Super Resolution 8.6 (3D, Auto)                                                                                                                            |
| 3D Maximum Projection | Precise                                                                                                                                                    |
| Appearance            | Threshold 2% (680), Ramp 0% (647), Maximum 100% (680)                                                                                                      |
| Background            | Black                                                                                                                                                      |
| Light                 | Brightness 86%, Enabled Tone Mapping                                                                                                                       |
| Projection            | View Angle 54°, Scale Z 110%, 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™ 647 Affinity Purified Rabbit Anti-Mouse F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

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, alpha Tubulin Monoclonal Antibody (DM1A), 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.

### 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 = 65 Slices (3.2µm)

Channels = 1

Scaling (Per Pixel) = 42.52nm X 42.52nmµm X 50.00nm

Image Size (Pixels) = 3383 X 3383

Image Size (Scaled) = 143.85µm X 143.85µm

Bit Depth = 16 Bit

Image Center Position = X: 75.84mm, Y: 48.21mm

Stage Position = X: 75.84mm, Y: 48.21mm

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

## 647 -

Reflector = None  
 Contrast Method = Fluorescence  
 Pinhole = 5.00AU / 280µm  
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)  
 Laser Wavelength = 640nm @0.6%  
 Laser Blanking = Enabled  
 Scan Mode = Frame  
 Scan Zoom = 0.70  
 Rotation = 0°  
 Sampling = 2.00  
 Pixel Time = 0.62µs  
 Frame Time = 16.86s  
 LSM Scan Speed = 6  
 Scan Direction = Bidirectional  
 Line Step = 1  
 Averaging - 2  
 Excitation Wavelength = 653  
 Emission Wavelength = 668  
 Effective NA = 1.4  
 Detection Wavelength = 630 - 700  
 Imaging Device = Airyscan  
 Detector Type = GaAsP-PMT  
 Detector Gain = 800 V  
 Detector Offset = 0  
 Detector Digital Gain = 1.0  
 AiryScan Mode = Super Resolution 8.6 (3D, Auto)

## 3D Image Processing

3D Maximum Projection = Precise  
 Appearance = Threshold 2% (680), Ramp 0% (647), Maximum 100% (680)  
 Background = Black  
 Light = Brightness 86%, Enabled Tone Mapping  
 Projection = View Angle 54°, Scale Z 110%, 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

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### SOURCE PROVENANCE

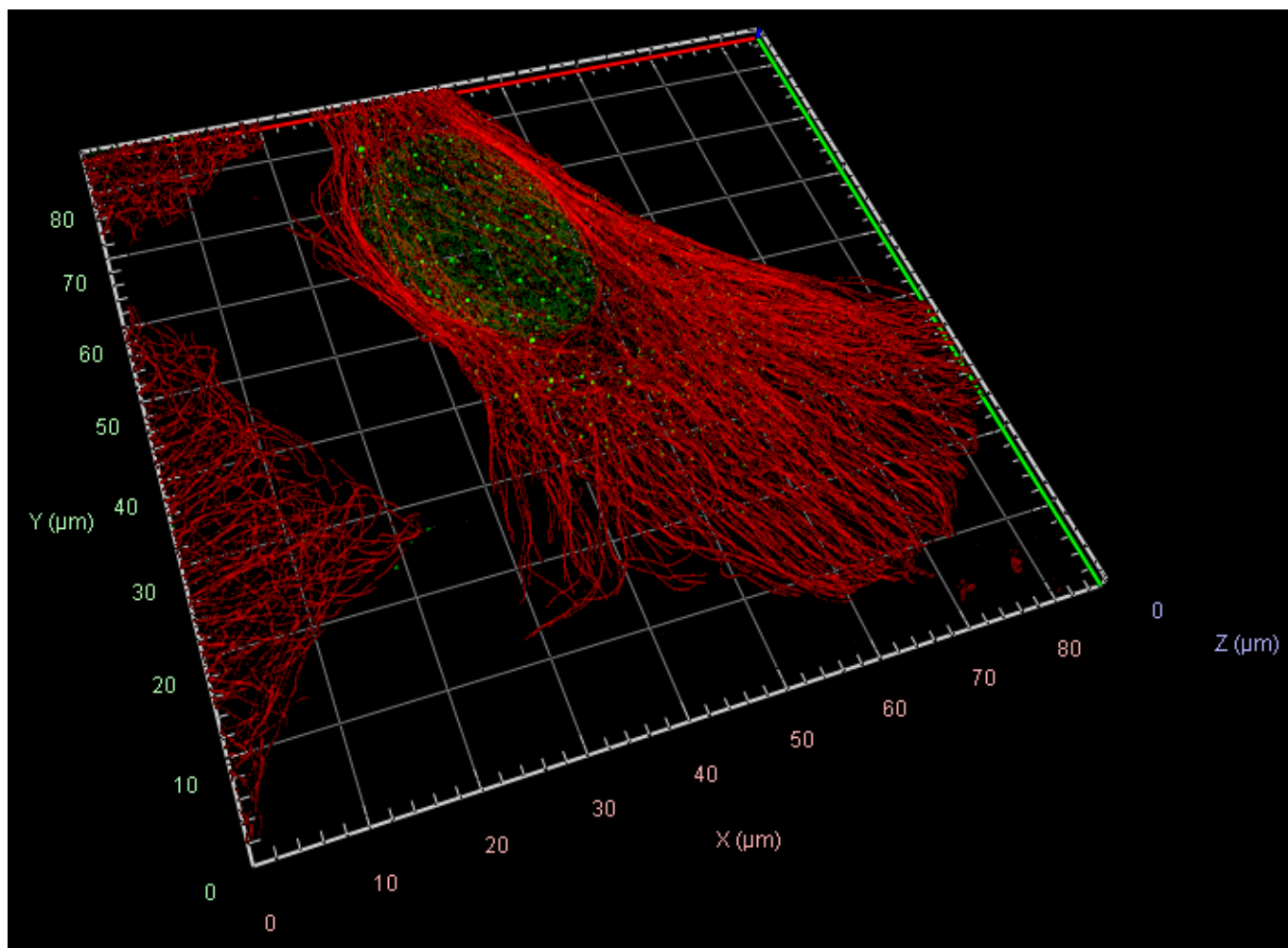
Catalog

SA-000066

## SOURCE PROVENANCE

|                                   |                                                                                                                                                    |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| Stage                             | Airyscan                                                                                                                                           |
| 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 | 39                                                                                                                                                 |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                   |
| Cell model                        | HeLa                                                                                                                                               |
| Image SHA-256                     | 14CCABF50A3EA28EF2BF9B25DC6AE0A2307A512AC71AE4845E6FD4DC3B740AEE                                                                                   |
| Method PDF SHA-256                | 43C1E70C9006386BC7BF2B07F07C756F69F1B495A27BED142FA513F1E13592BC                                                                                   |

## ORIGINAL IMAGE EVIDENCE / SIM



|                 |                    |
|-----------------|--------------------|
| EVIDENCE TIER   | SIM                |
| INSTRUMENT      | ZEISS Elyra        |
| CELL MODEL      | HeLa               |
| DETECTED DYES   | 405/DAPI; 488; 647 |
| METADATA VALUES | 53                 |

## STRUCTURED ACQUISITION METADATA / SIM

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

| FIELD                        | SOURCE-DOCUMENTED VALUE                                                                                                                                     |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Platform / configuration     | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye: |
| Objective                    | Plan-Apochromat 63X/1.40 oil DIC M27                                                                                                                        |
| Z-Stack                      | 47 Slices (2.3µm)                                                                                                                                           |
| Channels                     | 2   1                                                                                                                                                       |
| Scaling (Per Pixel)          | 0.01689 µm X 0.01689 µm X 0.05000 µm                                                                                                                        |
| Image Size (Pixels)          | 5056 X 5056                                                                                                                                                 |
| Image Size (Scaled)          | 85.40µm X 85.40µm                                                                                                                                           |
| Bit Depth                    | 16 Bit                                                                                                                                                      |
| Image Center Position        | X: 59.96mm, Y: 37.72mm                                                                                                                                      |
| Stage Position               | X: 59.96mm, Y: 37.72mm                                                                                                                                      |
| ROI Center Offset            | X: 0.00µm, Y: 0.00µm                                                                                                                                        |
| Reflectors                   | SR HE LBF 405/488/561/642                                                                                                                                   |
| Beam Splitter                | 405, 488, 561, 642                                                                                                                                          |
| Filter Excitation Wavelength | 200 - 405, 200 - 488, 200 - 561, 200 - 642                                                                                                                  |
| Filter Emission Wavelength   | 419 - 470, 503 - 546, 576 - 617, 657 - 800                                                                                                                  |
| Contrast Method              | Fluorescence                                                                                                                                                |
| Illumination Wavelength      | 640   488                                                                                                                                                   |
| Channel Name                 | T1-Axiocam 820 -SR   T1-Axiocam 820-SR                                                                                                                      |
| Excitation Wavelength        | 640   488                                                                                                                                                   |
| Emission Wavelength          | 729   525                                                                                                                                                   |
| Effective NA                 | 1.4                                                                                                                                                         |
| Detection Wavelength         | 419 - 470, 503 - 546, 576 - 617, 657 - 800                                                                                                                  |
| Imaging Device               | Axiocam 820                                                                                                                                                 |
| Camera Adapter               | 1X                                                                                                                                                          |
| Exposure Time                | 120ms                                                                                                                                                       |
| Depth of Focus               | 1.13µm   0.81µm                                                                                                                                             |
| Binning Mode                 | 2,2                                                                                                                                                         |
| Laser Wavelength             | 640nm @2.5%   561nm @3.0%                                                                                                                                   |
| Frame Time                   | 1.59s                                                                                                                                                       |
| Scan Direction               | Unidirectional                                                                                                                                              |
| Result Sampling              | 4                                                                                                                                                           |
| Process Sampling             | 2                                                                                                                                                           |
| Algorithm                    | SIM                                                                                                                                                         |

| FIELD                 | SOURCE-DOCUMENTED VALUE                                                                                                                                   |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| SIM Settings          | Standard                                                                                                                                                  |
| Auto Sharpness        | On                                                                                                                                                        |
| Sharpness             | 10.3893                                                                                                                                                   |
| Input SNR             | N/A                                                                                                                                                       |
| Regularization Weight | N/A                                                                                                                                                       |
| Iterations            | N/A                                                                                                                                                       |
| Filter                | N/A                                                                                                                                                       |
| Fitting               | N/A                                                                                                                                                       |
| Normalization         | Automatic                                                                                                                                                 |
| Experimental PSF      | N/A                                                                                                                                                       |
| 3D Maximum Projection | Precise                                                                                                                                                   |
| Appearance            | Threshold 4% (647), Threshold 3% (488), Ramp 0% all channels, Maximum 100% all channels                                                                   |
| Background            | Black                                                                                                                                                     |
| Light                 | Brightness 126%, Enabled Tone Mapping                                                                                                                     |
| Projection            | View Angle 42°, Scale Z 60%, 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 Primary Antibody

Rabbit anti-Human  $\gamma$ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate  
Cat ID# - DC 000014

#### Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (H + L)  
Cat ID# - SA 000066

Gamma H2AX ( $\gamma$ H2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of DSBs. When DSBs occur in chromatin,  $\gamma$ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or  $\gamma$ -phosphorylation) near the site of the break. This phosphorylation event forms  $\gamma$ H2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

#### Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 $\mu$ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human  $\gamma$ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

#### Microscope

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

#### Z Stack - (3D)

##### Z-Stack = 47 Slices (2.3 $\mu$ m)

Channels = 2

Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm

Image Size (Pixels) = 5056 X 5056

Image Size (Scaled) = 85.40 $\mu$ m X 85.40 $\mu$ m

Bit Depth = 16 Bit

Image Center Position = X: 59.96mm, Y: 37.72mm

Stage Position = X: 59.96mm, Y: 37.72mm

ROI Center Offset = X: 0.00 $\mu$ m, Y: 0.00 $\mu$ m

## 647 -

Reflectors = SR HE LBF 405/488/561/642  
 Beam Splitter = 405, 488, 561, 642  
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642  
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Contrast Method = Fluorescence  
 Illumination Wavelength = 640  
 Channel Name = T1-Axiocam 820 -SR  
 Excitation Wavelength = 640  
 Emission Wavelength = 729  
 Effective NA = 1.4  
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Imaging Device = Axiocam 820  
 Camera Adapter = 1X  
 Exposure Time = 120ms  
 Depth of Focus = 1.13µm  
 Binning Mode = 2,2  
 Laser Wavelength = 640nm @2.5%  
 Frame Time = 1.59s  
 Scan Direction = Unidirectional  
 Grating 36.5µm grating

## 488 -

Reflectors = SR HE LBF 405/488/561/642  
 Beam Splitter = 405, 488, 561, 642  
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642  
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Contrast Method = Fluorescence  
 Illumination Wavelength = 488  
 Channel Name = T1-Axiocam 820-SR  
 Excitation Wavelength = 488  
 Emission Wavelength = 525  
 Effective NA = 1.4  
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Imaging Device = Axiocam 820  
 Camera Adapter = 1X  
 Exposure Time = 120ms  
 Depth of Focus = 0.81µm  
 Binning Mode = 2,2  
 Laser Wavelength = 561nm @3.0%  
 Frame Time = 1.59s  
 Scan Direction = Unidirectional  
 Grating 36.5µm grating

## Post Processing - SIM Processing

Processing Dimension - 3D  
Result Sampling = 4  
Process Sampling = 2  
Channels = 1  
Algorithm = SIM  
SIM Settings = Standard  
Auto Sharpness = On  
Sharpness = 10.3893  
Input SNR = N/A  
Regularization Weight = N/A  
Iterations = N/A  
Filter = N/A  
Fitting = N/A  
Normalization = Automatic  
Experimental PSF = N/A

## 3D Image Processing

3D Maximum Projection = Precise  
Appearance = Threshold 4% (647), Threshold 3% (488), Ramp 0% all channels, Maximum 100% all channels  
Background = Black  
Light = Brightness 126%, Enabled Tone Mapping  
Projection = View Angle 42°, Scale Z 60%, 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.

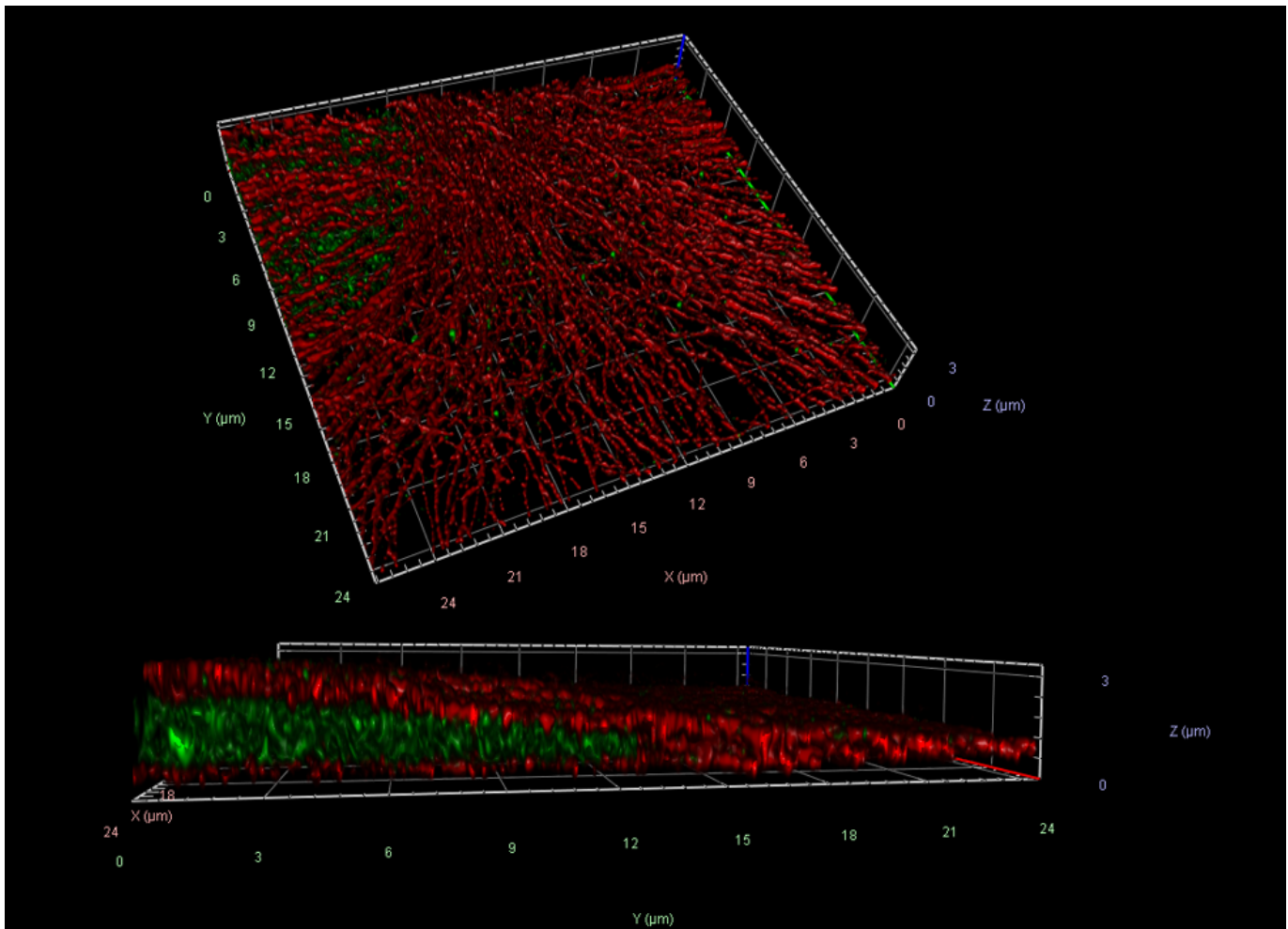
### SOURCE PROVENANCE

|                                   |                                                                                                                                                             |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                                   |
| Stage                             | SIM                                                                                                                                                         |
| Instrument                        | ZEISS Elyra                                                                                                                                                 |
| Microscope configuration          | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye: |
| Structured source metadata values | 53                                                                                                                                                          |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                            |
| Cell model                        | HeLa                                                                                                                                                        |
| Image SHA-256                     | C4FA1E272DE924A8CDCA66D59348B1D6DC693AD8C6018C57BB90B19AE44BA60A                                                                                            |

**SOURCE PROVENANCE**

Method PDF SHA-256

9F73B5AB60ACAA39ACCD3AB67696CCAA8B727780D86D7E836FC17D50898337

ORIGINAL IMAGE EVIDENCE / SIM<sup>2</sup>

|                 |                    |
|-----------------|--------------------|
| EVIDENCE TIER   | SIM <sup>2</sup>   |
| INSTRUMENT      | ZEISS Elyra        |
| CELL MODEL      | HeLa               |
| DETECTED DYES   | 405/DAPI; 488; 647 |
| METADATA VALUES | 53                 |

## STRUCTURED ACQUISITION METADATA / SIM<sup>2</sup>

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

| FIELD                        | SOURCE-DOCUMENTED VALUE                                                                                                                                     |
|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Platform / configuration     | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye: |
| Objective                    | Plan-Apochromat 63X/1.40 oil DIC M27                                                                                                                        |
| Z-Stack                      | 67 Slices (3.3µm)                                                                                                                                           |
| Channels                     | 2   1                                                                                                                                                       |
| Scaling (Per Pixel)          | 0.01689 µm X 0.01689 µm X 0.05000 µm                                                                                                                        |
| Image Size (Pixels)          | 1569 X 1455                                                                                                                                                 |
| Image Size (Scaled)          | 26.50µm X 24.58µm                                                                                                                                           |
| Bit Depth                    | 16 Bit                                                                                                                                                      |
| Image Center Position        | X: 60.89mm, Y: 36.73mm                                                                                                                                      |
| Stage Position               | X: 60.89mm, Y: 36.73mm                                                                                                                                      |
| ROI Center Offset            | X: 0.00µm, Y: 0.00µm                                                                                                                                        |
| Reflectors                   | SR HE LBF 405/488/561/642                                                                                                                                   |
| Beam Splitter                | 405, 488, 561, 642                                                                                                                                          |
| Filter Excitation Wavelength | 200 - 405, 200 - 488, 200 - 561, 200 - 642                                                                                                                  |
| Filter Emission Wavelength   | 419 - 470, 503 - 546, 576 - 617, 657 - 800                                                                                                                  |
| Contrast Method              | Fluorescence                                                                                                                                                |
| Illumination Wavelength      | 640   488                                                                                                                                                   |
| Channel Name                 | T1-Axiocam 820 -SR   T1-Axiocam 820-SR                                                                                                                      |
| Excitation Wavelength        | 640   488                                                                                                                                                   |
| Emission Wavelength          | 729   525                                                                                                                                                   |
| Effective NA                 | 1.4                                                                                                                                                         |
| Detection Wavelength         | 419 - 470, 503 - 546, 576 - 617, 657 - 800                                                                                                                  |
| Imaging Device               | Axiocam 820                                                                                                                                                 |
| Camera Adapter               | 1X                                                                                                                                                          |
| Exposure Time                | 120ms                                                                                                                                                       |
| Depth of Focus               | 1.13µm   0.81µm                                                                                                                                             |
| Binning Mode                 | 2,2                                                                                                                                                         |
| Laser Wavelength             | 640nm @2.5%   561nm @3.0%                                                                                                                                   |
| Frame Time                   | 1.59s                                                                                                                                                       |
| Scan Direction               | Unidirectional                                                                                                                                              |
| Result Sampling              | 4                                                                                                                                                           |
| Process Sampling             | 4                                                                                                                                                           |
| Algorithm                    | SIM2                                                                                                                                                        |

| FIELD                 | SOURCE-DOCUMENTED VALUE                                                                                                                                    |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SIM Settings          | Standard                                                                                                                                                   |
| Auto Sharpness        | On                                                                                                                                                         |
| Sharpness             | 10.4378                                                                                                                                                    |
| Input SNR             | N/A                                                                                                                                                        |
| Regularization Weight | N/A                                                                                                                                                        |
| Iterations            | N/A                                                                                                                                                        |
| Filter                | N/A                                                                                                                                                        |
| Fitting               | N/A                                                                                                                                                        |
| Normalization         | Automatic                                                                                                                                                  |
| Experimental PSF      | N/A                                                                                                                                                        |
| 3D Volume Projection  | Precise                                                                                                                                                    |
| Appearance            | Threshold 2% (647), Threshold 2% (488), Ramp 98% all channels, 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 Primary Antibody

Rabbit anti-Human  $\gamma$ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate  
Cat ID# - DC 000014

#### Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (H + L)  
Cat ID# - SA 000066

Gamma H2AX ( $\gamma$ H2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of DSBs. When DSBs occur in chromatin,  $\gamma$ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or  $\gamma$ -phosphorylation) near the site of the break. This phosphorylation event forms  $\gamma$ H2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

#### Method

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 $\mu$ M Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human  $\gamma$ H2Ax Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488 - Direct Conjugate, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody followed this, Alpha Tubulin Monoclonal Antibody (DM1A), which was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

#### Microscope

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

#### Z Stack - (3D)

##### Z-Stack = 67 Slices (3.3 $\mu$ m)

Channels = 2

Scaling (Per Pixel) = 16.89nm X 16.89nm X 50.00nm

Image Size (Pixels) = 1569 X 1455

Image Size (Scaled) = 26.50 $\mu$ m X 24.58 $\mu$ m

Bit Depth = 16 Bit

Image Center Position = X: 60.89mm, Y: 36.73mm

Stage Position = X: 60.89mm, Y: 36.73mm

ROI Center Offset = X: 0.00 $\mu$ m, Y: 0.00 $\mu$ m

## 647 -

Reflectors = SR HE LBF 405/488/561/642  
 Beam Splitter = 405, 488, 561, 642  
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642  
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Contrast Method = Fluorescence  
 Illumination Wavelength = 640  
 Channel Name = T1-Axiocam 820 -SR  
 Excitation Wavelength = 640  
 Emission Wavelength = 729  
 Effective NA = 1.4  
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Imaging Device = Axiocam 820  
 Camera Adapter = 1X  
 Exposure Time = 120ms  
 Depth of Focus = 1.13µm  
 Binning Mode = 2,2  
 Laser Wavelength = 640nm @2.5%  
 Frame Time = 1.59s  
 Scan Direction = Unidirectional  
 Grating 36.5µm grating

## 488 -

Reflectors = SR HE LBF 405/488/561/642  
 Beam Splitter = 405, 488, 561, 642  
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642  
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Contrast Method = Fluorescence  
 Illumination Wavelength = 488  
 Channel Name = T1-Axiocam 820-SR  
 Excitation Wavelength = 488  
 Emission Wavelength = 525  
 Effective NA = 1.4  
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
 Imaging Device = Axiocam 820  
 Camera Adapter = 1X  
 Exposure Time = 120ms  
 Depth of Focus = 0.81µm  
 Binning Mode = 2,2  
 Laser Wavelength = 561nm @3.0%  
 Frame Time = 1.59s  
 Scan Direction = Unidirectional  
 Grating 36.5µm grating

## Post Processing - SIM2 Processing

Processing Dimension - 3D  
 Result Sampling = 4  
 Process Sampling = 4  
 Channels = 1  
 Algorithm = SIM2  
 SIM Settings = Standard  
 Auto Sharpness = On  
 Sharpness = 10.4378  
 Input SNR = N/A  
 Regularization Weight = N/A  
 Iterations = N/A  
 Filter = N/A  
 Fitting = N/A  
 Normalization = Automatic  
 Experimental PSF = N/A

## 3D Image Processing

3D Volume Projection = Precise  
 Appearance = Threshold 2% (647), Threshold 2% (488), Ramp 98% all channels, Maximum 100% all channels  
 Background = Black  
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping  
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

## Image Corrections

None

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## Image by P. Raut

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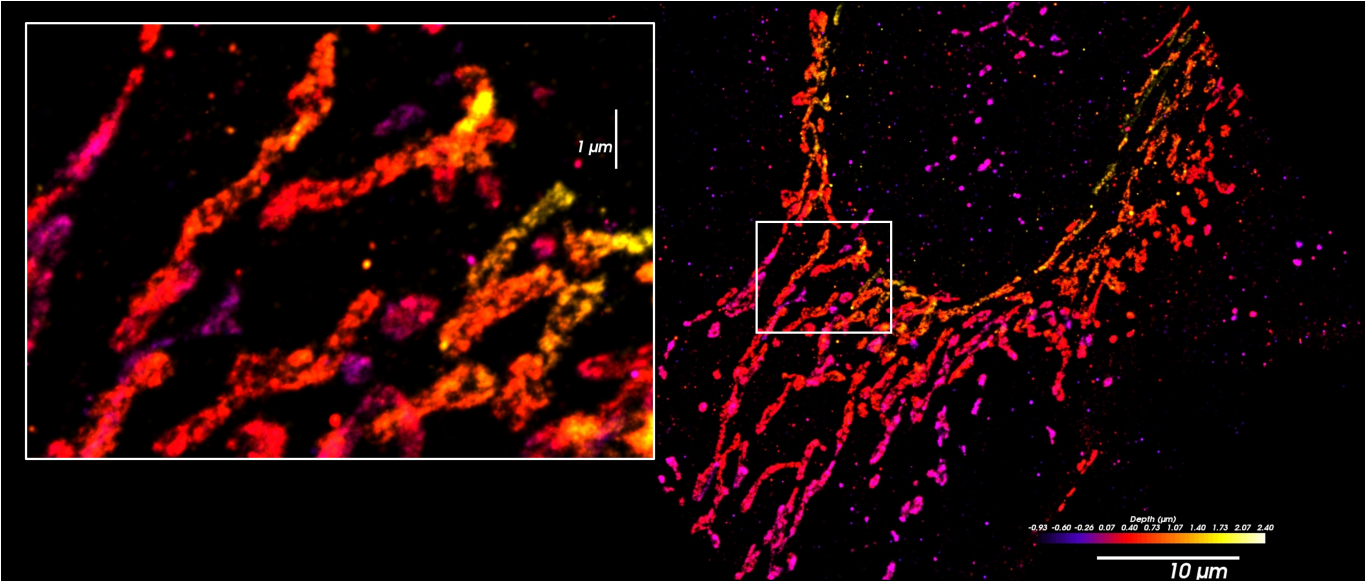
| SOURCE PROVENANCE                 |                                                                                                                                                             |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                                   |
| Stage                             | SIM <sup>2</sup>                                                                                                                                            |
| Instrument                        | ZEISS Elyra                                                                                                                                                 |
| Microscope configuration          | Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1X Tubelens, was used with the following parameters for each dye: |
| Structured source metadata values | 53                                                                                                                                                          |
| Metadata source                   | PRESERVED_SOURCE_METHOD_PDF_TEXT                                                                                                                            |
| Cell model                        | HeLa                                                                                                                                                        |
| Image SHA-256                     | 60A815F49FA15B568CE3D9B5AAFD65F39A4A9E34DE35D852D2877E430594EF5D                                                                                            |

**SOURCE PROVENANCE**

Method PDF SHA-256

F5A61DE07EC66C3B7362EE5487BBEF06D3186FE3AA15C6998D6780A6183574FB

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



|                 |                       |
|-----------------|-----------------------|
| EVIDENCE TIER   | Single-molecule STORM |
| INSTRUMENT      | Bruker Vutara VXL     |
| CELL MODEL      | HeLa                  |
| DETECTED DYES   | 647                   |
| 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)                                                                                                                                  |
| 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: 640 nm (635 nm Dichroic); First reference probe: 640 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                         | 185.5 µm                                                                                                                                       |

| FIELD                                                      | SOURCE-DOCUMENTED VALUE                                                         |
|------------------------------------------------------------|---------------------------------------------------------------------------------|
| Widefield 640nm                                            | 0.1X Neutral Density Filter: Selected, @0.2% Laser Input                        |
| Reference Probe 1 Laser control (640 nm (635 nm Dichroic)) | Not Selected                                                                    |
| Widefield camera Exposure Time                             | 100ms                                                                           |
| Widefield Laser                                            | "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected. |
| Current Intensity                                          | 4.5                                                                             |
| Calibration Value                                          | -5070 nm/unit                                                                   |
| Autofocus Mode                                             | Pre-Capture                                                                     |
| SuperRes                                                   | 50µm is Selected                                                                |
| Piezo Record                                               | Begin: 185.0; End: 186.6                                                        |
| SuperRes 640nm                                             | 0.1X Neutral Density Filter: Selected, @0.02% Laser Input                       |
| Record Workflow - Record Tab                               | Capture Parameters                                                              |
| Frames                                                     | Probe 1: 500                                                                    |
| Z Positions                                                | 8 using Steps: 0.1µm (100nm)                                                    |
| Cycles                                                     | 8                                                                               |
| Time Points                                                | 1                                                                               |
| Pause Between Cycles                                       | Not Selected                                                                    |
| Automatic Photoactivation Laser Control                    | Not Selected                                                                    |
| Laser Power                                                | 50%                                                                             |
| 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: 20                                                                |
| Sizing Mode                                                | Radial precision                                                                |
| Particle size                                              | 5nm                                                                             |
| Color by                                                   | Depth                                                                           |
| Color Table                                                | Single                                                                          |
| Opacity Mode                                               | Constant; Opacity 1: 0.04                                                       |
| Front-to-back Attenuation                                  | Not Selected                                                                    |
| Method                                                     | Particle (direct)                                                               |
| Time Intervals                                             | 10 Intervals                                                                    |

| FIELD            | SOURCE-DOCUMENTED VALUE |
|------------------|-------------------------|
| Pixel Size       | 50nm                    |
| Smoothing        | 8.00                    |
| PSF Z offset     | -450 to +450            |
| Radial precision | 25                      |
| Axial precision  | 70                      |

## 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™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (H + L)

Cat ID# - SA 000066

Hela cells were grown on μ-Slide 8 Well high Glass Bottom Ibidi chamber, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Mitochondria Antibody (MA5-12017), 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® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:50. 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)  
 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: 640 nm (635 nm Dichroic); First reference probe: 640 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: 185.5 µm

### Probe 1 Laser control (640 nm (635 nm Dichroic))

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input  
 Reference Probe 1 Laser control (640 nm (635 nm Dichroic): Not Selected

## Advanced Tools Option

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

Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.

## Autofocus - Calibration Values

Current Position" -0.979

Current Intensity: 4.5

Calibration Value: -5070 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: 185.0; End: 186.6**

## Probe 1 Laser control (640 nm (635 nm Dichroic)

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

## Reference Probe 1 Laser control (640 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: 500

Z Positions: 8 using Steps: 0.1µm (100nm)

Cycles: 8

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

## Probe 1 Laser control (640 nm (635 nm Dichroic)

**Laser Power: 50%**

## 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: 20**

### 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: 5nm  
 Color by: Depth  
 Color Table: Single  
 Opacity Mode: Constant; Opacity 1: 0.04  
 Front-to-back Attenuation: Not Selected

## Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)  
 Time Intervals: 10 Intervals  
 Pixel Size: 50nm  
 Smoothing: 8.00

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

**PSF Z offset: -450 to +450**

**Radial precision: 25**

**Axial precision: 70**

The other parameters were not applied.

**Statistical analysis - NA**

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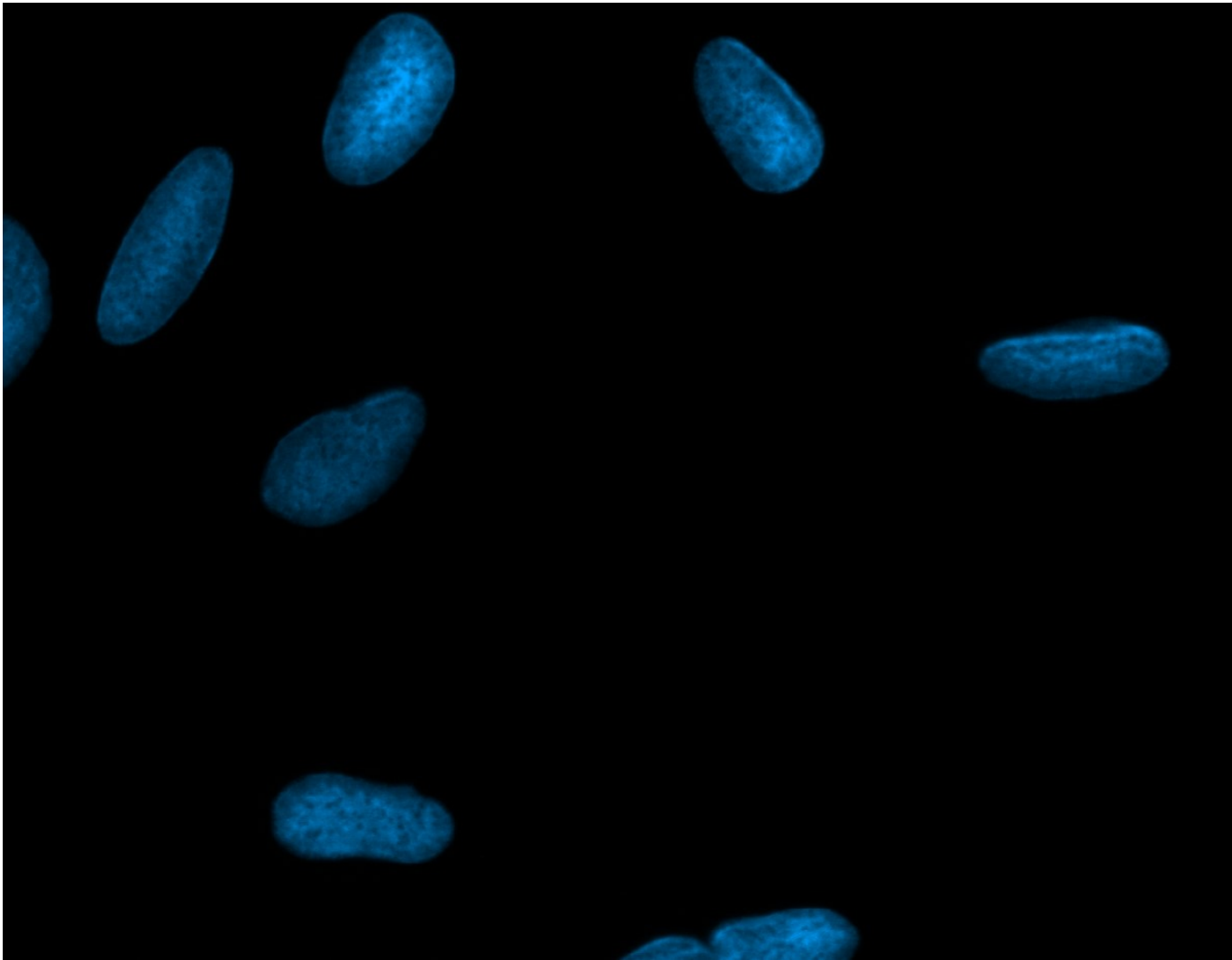
**Image by S. Thakur**

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## SOURCE PROVENANCE

|                                   |                                                                                                                                                |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                      |
| 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                     | 587F4A615836BF0A8F09AD9C38AFC6BD70E4A9EC81C03BBADA9172ACB220F5F5                                                                               |
| Method PDF SHA-256                | F5D517AF13A26B661459FAB974015955EDEDEB01912514869CEE26B73673696D                                                                               |

ORIGINAL IMAGE EVIDENCE / CONTROL



|                 |                                  |
|-----------------|----------------------------------|
| EVIDENCE TIER   | Control                          |
| INSTRUMENT      | ZEISS Axio Observer.Z1/7 Apotome |
| CELL MODEL      | HeLa                             |
| DETECTED DYES   | 405/DAPI; 647                    |
| METADATA VALUES | 32                               |

## STRUCTURED ACQUISITION METADATA / CONTROL

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

| FIELD                        | SOURCE-DOCUMENTED VALUE                                                                                                                                                   |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Platform / configuration     | Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| Objective                    | EC Plan-Neofluar 63X/1.25 oil M27                                                                                                                                         |
| Channels                     | 2                                                                                                                                                                         |
| Scaling (Per Pixel)          | 0.143µm X 0.143µm                                                                                                                                                         |
| Image Size (Pixels)          | 1261 X 980                                                                                                                                                                |
| Image Size (Scaled)          | 180.14µm X 140.00µm                                                                                                                                                       |
| Bit Depth                    | 14 Bit                                                                                                                                                                    |
| Image Center Position        | X: 47.62mm, Y: 45.81mm                                                                                                                                                    |
| Stage Position               | X: 47.62mm, Y: 45.81mm                                                                                                                                                    |
| ROI Center Offset            | X: 1.14µm, Y: 0.00µm                                                                                                                                                      |
| Reflector                    | 50 Cy5   49 DAPI                                                                                                                                                          |
| Beam Splitter                | 660   395                                                                                                                                                                 |
| Filter Excitation Wavelength | 625 - 655   335 - 383                                                                                                                                                     |
| Filter Emission Wavelength   | 665 - 715   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        | 653   353                                                                                                                                                                 |
| Emission Wavelength          | 668   465                                                                                                                                                                 |
| Effective NA                 | 1.25                                                                                                                                                                      |
| Imaging Device               | Axiocam 807 mono                                                                                                                                                          |
| Camera Adapter               | 1X                                                                                                                                                                        |
| Depth of Focus               | 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™ 647 Affinity Purified Rabbit Anti-Mouse, F(ab')<sub>2</sub> (H + L)  
Cat ID# - SA 000066

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® 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 = 2  
Scaling (Per Pixel) = 0.143µm X 0.143µm  
Image Size (Pixels) = 1261 X 980  
Image Size (Scaled) = 180.14µm X 140.00µm  
Bit Depth = 14 Bit  
Image Center Position = X: 47.62mm, Y: 45.81mm  
Stage Position = X: 47.62mm, Y: 45.81mm  
ROI Center Offset = X: 1.14µm, Y: 0.00µm

### 647 -

Reflector = 50 Cy5  
Beam Splitter = 660  
Filter Excitation Wavelength = 625 - 655  
Filter Emission Wavelength = 665 - 715  
Contrast Method = Fluorescence  
Light source = X-Cite Xylis Lamp Intensity @25%  
Exposure Time = 200ms  
Excitation Wavelength = 653  
Emission Wavelength = 668  
Effective NA = 1.25  
Imaging Device = Axiocam 807 mono  
Camera Adapter = 1X  
Depth of Focus = 1.30µm  
Binning Mode = 2,2

## DAPI -

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

## Post Processing - SIM Processing

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

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

## Image Corrections

None

## Control Summary -

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

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

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## Image by J.K. Bennett

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### SOURCE PROVENANCE

|                                   |                                                                                                                                                                           |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Catalog                           | SA-000066                                                                                                                                                                 |
| Stage                             | Control                                                                                                                                                                   |
| Instrument                        | ZEISS Axio Observer.Z1/7 Apotome                                                                                                                                          |
| Microscope configuration          | Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: |
| Structured source metadata values | 32                                                                                                                                                                        |

## SOURCE PROVENANCE

|                    |                                                                  |
|--------------------|------------------------------------------------------------------|
| Metadata source    | PRESERVED_SOURCE_METHOD_PDF_TEXT                                 |
| Cell model         | HeLa                                                             |
| Image SHA-256      | 6FA8C0F087A78ED2D3229745EB20F0366E8D099EF8536142215405E41FC971D7 |
| Method PDF SHA-256 | A89E614333B5C72DB3B0AABDF8A424F4CC03553DE67E78BF80F21BE9FDDA6DF9 |